

This electronic thesis or dissertation has been downloaded from the King's Research Portal at <https://kclpure.kcl.ac.uk/portal/>



Effectiveness of Iron Therapy During Pregnancy and Early Childhood on Infant Developmental Outcomes

Stelle, Isabella

Awarding institution:
King's College London

The copyright of this thesis rests with the author and no quotation from it or information derived from it may be published without proper acknowledgement.

END USER LICENCE AGREEMENT



Unless another licence is stated on the immediately following page this work is licensed

under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International

licence. <https://creativecommons.org/licenses/by-nc-nd/4.0/>

You are free to copy, distribute and transmit the work

Under the following conditions:

- Attribution: You must attribute the work in the manner specified by the author (but not in any way that suggests that they endorse you or your use of the work).
- Non Commercial: You may not use this work for commercial purposes.
- No Derivative Works - You may not alter, transform, or build upon this work.

Any of these conditions can be waived if you receive permission from the author. Your fair dealings and other rights are in no way affected by the above.

Take down policy

If you believe that this document breaches copyright please contact librarypure@kcl.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

Effectiveness of Iron Therapy During Pregnancy and Early Childhood on Infant Developmental Outcomes

Isabella Stelle

A Thesis submitted to King's College London for the degree of
Doctor of Philosophy

Department of Women and Children's Health

King's College London

November 2023

Abstract

Iron deficiency anaemia (IDA) is the most common nutrition deficiency worldwide and the leading cause of morbidity globally from a micronutrient deficiency. Women of reproductive age are more susceptible to iron deficiency due to blood loss and increased iron requirements in pregnancy, while about 43% of infants between six and 59 months of age are reported to be anaemic. Additionally, iron deficiency (ID) is five times more common in low- and middle- income countries (LMICs) and is the leading cause of years lived with disability in these contexts. ID is multifactorial, can be impacted by infection and inflammation, and is more commonly seen in infants in children in LMICs where iron rich foods are less frequently consumed.

Healthy, full-term infants should be born with an iron endowment acting as a buffer for growth and development in the first six months of life, but recent evidence suggests that in areas with high rates of ID this may not be sufficient during the period of exclusive breastfeeding (EBF). While maternal supplementation with iron during pregnancy is recommended by the World Health Organization (WHO), infants may be at risk during the first six months of life when the only practices recommended to prevent anaemia are EBF and delayed cord clamping.

This Thesis explores the gap of iron interventions in early infancy, specifically around impacts to feeding and growth, as well as exploring caregiver acceptability of nutrition interventions in young infants.

This Thesis starts with a systematic review of iron and/or multiple micronutrient (MMN) supplements in infants under six months of age. The objective was to systematically review iron and/or MMN supplementation in healthy, term, normal birthweight infants under six months of age to summarise the evidence and identify any gaps in the literature. Twenty- four trials across 25 locations were included (19 iron and five MMN supplementation trials). Biochemical, growth, morbidity and/or mortality, and neurobehavioral development outcomes were reviewed. LMICs made up 88% (22/25) of the total trial locations. This review found that infants less than six months of age benefit biochemically from early supplementation with iron, but the effect of additional nutrients or MMNs, along with the impacts on growth, morbidity and/or mortality, and neurobehavioral outcomes remain unclear.

Next, a second systematic review exploring the evidence of caregiver perceptions of nutrition interventions in infants and young children under 24 months of age, as well as any side effects and impacts on infant feeding, was undertaken. The objective was to systematically review caregiver acceptability of nutrition interventions in their infants and children (under 24 months of age) to inform the feasibility of future proposed nutrition interventions in infants. While efficacy studies in infants

have shown that early nutrition interventions improve infant nutrition status, to examine whether such interventions can be effectively taken to scale, a better understanding of caregiver acceptability is required. Thirty-seven publications were included with interventions including oral supplementation, food fortification, and nutrition counselling. Caregivers included mothers (83%), fathers, grandparents, and aunts. Eighty-nine percent of studies noted high acceptability (N=33), most notably increased appetite (N=17). Fifty seven percent of studies (N=21) cited low acceptability, commonly from side effects (N=13) such as gastrointestinal, appetite loss and stained teeth. This review supports the need for implementation focused interventions to improve the nutritional status of infants and young children to strengthen sustainability and acceptability, especially in settings with high rates of deficiencies.

A pilot trial conducted among breastfed, young infants (N=101) in rural Gambia (The “Iron Babies” trial) found that being supplemented daily with iron from six to ten weeks of age for 98 days showed improvements in biomarkers, with no difference in adverse events and no reported deaths versus placebo. Work presented in this Thesis went on to explore the relationship between infant feeding and growth within the Iron Babies trial. The objective was to analyse the impacts on nutrition specific outcomes from *Iron Babies* such as the link between supplementation, breastfeeding rates, and anthropometry. Thirty-three percent of infants (N=31) were exclusively breastfed throughout the study. The mean age of cessation of EBF in the iron group was not different to the placebo group and supplementation with iron versus placebo had no impact on whether infants were exclusively breastfed throughout the study duration. There was no difference in mean infant weight or length at baseline, weight or length gains over the study duration, or in the proportion of infants who were underweight, stunted, or wasted between trial arms. There were no negative impacts of early iron intervention on mode of feeding or growth parameters.

To support the quantitative data from the Iron Babies trial, an adjunct qualitative study was conducted to explore acceptability of early iron interventions to infants. The objective was to assess the acceptability of early administration of iron, through qualitative research with local stakeholders and mothers from *Iron Babies*, to inform future scale up and implementation of nutrition interventions in young infants. Generally, caregivers and stakeholders understood the benefits of the early iron intervention and were supportive. However, some concerns were raised around confusion around nutritional messaging and practicalities of taking such an intervention to scale.

The overall aim of this Thesis was to examine if iron supplementation before six months of age in healthy, term, normal birthweight infants, with a focus on low- and middle- income countries,

improved infant outcomes, specifically the health impacts for the infant (breastfeeding and growth) as well as the barriers and associated practicalities of implementing an early intervention.

The research presented in this Thesis highlights the importance of iron interventions in young infants, during a critical period of growth and development, when foetal endowments and nutrition provided through EBF may not be sufficient in areas with high rates of maternal nutritional deficiencies. Additionally, implementation factors, such as maternal acceptability are key for consideration for sustainability and effectiveness of interventions. These findings are a particularly valuable contribution to the literature of iron supplementation in infants under six months of age living in West Africa, where data in this field is scarce.

Isabella Stelle, November 2023

Preface

This Thesis is the result of my own work and does not include any work from collaborations, unless specifically stated. It is not substantially the same as any of my previously submitted work or any work I am concurrently submitting or plan to submit for another qualification, degree, or diploma. It falls within the King's College London (KCL) 100,000 word limit.

The data and material analysed within this Thesis includes data collected from the Iron Babies study, a double blind placebo controlled randomised supplementation trial taking place in rural Gambia from August 2021 until March 2022. My contributions to the Iron Babies trial as well as the other work of this Thesis consisted of:

1. Various courses across the three years to prepare for my systematic reviews, publishing scientific papers, scientific posters, qualitative data collection and analysis, and quantitative data analysis, as well as courses for scientific writing and speaking, and good clinical practice certification.
2. Systematically reviewing the literature on iron (and/or MMN) supplementation in infants under six months of age as well as publishing this manuscript in Wellcome Open and supervising two intercalated Bachelor of Science Medical (iBSc) students in their student projects (Sruthi Venkatesan and Theresa Eke).
3. Preparation leading up to the commencement of the Iron Babies trial at the Medical Research Council (MRC) Unit The Gambia at the London School of Hygiene and Tropical Medicine (MRCG@LSHTM) in August 2021 consisting of the following:
 - a. Drafting and taking the Iron Babies protocol manuscript through to publication in Wellcome Open Research.
 - b. Preparation of full paperwork for the joint Gambian/MRCG@LSHTM and KCL Ethics Committees and for the Scientific Coordinating Committee (SCC) at MRCG@LSHTM for Iron Babies and the qualitative adjunct study (see below).
 - c. Preparation of the paperwork for The Gambian Medical Control Agency application and approval.
 - d. Sourcing, procuring, and delivering the investigational products.
 - e. Developing the Standard Operating Procedures for all Iron Babies related clinical trial activities.
 - f. Working with the data team and my co-sub investigator Mamadou Bah on developing the REDCap database in The Gambia from May-July 2021.
 - g. Staff training in The Gambia along with Mamadou Bah from May-July 2021.

- h. Village sensitisation in The Gambia along with Mamadou Bah from May-July 2021.
- i. Creating and managing the Trial Master File as well as all ensuring all clinical monitoring procedures and checks were sufficient before and throughout the study.
- 4. Remotely supporting the Iron Babies study with Mamadou Bah (based in The Gambia).
- 5. Preparation and management of the Iron Babies qualitative adjunct study consisting of the following:
 - a. SCC and Ethics Committee applications
 - b. Writing, piloting, and revising the qualitative focus group discussion (x4) and interview guides.
 - c. Conducting the focus group data collection (x3) in The Gambia with the qualitative interviewer Mariama Saidykhan (May-July 2021).
 - d. Transcribing the focus group data (x3).
 - e. Remotely supervising Mariama Saidykhan (based in The Gambia) in collecting the maternal focus group discussion and interview data.
 - f. Overseeing the MRCG@LSHTM data services maternal focus group discussion and interviews translation and transcription process.
- 6. Systematically reviewing the literature on primary caregiver's acceptability of nutrition interventions in infants and young children under 24 months of age as well as publishing this manuscript in Public Health Nutrition.
- 7. Analysing and interpreting the qualitative Iron Babies focus group discussion and maternal interview data, as well as publishing these results in Reproductive, Female, and Child Health.
- 8. Checking the baseline demographic, feeding, and anthropometric data for Iron Babies as well as data analysis and statistical interpretation of all data presented.

Papers published from this Thesis:

Stelle I, Venkatesan S, Edmond K and Moore SE. Acknowledging the gap: a systematic review of micronutrient supplementation in infants under six months of age. *Wellcome Open Res.* 2020, 5:238. (See Chapter 3)

Stelle I, Bah M, Silverio SA, Verhoef H, Comma E, Prentice AM, Moore SE and Cerami C. Iron supplementation of breastfed Gambian infants from 6 weeks to 6 months of age: protocol for a randomised controlled trial. *Wellcome Open Res.* 2022, 7:16. (See Chapter 6)

Stelle I, Bah M, Saidykhan M, Verhoef H, Prentice AM, Cerami C, Silverio SA and Moore SE. Maternal acceptability of iron supplementation in young breastfed rural Gambian infants. *Reproductive, Female and Child Health.* 2023. (See Chapter 8)

Stelle I, Kinshella MLW and Moore SE. Caregiver perceptions of nutrition interventions in infants and children under 24 months of age: a systematic review. *Public Health Nutrition*. 2023. (See Chapter 4)

Papers published in conjunction to this Thesis:

Bah M, **Stelle I**, Verhoef H, Saidykhan A, Moore SE, Susso B, et al. Early iron supplementation of exclusively breastfed African infants: a proof-of-principle, placebo-controlled, randomised, double-blinded efficacy trial. medRxiv. 2023:2023.01.12.22284059. (See Appendix 7)

Papers under review for publication for this Thesis:

Stelle I, Bah M, Verhoef H, Moore SE and Cerami C. Daily iron supplementation does not impact on prevalence of exclusive breastfeeding or growth in young breastfed Gambian Infants. Submitted to: Nutrition, Prevention and Health (BMJ).

Related work not included in this thesis:

Stelle I, Kalea AZ, Pereira DIA. Iron deficiency anaemia: experiences and challenges. *Proc Nutr Soc*. 2019;78(1):19-26.

Stelle I, McDonagh LK, Hossain I, Kalea AZ, Pereira DIA. The IHAT-GUT Iron Supplementation Trial in Rural Gambia: Barriers, Facilitators, and Benefits. *Nutrients*. 2021;13(4).

Acknowledgments

The work of this PhD goes beyond the words on this page or papers published, but to an experience grounded in sound and ethical scientific research and one that will shape my future and the future of the study participants thanks to none other than my supervisor, Prof Sophie Moore. Sophie, thank you first and foremost for teaching me what it means to have a passion for what one does. You lead by example, and it inspires your students and colleagues more than you can imagine. Thank you for teaching me what ethical scientific practices look like, you never take for granted how important your work is and what it means to be working with human lives. Thank you for being the role model you are- patient and giving me space to form my own ideas and path, but also guiding me and pushing me when I needed it. Few people could have led my transition into motherhood while completing a PhD like you did. Thank you for being an example that mothers really can do it all, for making juggling work, travel, kids, school pickups, and endless sports games while also being a team leader look easy. I hope to achieve what you have and continue to achieve. Thank you for trusting in me from our first meeting and laying a foundation for me. I would not be here without your unwavering support and guidance.

I am indefinitely grateful to Dr Carla Cerami, the Iron Babies PI and my Gambian tour guide and mother. Thank you for letting me nurture your baby that was Iron Babies and for taking me under your wing while in The Gambia. Your passion for your work and helping others shows no bounds. You care deeply for what you do, and you touch the lives of everyone who crosses your path. Thank you for helping me laugh and have fun along the way when it felt like things were slowly crumbling around us. I will forever cherish working on Iron Babies with you, my time in The Gambia, and our friendship.

I would not be where I am today if it were not for my fearless female scientist role models from my MSc: Dr Dora Pereira and Dr Anastasia Kalea. Thank you for being my steppingstones into the realm of public health and The Gambia. Thank you for paving the way for me. I admire you both endlessly.

A big thank you to Mamadou Bah, my co sub-investigator in Iron Babies and friend. Thank you for welcoming me into your home with open arms and giving me a taste of your beautiful culture - your wife's benachin is second to no other. Thank you for making Iron Babies a success. I will always look back on our time in Keneba with fondness. I look forward to returning the favour of your hospitality.

To Prof Andrew Prentice. I will never forget the first time we met in 2018 in Basse. Thank you for all you do in the field of global health and nutrition. Thank you for taking me under your wing in The Gambia – you and Carla are the greatest of hosts. Santamba is a special place on this earth!

To Prof Hans Verhoef. Thank you for keeping our statistics in line. You care deeply about Iron Babies and your students and your insight and thought into the project made all the difference.

Thank you to the entire Iron Babies team: Dr Susso; Mariama Saidykhan - for your fabulous knowledge and connection to your culture, the qualitative study would not have been possible without you; the Iron Babies nurses: Morris and Abdou; to our field team: Kebba, Lamin, Buba and Kebba, Fatou and, Foday; Mortala Ndow, Babubcarr Touray, Ebrima Comma, Amie Demba, the Clinical Trial Support Office, the Transport Team, Data Services, the Keneba Lab team, and all the MRCG teams (IT, mechanics, security, groundsmen, cleaning, and hospitality among others) that helped our trial become a reality and for your dedication to improving the health and lives of your communities. Finally, thank you to all our Iron Babies as well as all the qualitative data participants (mothers, the local Iron Babies staff, the local non- Iron Babies staff, and the National Nutrition Agency). Thank you especially to all the mothers for entrusting us with your infants.

Thank you to my Thesis Advisory Committee: Prof Peter Von Dadelzen - for making sure my PhD process was smooth and successful and for being an inspiration in the realm of Women and Children's Health; Prof Jane Sandall and Dr Phil James - you are both enormous inspirations, your knowledge and willingness to help students learn and excel shows no bounds, and I am eternally grateful for your guidance. Phil, I could not have created the Trial Master File and gotten MCA approval without you!

Thank you to Dr Karen Edmond, my second supervisor, while our time at KCL together was short, your work in the realm of Global Health is an inspiration.

To Sergio A Silverio, the qualitative guru. I could not have done the Iron Babies qualitative component if it were not for your knowledge and guidance, thank you.

Thank you to the KCL Global Health Nutrition team: Jenny, Sam M, Andy, Sam B, Jia Dai, and Maggie. You have made work a joy. Thank you for always being willing to lend a helping hand.

To my Becket house girls – Claire, Mel, Reena. I came into work excited knowing it meant having lunch with you all. I will always cherish our chats and catch ups.

To all those that I failed to mention but came across during my PhD work. The amount of guidance and generosity with others time that I experienced is something I will always remember and has inspired me to help other students and colleagues to the best of my ability.

And lastly, to my family – Mom, Dad, Tom, Sissy. You all are my biggest cheerleaders and have been patient with me while I continued my studies. To my grandmother, Nonna, whom my interest in Sub-Saharan Africa is thanks to. And to my husband, our little boy - Charles and Oliver, and our soon to be daughter. Thank you for bringing joy and purpose to my life. Charles your ability to consistently be the best dad made this PhD possible.

Contents

1	Introduction	18
1.1	Positioning the thesis	18
1.1.1	Importance of iron	19
1.1.2	The outstanding issue.....	20
1.1.3	Research question	20
1.2	Thesis outline	21
1.2.1	Chapter 1: Introduction	21
1.2.2	Chapter 2: Background	21
1.2.3	Chapter 3: Quantitative systematic review	21
1.2.4	Chapter 4: Qualitative systematic review.....	21
1.2.5	Chapter 5: Setting.....	22
1.2.6	Chapter 6: Methods - protocol paper.....	22
1.2.7	Chapter 7 & 8: Results- quantitative and qualitative research; the <i>Iron Babies</i> trial...	22
1.2.8	Chapter 9: Discussion	23
1.3	Covid-19 impact statement	24
2	Background	25
2.1	Definition of iron deficiency	25
2.1.1	Anaemia.....	25
2.1.2	Iron deficiency anaemia.....	25
2.2	The global toll of iron deficiency and anaemia	26
2.3	Biochemistry of iron metabolism	26
2.3.1	Iron absorption and metabolism.....	26
2.3.2	Hepcidin.....	27
2.3.3	Iron overload.....	28
2.4	Biomarkers of iron deficiency and anaemia	28
2.4.1	Markers of iron deficiency anaemia.....	29
2.4.2	Markers of iron deficiency erythropoiesis.....	30
2.4.3	Iron depletion.....	32
2.4.4	Other biomarkers of iron deficiency and anaemia.....	33
2.4.5	Measuring iron status in early infancy	33
2.5	Iron and physiology	34
2.5.2	Iron interactions with other micronutrients.....	39
2.6	Iron requirements in women, infants, and children	40
2.6.1	Women of reproductive age and in pregnancy	40

2.6.2	Infants and children.....	40
2.6.3	Early infancy	41
2.6.4	Early childhood.....	42
2.7	Iron interventions in women, infants, and children	43
2.7.1	Population level – World Health Organisation	43
2.7.2	High income regions - United Kingdom, Europe and the United States	43
2.7.3	Low- and middle- income regions – The Gambia and India	44
2.7.4	Women of reproductive age	50
2.7.5	Pregnancy	51
2.7.6	Early infancy (0-6 months of age).....	51
2.7.7	Six months to five years of age	52
2.7.8	Interventions to improve iron status.....	53
2.8	The outstanding issue	56
2.9	Aims and objectives	57
3	Acknowledging the gap: a systematic review of micronutrient supplementation in infants under six months of age	58
3.1	Introduction	58
3.2	Author roles	59
3.3	Published paper 1: Acknowledging the gap: a systematic review of micronutrient supplementation in infants under six months of age	59
4	Caregiver perceptions of nutrition interventions in infants and children under 24 months of age: a systematic review	79
4.1	Introduction	79
4.2	Author roles	79
4.3	Published paper 2: Caregiver perceptions of nutrition interventions in infants and children under 24 months of age: a systematic review	80
5	Setting.....	96
5.1	The Gambia	96
5.1.1	Demographics	96
5.1.2	History.....	97
5.1.3	Economy	97
5.1.4	Education	98
5.1.5	Climate.....	98
5.1.6	Maternal health.....	99
5.1.7	Child health	100
5.2	Study setting	103
5.2.1	MRCG@LSHTM Keneba	103

5.2.2	Soma	103
6	Methods	105
6.1	Introduction	105
6.2	Author roles	106
6.3	Published paper 3: Iron supplementation of breastfed Gambian infants from 6 weeks to 6 months of age: a randomised controlled trial	106
7	Results: Daily iron supplementation does not impact on rates of exclusive breastfeeding or growth in young breastfed Gambian infants	122
7.1	Introduction	122
7.2	Author roles	123
7.3	Abstract:	124
7.4	Background	125
7.5	Methods	127
7.5.1	Study setting	127
7.5.2	Study design and field implementation.....	127
7.5.3	Consent and ethics	128
7.5.4	Data processing and analysis	128
7.6	Results	129
7.6.1	Infant feeding practices	131
7.6.2	Anthropometry	133
7.6.3	Impact of mode of feeding on infant growth.....	135
7.7	Discussion	136
7.8	Conclusion	137
8	Results: Maternal acceptability of iron supplementation in young breastfed rural Gambian infants	139
8.1	Introduction	139
8.2	Author roles	139
8.3	Published paper (4): Maternal acceptability of iron supplementation in young breastfed rural Gambian infants	140
9	Discussion.....	155
9.1	Key findings	155
9.2	Cautions around early interventions	156
9.3	Caregiver acceptability of early interventions	157
9.4	Continuum of care	158
9.4.1	Integrating nutrition programmes	159
9.5	Remaining questions	160
9.5.1	Infants under six months of age.....	160

9.5.2	Acceptability of iron interventions in young infants	161
9.5.3	Across the life course.....	162
9.6	Strengths and limitations	163
9.7	Recommendations for programmes, policy, and research	164
9.8	Conclusion	165
Appendices.....		166
Appendix 1 Additional files from Published paper 1 (Chapter 3): Acknowledging the gap: a systematic review of micronutrient supplementation in infants under six months of Age		166
Appendix 2 Additional files from Published Paper 2 (Chapter 4): Caregiver perceptions of nutrition interventions in infants and children under 24 months of age: a systematic Review		187
Appendix 3 Additional files from Published Paper 3 (Chapter 6): Iron supplementation of breastfed Gambian infants from 6 weeks to 6 months of age: protocol for a randomised controlled trial		236
Appendix 4 Additional files from Published Paper 4 (Chapter 8): Maternal acceptability of iron supplementation in young breastfed rural Gambian infants.		249
Appendix 5 Micronutrient Forum (2020) and School of Life Course Sciences student poster (year 1)		262
Appendix 6 School of Life Course Sciences student poster (year 2)		263
Appendix 7 MedRxiv Iron Babies main outcomes manuscript (pre-print)		264
REFERENCES.....		293

Figure 1. Serum iron levels in breastfed Gambian infants.	19
Figure 2. <i>Iron absorption and metabolism.</i>	27
Figure 3. Calculating transferrin saturation.....	31
Figure 4. <i>The Gambia (red) within West Africa.</i>	96
Figure 5. Population pyramid, The Gambia	97
Figure 6. Monthly trends of small for gestational age and preterm birth, The Gambia.	99
Figure 7. Three main MRCG@LSHTM study sites and Soma, The Gambia	103
Figure 8. Jarra Soma Regional Hospital and Soma.	104

Table 1. Iron biomarkers and levels of deficiency.	29
Table 2. Human haemoglobin cut-offs to diagnose anaemia.	30
Table 3. Other biomarkers of iron deficiency and anaemia.	33
Table 4. Iron requirements in women.	40
Table 5. Iron requirements in infants and children.	41
Table 6. Iron supplementation recommendations.	46
Table 7. Strategies to prevent and rectify iron deficiency in women of reproductive age, infancy, and childhood.	50
Table 8. Expanded Programme on Immunisation vaccine schedule, The Gambia.	100

Abbreviations

AGP	Alpha-1-acid glycoprotein
ANOVA	Analysis of variance
BF	Breastfeeding
CASP	Critical Appraisal Skills Programme
CDC	Centre for Disease Control and Prevention
CFs	Complementary foods
CHr	Reticulocyte Hb content
CRP	C-reactive protein
DCytB	Duodenal cytochrome B
DHS	Demographic and Health Survey
DMT-1	Divalent metal ion transporter 1
DSMB	Data Safety Monitoring Board
EBF	Exclusive breastfeeding
EED	Environmental enteric dysfunction
EFRE	Erythroferrone
EPI	Expanded Programme on Immunisation
EPO	Erythropoietin
ESPGHAN	European Society for Paediatric Gastroenterology Hepatology and Nutrition
Fe II	Ferrous iron
Fe III	Ferric iron
FGD	Focus group discussion
GEMS	Global Enteric Multicenter Study
GDP	Gross domestic profit
HAZ ¹	Height-for-age z score
Hb	Haemoglobin
HCP-1	Haem carrier protein 1
HIC	High-income country
IB	Iron Babies
iBSc	Intercalated Bachelor of Science
ID	Iron deficiency
IDA	Iron deficiency anaemia
IDI	Individual interview
IFA	Iron folic acid
IHAT	Iron hydroxide adipate tartrate
IL-6	Interleukin-6
IPT	Intermittent preventative treatment
IRN	Iron
IV	Intravenous
KCL	King's College London
kg	Kilogram
LAZ ¹	Length-for-age z-score
LBW	Low birth weight
LMICs	Low-and-middle-income countries
LNS	Lipid-based nutrient supplement
LRR	Lower-River Region
MCH	Mean corpuscular haemoglobin
MCV	Mean corpuscular volume

mg	Milligram
MMN	Multiple micronutrient
MNPs	Micronutrient powders
MRC	Medical Research Council
MRCG@LSHTM	Medical Research Council Unit The Gambia, at the London School of Hygiene and Tropical Medicine
MSD	Moderate to severe diarrhoea
NaNA	National Nutrition Agency
PI	Principal Investigator
PRISMA	Preferred reporting items for systematic reviews and meta-analysis
PT(B)	Preterm (birth)
PX	Participant (followed by a number)
RCT	Randomised controlled trial
RoB	Risk of bias
SCC	Scientific Coordinating Committee
SD	Standard deviation
SDG	Sustainable Development Goal
SF	Serum ferritin
sTfR	Soluble transferrin receptor
TSAT	Transferrin saturation
TIBC	Total iron binding capacity
UIBC	Unsaturated iron binding capacity
UK	United Kingdom
UK GDPR	United Kingdom General Data Protection Regulation
UNICEF	United Nations Children's Fund
UNFPAF	United Nations Population Fund
US(A)	United States (of America)
VLBW	Very low birth weight
WAZ	Weight-for-age z score
WHO	World Health Organisation
WHZ	Weight-for-height z score
WLZ	Weight-for-length z score
ZPP	Zinc protoporphyrin

¹ Both LAZ/HAZ refer to a head to foot measurement for age. LAZ is used from zero to two years of age when the infant/child is measured lying down (recumbent), referred to as length. HAZ is used from two years of age onwards, when the child is measured standing. Both WHZ/WLZ refer to a weight for head to foot measurement. WLZ is used from zero to two years of age when the infant/child's height is measured lying down.

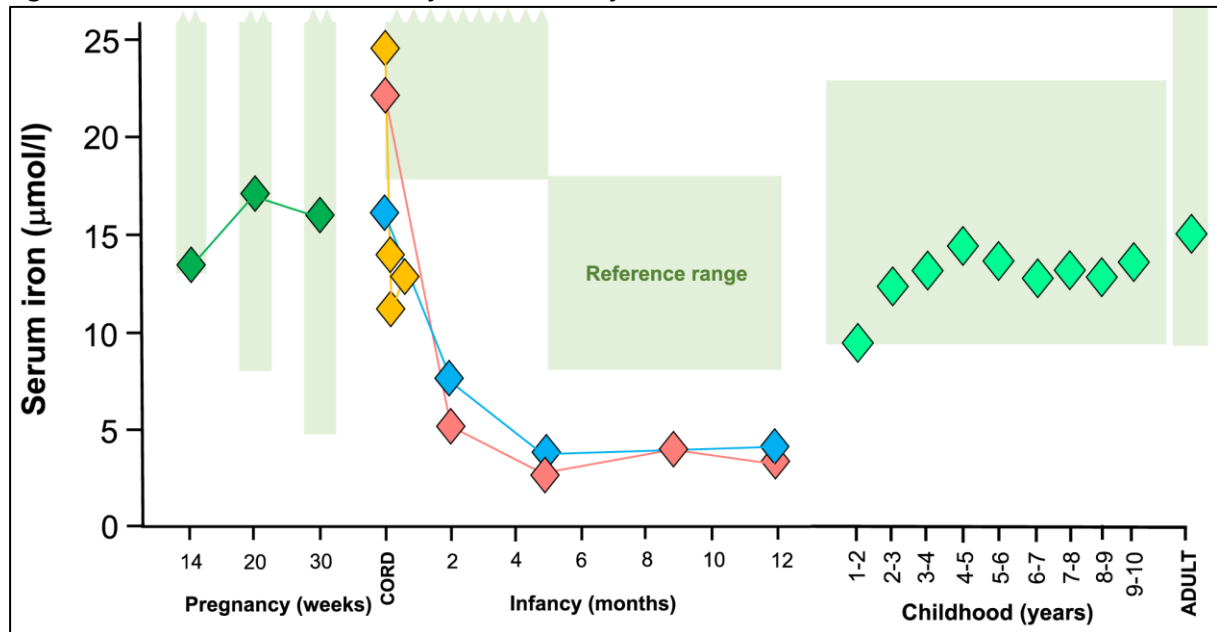
1 INTRODUCTION

1.1 POSITIONING THE THESIS

IDA is estimated to affect 1.2 billion people worldwide, with about 43% of children between the ages of six to 59 months reported to be anaemic (1). This makes IDA the largest nutritional deficiency disorder worldwide. The third Sustainable Development Goal (SDG) set out by the United Nations in 2015 is to “*ensure healthy lives and promote wellbeing for all at all ages*”. However, despite targeted global policies focused on improving iron status, ID remains the leading cause of years lived with disability in LMICs and is responsible for more than 120,000 maternal deaths per year (1). Anaemia is five times more common in LMICs than high-income countries (HICs) and IDA remains in the top five leading causes of death worldwide since 1990 (1, 2). One of the six global nutrition targets for 2025 by the World Health Assembly Resolution is a 50% reduction of anaemia in women of reproductive age (3).

Healthy, full-term infants are born with an iron endowment, accumulated in the foetal liver during gestation and acting as a buffer for growth and development in the first six months of life (4). However, recent evidence suggests that this endowment may not always be sufficient to meet the infant’s needs across the period of EBF, especially in contexts where rates of maternal anaemia are high. Additionally, breastmilk concentrations alone cannot meet infant iron requirements at any stage of lactation (5). In an analysis of 317 breastfed rural Gambian infants, there was a rapid decline in haemoglobin (Hb) and serum ferritin, both markers of iron status, within the first few weeks of life (Figure 1, showing serum iron) (6, 7). This leaves these infants vulnerable as EBF is often the only practice recommended by the WHO to prevent anaemia in infants under six months of age (8).

Figure 1. Serum iron levels in breastfed Gambian infants.



Mean values. Shaded green = reference ranges (9). Diamonds: dark green = pregnancy (10); yellow = neonates (11); blue = infancy (6); red = infancy (analysis of stored samples) (12); light green = childhood and adulthood (13).

From: Stelle I, Bah M, Silverio SA, et al. Iron supplementation of breastfed Gambian infants from 6 weeks to 6 months of age: protocol for a randomised controlled trial. *Wellcome Open Res.* 2022. <https://doi.org/10.12688/wellcomeopenres.17507.1> (14)

1.1.1 Importance of iron

In women of reproductive age, iron is required for tissue homeostasis and adaptation to the changing environment such as blood loss, infection, pregnancy, and nutritional deficiencies (15). Iron is important in pregnancy to prevent low birth weight (under 2,500 grams) (LBW) and preterm birth (less than 37 weeks gestation) (PTB) (16). Additionally, low levels of iron in pregnancy are associated with an increased risk of maternal and child mortality from infection (16). In early childhood, iron is required to support normal physiology, with the demand for iron at levels much higher than in adulthood due to rapid increases in blood volume and muscle and tissue mass, and for normal cognitive, behavioural, and motor function development (4, 17-20). From infancy to childhood, limited iron leads to a decrease in growth related blood volume expansion, causing iron restricted erythropoiesis, a common outcome in LMICs (4). Additionally, in such settings, high risk of infection often co-exists with nutritional deficiencies, such as ID (4). Overall, IDA is associated with increased risk of impaired immunity, serious morbidity, poor pregnancy outcomes, and poor motor function and mental development in children leading to lower educational attainment and career progression (21, 22).

1.1.2 The outstanding issue

During pregnancy the use of daily oral iron and folic acid (IFA) supplementation (30 - 60 milligrams [mg] of elemental iron and 0.4 mg of folic acid, daily) is recommended by the WHO to prevent maternal anaemia, puerperal sepsis, LBW, and PTB (23). Further details on iron supplementation in maternal and child health are given in Section 2.7 of Chapter 2. However, the long-term efficacy of maternal supplementation during pregnancy for infant iron status is less clear (16). For young children between the ages of six and 23 months, in areas with an IDA prevalence over 40%, the WHO recommends daily iron supplementation (10 – 12.5 mg elemental iron) for three consecutive months per year (24). This may miss a critical window for development from birth to six months of age, especially in countries where prolonged EBF is an infant's main source of nutrition, as breastmilk is low in iron, and previous studies have shown deficiencies at this young age (4, 25).

This Thesis sets out to explore the efficacy of iron interventions in early infancy and the acceptability of these interventions from a caregiver's perspective. The field-based research presented here was conducted in The Gambia, West Africa, a low-resource setting with high rates of ID.

1.1.3 Research question

The overarching research question addressed in this Thesis is: does early initiation of iron supplementation before six months of age in term infants, with a specific focus on low-resource settings, improve infant outcomes and are such interventions acceptable to caregivers? The field work for this Thesis examined the health impacts of an early iron intervention for the infant (breastfeeding and growth) as well as the barriers and associated practicalities of implementing such an intervention.

1.2 THESIS OUTLINE

This Thesis uses a mix of a traditional PhD Thesis writeup along with an “*alternative but equivalent route of submission*”¹ by including peer-reviewed publications. Each published paper includes an Introduction, Methods, Results, and Discussion section, except for the published protocol paper which consists of an Introduction, Protocol, and Discussion section.

This Thesis consists of nine Chapters. The first of which is this current Chapter, the Introduction.

1.2.1 Chapter 1: Introduction

This first Chapter introduces the thesis by positioning it and contains a Covid-19 impact statement.

1.2.2 Chapter 2: Background

This first research Chapter provides the background information on iron, its importance, consequences of its deficiency, and its global impact, as well as supplementation guidelines. It concludes with the aims and objectives of this PhD.

1.2.3 Chapter 3: Quantitative systematic review

This Chapter includes a peer-reviewed quantitative systematic review published in Wellcome Open Research, titled *Acknowledging the gap: a systematic review of micronutrient supplementation in infants under six months of age* (Stelle et al 2020). It reviews micronutrient interventions in term infants under six months of age, with a specific focus on iron supplementation.

1.2.4 Chapter 4: Qualitative systematic review

This Chapter includes a peer-reviewed qualitative systematic review published in Public Health Nutrition, titled *Caregiver perceptions of nutrition interventions in infants and children under 24 months of age: a systematic review* (Stelle et al 2023). It examines caregivers’ perceptions of nutrition interventions delivered to infants and children under 24 months of age.

The following Chapters (5-8) focus around a pilot intervention trial in The Gambia, the Iron Babies Trial. The primary outcome of Iron Babies (iron status) is not included as a Chapter in this Thesis, but can be found in Appendix 7. The secondary outcomes of breastfeeding and growth, analysed for this Thesis (Chapter 7), consists of data from the infants enrolled in Iron Babies. In addition to this, a

¹ See King’s College London *Guidelines on Submitting a Thesis Incorporating Publications* available at <https://www.kcl.ac.uk/campuslife/acservices/researchdegrees/students/guidelines-on-submitting-a-thesis-incorporating-publications.pdf>

qualitative study assessed the acceptability of the iron intervention in Iron Babies, through focus group discussions with key stakeholders (not enrolled in Iron Babies), as well as individual interviews with mothers whose infants were enrolled in Iron Babies (Chapter 8). Iron Babies is a proof of concept trial, so if successful, the aim is to conduct a larger, long term trial including behavioural, cognitive, and immune assessments (not included in this Thesis).

1.2.5 Chapter 5: Setting

This Chapter consists of the settings for the quantitative and qualitative components of the results of this Thesis. Data for this PhD was collected during a double-blind, placebo controlled, pilot randomised trial, known as the 'Iron Babies' trial, based at the MRCG@LSHTM.

1.2.6 Chapter 6: Methods - protocol paper

This Chapter includes a peer-reviewed protocol paper published in Wellcome Open Research, titled *Iron supplementation of breastfed Gambian infants from 6 weeks to 6 months of age: protocol for a randomised controlled trial* (Stelle et al 2022). It is the published protocol paper for the Iron Babies trial and includes both the quantitative and qualitative methods used for the results of this Thesis.

1.2.7 Chapter 7 & 8: Results- quantitative and qualitative research; the *Iron Babies* trial

The results section consists of Chapters 7 and 8 of this Thesis. A portion of the quantitative results of the Iron Babies trial make up Chapter 7, and the qualitative study of Iron Babies makes up Chapter 8 of this Thesis. The primary outcomes of Iron Babies are published elsewhere (See Appendix 7). All data for these two Chapters was collected in The Gambia through MRCG@LSHTM.

1.2.7.1 Chapter 7: Quantitative

This Chapter looks at a portion of the nutrition outcomes from the pilot trial outlined in Chapter 6. Specifically, if early iron supplementation with daily paediatric drops in six to ten week old infants for 98 days impacts breastfeeding rates and anthropometry. This manuscript is under review for publication.

1.2.7.2 Chapter 8: Qualitative

This Chapter includes a peer-reviewed original research article published in Reproductive, Female and Child Health, titled *Maternal acceptability of iron supplementation in young breastfed rural Gambian infants* (Stelle et al 2023). This Chapter looks at qualitative data collected alongside the pilot trial. Given the nature of the main study being an efficacy trial, it was not possible to assess the full implementation and scale up of such a trial to the real world setting. Instead we assess whether the intervention proposed in the pilot trial is acceptable through focus group discussions (FGDs) with local stakeholders and individual interviews (IDIs) with mothers (the main care providers).

1.2.8 Chapter 9: Discussion

The final Chapter summarises, critiques, and contextualises this Thesis in relation to other literature in the field. It provides considerations for policy recommendations as well as looking ahead to future practice and research.

1.3 COVID-19 IMPACT STATEMENT

Early 2020 brought about significant changes to all our lives that were unexpected due to the onset of the Covid-19 pandemic. Covid-19 began impacting our lives here in the United Kingdom (UK) from about month nine of my PhD. The original research plan for my PhD was to live in rural Gambia about a year into my project and run a double-blinded, placebo controlled pilot trial at MRCG@LSHTM. This would have placed me on the ground for about nine months, overseeing the day to day activities of the trial. From March 2020, the UK went into a national lock-down with all but essential travel banned. Shortly after, The Gambia locked its borders to all foreigners to mitigate the pandemic. At the time, MRCG@LSHTM paused all non-essential research, and their resources were shifted towards the Covid-19 response and Covid-specific research in The Gambia. Not only was my travel to The Gambia uncertain, but likewise, when we would be able to begin the trial. As a study team, we decided the most logical decision was to take on another locally based PhD student (Mamadou Bah, MB) to oversee the day to day activities of our trial for when it did finally commence due to the uncertainty around the boarders opening. At this point, I had already written the trial protocol and began the procurement of the iron, the local and UK ethics applications, approval to supplement infants from The Gambian Medicines Control Agency, drafting the staff training and protocol procedure documents, and the process of MRCG@LSHTM clinical trial study start approvals. MB was then charged with tasks such as the database development, and later staff recruitment and training, as well as daily management of the field activities. As time progressed it became evident that Covid-19 would be impacting us for months to come, and that if done safely with respect to the pandemic, our research should go ahead. In May 2021, I was able to travel to The Gambia to meet the study team and get the final pieces in place for our study to start. In July, we received the final study start approval and in August, we recruited our first infants to the study. Working closely with MB has allowed this study to progress in these unforeseen circumstances and without his help, we would not be where we are today. Given that the primary endpoint of the pilot trial is now used for MB's PhD project, we have added a much more in-depth qualitative aspect to this PhD and conducted two systematic reviews during the time that I was originally intended to be in The Gambia. Additionally, I more specifically focus on the secondary outcomes from the pilot trial: breastfeeding and anthropometry. While the circumstances around my time in The Gambia are unfortunate, with much guidance from Dr Moore as well as my supervisory team and the Thesis Advisory Committee, we were able to pivot this PhD in a logical way that still allowed me to complete my PhD with relevant outputs.

2 BACKGROUND

2.1 DEFINITION OF IRON DEFICIENCY

ID (full definition provided below) can be caused by inadequate iron intake or absorption, increased requirements during growth, and excessive losses (26). ID creates a prolonged negative iron balance. Women of reproductive age are specifically at risk due to blood loss from menstruation and the iron loss from pregnancy and childbirth (26). Additionally, infants and children are at heightened risk of ID due to rapid growth, inadequate iron supplies during the period of EBF, and often low iron content in weaning diets.

ID commonly develops in three stages: iron depletion, iron-deficient erythropoiesis, and lastly, IDA (20). Iron depletion is commonly measured by serum ferritin, indicating iron stores (20). However, this is an acute phase reactant and makes measurement in the presence of inflammation or infection difficult. During iron-deficient erythropoiesis, soluble transferrin receptor (sTfR) increases due to increased need for iron stores, as well as zinc protoporphyrin (ZPP), whereas reticulocyte Hb decreases (20). With IDA, Hb concentration decreases, therefore decreasing red blood cell concentration (20). In children under five years of age, ID is defined as a serum ferritin concentration of less than 12 µg/L (27). These and other biomarkers of ID are further discussed in Section 2.4.

2.1.1 Anaemia

Anaemia, as defined by the WHO, is a condition in which the number of red blood cells or the Hb concentration within them is lower than normal (28). This leads to a decreased capacity to transport oxygen to the body's tissues. Anaemia causes fatigue, weakness, dizziness, drowsiness, and shortness of breath among other symptoms. In children, consequences include slower growth, reduced school achievement, impaired motor and cognitive development, and increased morbidity from causes such as diarrhoea and acute respiratory infections (29). Anaemia is multifaceted in its pathology. It is most commonly caused by nutritional ID, but also deficiencies of folate and vitamins B12 and A, as well as haemoglobinopathies and infectious diseases such as malaria, tuberculosis, human immunodeficiency virus and parasitic infections (28). Women and children, particularly in LMICs, are at higher risk of anaemia.

2.1.2 Iron deficiency anaemia

IDA is the most common form of anaemia. When iron stores are being used at a faster rate than they are being replenished, the supply of iron to the tissues becomes diminished, leading to IDA (30). IDA

can be diagnosed clinically through fatigue and pallor (30). The WHO diagnoses IDA in children between six and 59 months of age as a Hb level below 11.0 g/dL (27).

2.2 THE GLOBAL TOLL OF IRON DEFICIENCY AND ANAEMIA

The WHO estimates 42% of pregnant women, 30% of women of reproductive age, and 47% of children from birth to five years of age to be anaemic (31). In 2011, anaemia was estimated to affect 2.75 million children worldwide and ID is estimated to account for about half of this global toll (32-34). This burden is much higher in LMICs, with about 97% of deaths from IDA being in LMICs (34). The much greater burden in LMICs of anaemia, besides ID, is likely to reflect diverse aetiologies, such as inflammation and infection, inherited erythrocyte disorders such as sickle cell anaemia and thalassaemia, and other micronutrient deficiencies (4).

While the WHO considers serum ferritin the most useful index for ID in a global health context, ferritin is an acute phase reactant and may underestimate ID rates in areas with high rates of infection and inflammation (26). Serum ferritin and other biomarkers of ID are further discussed in Section 2.4.

2.3 BIOCHEMISTRY OF IRON METABOLISM

Iron is crucial for energy production and DNA synthesis in muscle cells (as myoglobin) and for oxygen transport. Iron's ability to switch between ferric and ferrous forms, discussed below, allows these processes to take place. However, excess iron can catalyse to form reactive oxygen species, damaging cellular components such as lipid membranes (35). Given that iron is not actively excreted by the human body, iron absorption is tightly regulated to avoid iron overload (25).

2.3.1 Iron absorption and metabolism

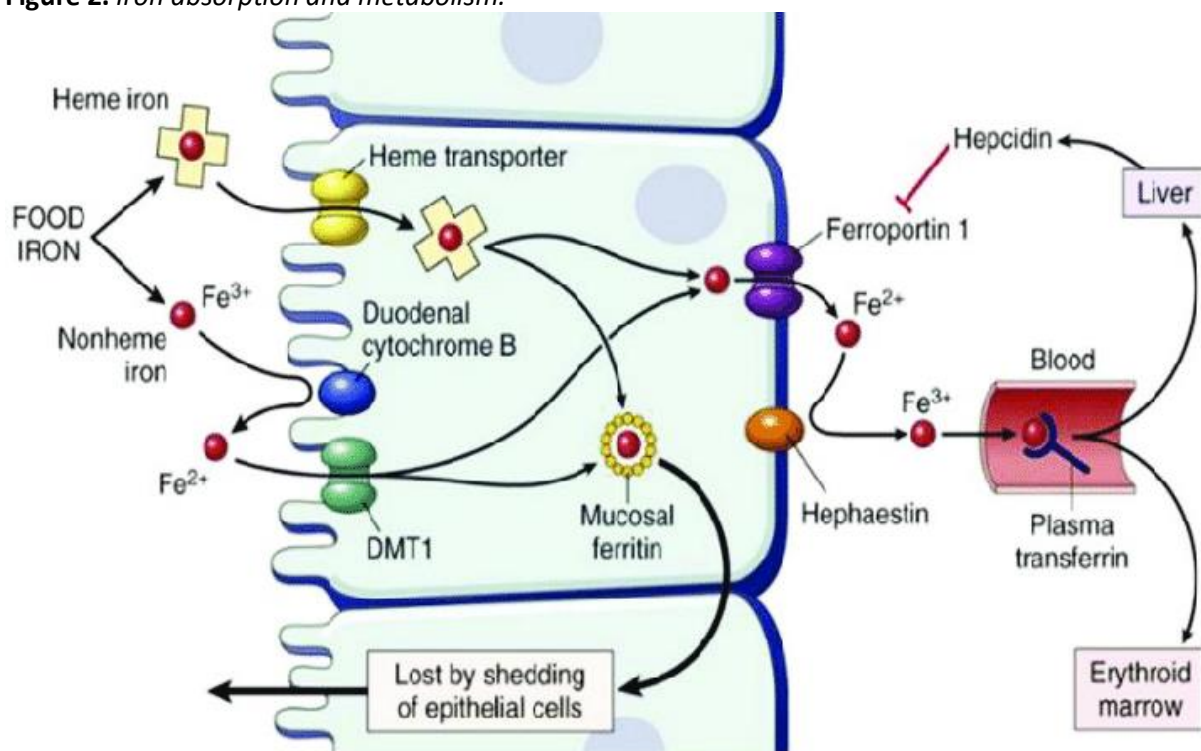
Iron homeostasis is maintained through iron storage, erythrocyte iron reutilisation, and regulation of iron absorption (30). When iron is deficient, absorption increases, and when it is sufficient, absorption is limited (36). If iron is required by the human body, it is released from enterocytes into the blood stream and if it is redundant, iron is stored as ferritin in enterocytes and as hemosiderin in the liver, spleen, and bone marrow (30).

Iron absorption begins at the apical side of enterocytes in the duodenum and upper jejunum at a rate of about 1-2mg of iron/day (Figure 2) (37). Ferric iron (Fe III) is reduced to ferrous iron (Fe II) via a protein known as duodenal cytochrome B (DCyB) (37). Fe II can then be absorbed into cells via divalent metal ion transporter 1 (DMT-1) (37). Additionally, haem iron (organic iron, found in animal based foods) can be directly absorbed via haem carrier protein 1 (HCP-1) (37). Fe II can either be stored as ferritin inside the enterocyte or can move out of storage and into circulation for absorption via an

iron transport protein, ferroportin (37). On the basolateral surface of the enterocyte, ferroxidase hephaestin oxidises Fe II back into Fe III, circulated via serum (plasma) transferrin which can bind two iron atoms (37).

The protein hepcidin can bind to ferroportin, which is then internalised and metabolised, therefore acting as the main inhibitor of iron transport from enterocytes into blood circulation for absorption (21, 38). The life expectancy of enterocytes is about one to two days, so during their physiological renewal, any iron locked in the cell travels through the gut as unabsorbed iron and is excreted in the faeces (39). Hepcidin is fast acting. In animal models, when hepcidin was injected, serum iron levels fell by approximately 80% within one hour (40).

Figure 2. Iron absorption and metabolism.



From: Abera Teka T. Factors Affecting Iron Absorption and Mitigation Mechanisms: A review. International Journal of Agricultural Science and Food Technology. 2019. (41)

2.3.2 Hepcidin

As mentioned, hepcidin acts as the main inhibitor of iron transport from cells into blood circulation for absorption (21, 38). Further details of hepcidin's role in iron homeostasis is detailed below.

2.3.2.1 Hepcidin and erythropoiesis

Erythropoiesis, the production of red blood cells, requires high amounts of iron (4). Erythropoietic expansion stimulates the production of erythroferrone, a hepcidin suppressive hormone found in immature red blood cells (42). An increase in iron requirements and consumption reduces the hepcidin-stimulatory signal from serum iron (43). Additionally, when circulating blood oxygen levels

are low, hepcidin can be suppressed (44). These mechanisms inhibit hepcidin production, allowing iron stores to be released to supply erythropoiesis (4). When hepcidin is low, duodenal iron absorption is increased due to the upregulation of some of the genes involved in iron absorption (44). Overall, multiple physiological signals are involved in hepcidin regulation to ensure adequate iron supply, while still managing iron loading and infection (4).

2.3.2.2 *Hepcidin and inflammation*

High levels of hepcidin are seen during chronic infection and inflammation (45, 46). Hepcidin is primarily regulated by interleukin-6 (IL-6), among other interleukin cytokines (47-49). During infection and inflammation, hepcidin blocks the release of iron into the blood stream. This can cause hypoferremia, a decrease in serum iron, which is protective against extracellular bacterial infections (50-52). Given that ferroportin is hepcidin mediated, during infection and inflammation the downregulation of ferroportin may further contribute to anaemia (53). Ferroportin inhibition leads to iron being locked inside enterocytes, unable to be absorbed (21, 37). These two responses contribute to anaemia of inflammation through limiting the iron supply to the erythron (54). Further details of inflammation and iron are discussed in Section 2.5.1.5.2.

2.3.3 Iron overload

Iron overload is when the rate of iron uptake exceeds that of release from iron stored in the liver, leading to increased hepatic storage. Additionally transferrin becomes saturated with excess iron.

Certain genetic conditions are known to cause enhanced iron absorption such as hereditary forms of hemochromatosis (over absorption of iron). Other clinical disorders of iron overload include those that are acquired such as thalassemia (low Hb levels), or chronic haemolytic anaemia (destruction of red blood cells at a rate quicker than production), dietary iron overload, or chronic liver disease (26, 55). Epidemiologic data for thalassemia in LMICs is incomplete and may underestimate the burden (56). However, hemoglobinopathies such as sickle cell disease, have been shown not to pose a risk of iron overload in pregnant women (57). While the WHO does not offer specific recommendations for iron supplementation in populations with hemoglobinopathies or other genetic red blood cell disorders, women and children are at lowest risk of iron overload in these groups, but safety should be monitored throughout clinical trials and any population based programmes (26).

2.4 BIOMARKERS OF IRON DEFICIENCY AND ANAEMIA

There are multiple biomarkers of iron status in humans. The WHO and Centres for Disease Control (CDC) consider Hb and (serum) ferritin to be the most effective measures of IDA within populations (58). The gold standard approach of measuring ID at its earliest stages of iron depletion is through

bone marrow iron, but this is challenging to access and measure (4). Other important biomarkers of iron status to assess ID are summarised in Table 1 and outlined below.

Table 1. *Iron biomarkers and levels of deficiency.*

Biomarkers	Bone marrow	Serum iron	Haemoglobin
	Ferritin (serum)	Transferrin saturation (ratio of serum iron to transferrin, ratio of serum iron to total iron binding capacity, or sum of serum iron and unsaturated iron binding capacity)	Haematocrit
		Protoporphyrin (zinc)	Reticulocytes
		Hepcidin Soluble transferrin receptor	
Level of deficiency	<i>Iron depletion</i>	<i>Iron deficiency erythropoiesis</i>	<i>Iron deficiency anaemia</i>

Data adapted from: Pfeiffer CM, Looker AC. Laboratory methodologies for indicators of iron status: strengths, limitations, and analytical challenges. The American Journal of Clinical Nutrition. 2017. https://apps.who.int/iris/bitstream/handle/10665/85839/WHO_NMH_NHD_MNM_11.1_eng.pdf?ua=1. (59)

2.4.1 Markers of iron deficiency anaemia

Anaemia, having multiple aetiologies, is often a late manifestation of ID (4). Below, the common biomarkers of IDA are described.

2.4.1.1 Haemoglobin

Hb is a standardized and easy to measure biomarker used to indicate anaemia. Its concentration is directly related to oxygen carrying capacity (4). However, it has low sensitivity as a biomarker of ID due to the late manifestation of anaemia, and Hb should be one of multiple biomarkers used to define ID (4). The Hb levels to diagnose anaemia at different stages of the life course, as defined by WHO, are found in Table 2 (60). The term mild anaemia may be misleading in relation to iron status as ID may appear well before anaemia. Domellof et al (2014) provided recommendations regarding iron requirements in infants and toddlers in his position paper and states that cut-offs for Hb to define non-anaemia at two months of age should be as low as > 9.0 g/dL (20). Additionally, before two years of age, Domellof et al's Hb cut-off for non-anaemia remains at > 10.5 g/dL and only increases in line to WHO cut-offs (>11.0 g/dL) by two years of age (20).

Table 2. *Human haemoglobin cut-offs to diagnose anaemia.*

Age group	Non-anaemia*	Mild anaemia*	Moderate anaemia*	Severe anaemia*
Under 5 years	>11.0	10.0-10.9	7.0-9.9	<7.0
5-11 years	>11.5	11.0-11.4	8.0-10.9	<8.0
12-14 years	>12.0	11.0-11.9	8.0-10.9	<8.0
Women of childbearing age	>12.0	11.0-11.9	8.0-10.9	<8.0
Pregnant women ¹	>11.0	10.0-10.9	7.0-9.9	<7.0
Men	>13.0	11.0-12.9	7.0-10.9	<8.0

* Expressed in grams per decilitre (g/dL)

¹This definition changes slightly with non-anaemia being defined as >10.5g/dL after the first trimester. Data adapted from: The World Health Organisation Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. 2011.

https://apps.who.int/iris/bitstream/handle/10665/85839/WHO_NMH_NHD_MNM_11.1_eng.pdf?ua=1. (60)

2.4.1.2 Haematocrit

Haematocrit, the volume of red blood cells in relation to total blood volume, provides no further information in relation to iron status than Hb (59). Haematocrit can be directly determined by microhematocrit centrifugation or calculated indirectly.

2.4.1.3 Reticulocytes

Reticulocytes, made in the bone marrow, are red blood cells that are still developing. As recommended by the American Academy of Paediatrics, a commonly used biomarker to define anaemia is reticulocyte Hb content (CHr) (61-63). It has been shown to be less sensitive to inflammation than other biomarkers of iron status, however there is no standardised cut-off point and it may be affected by conditions that cause iron restricted erythropoiesis (64). While reticulocyte Hb has been shown to be a strong predictor of IDA in children, it has not been evaluated systematically for defining nutritional anaemia in younger children and cut-offs are not yet universalised (62, 63).

2.4.2 Markers of iron deficiency erythropoiesis

ID erythropoiesis, often the second stage manifestation of ID has multiple biomarkers, described below.

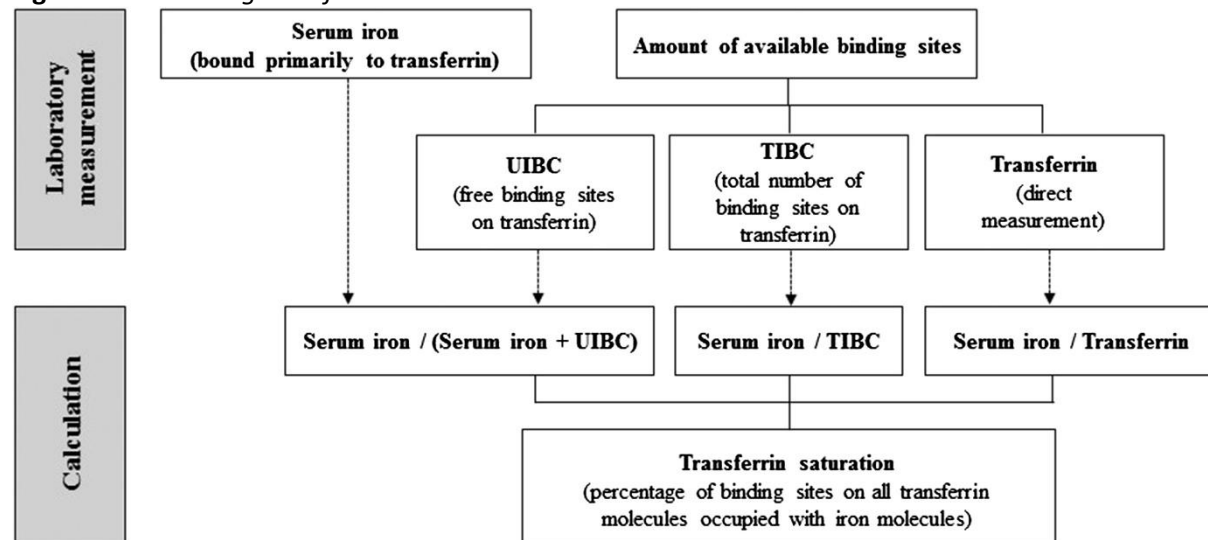
2.4.2.1 Serum Iron

More than 95% of serum iron is in the iron(III) (ferric) state and bound to transferrin (59). Serum iron on its own is an unreliable biomarker of iron status as it varies markedly even from hour to hour. It is heavily affected by iron supplementation and factors such as infection (59). Serum iron is used to measure iron status in combination with other biomarkers such as transferrin (see below).

2.4.2.2 Transferrin saturation

Transferrin saturation (TSAT) is the proportion of iron binding sites in the iron transport protein transferrin that are occupied by iron molecules (Figure 3). TSAT can be used to indicate iron availability for tissues, however, it is affected by circadian rhythms and decreases in the presence of infection and inflammation (62). TSAT is calculated as the ratio of serum iron to transferrin (which can be directly measured) or to total iron binding capacity (TIBC; total number of binding sites on transferrin). It can also be calculated as the ratio of serum iron to the sum of serum iron and unsaturated iron binding capacity (UIBC; free binding sites on transferrin) (65).

Figure 3. Calculating transferrin saturation.



TIBC: total iron binding capacity; UIBC: unsaturated iron binding capacity.

From: Namaste SM et al. Adjusting ferritin concentrations for inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anaemia (BRINDA) project. Am J Clin Nutr. (65)

2.4.2.3 Zinc protoporphyrin

Another biomarker of reduced iron levels is ZPP, which is seen in the end stages of iron depleted Hb synthesis (66). This is commonly seen as the ratio of ZPP to haem iron in red blood cells (66). During iron limited erythropoiesis, zinc can take the place of iron in the protoporphyrin ring, therefore making ZPP rather than haem protoporphyrin (66). While ZPP may be a better biomarker of iron status than ferritin in people with chronic inflammation, it has been shown to be in-effective in distinguishing ID during pregnancy. Therefore ZPP adds no diagnostic value to Hb concentration (67, 68). ZPP is also a weak biomarker of ID in paediatric settings (69).

2.4.2.4 Hepcidin

As highlighted in Section 2.3.2.2, hepcidin is an acute phase protein and is increasingly being used as a biomarker of ID (70). While it is raised in the presence of inflammation, its synthesis is directly

suppressed by erythropoiesis (4). Given hepcidin regulates iron uptake, it can be used to indicate response to oral iron (70-72).

2.4.2.5 Soluble transferrin receptor

When tissue iron is limited, sTfR is expressed (4). Other than in early infancy, when bone marrow most readily uses iron, sTfR is believed to reflect iron demand for erythroid precursors (4). Unlike ferritin, sTfR does not change concentrations in the presence of infection and inflammation, but may be affected by conditions such as thalassaemia, lower than normal Hb levels, and sickle cell anaemia (59). To improve reliability, the ratio of sTfR to log-ferritin is used to measure iron depletion (62). This has been validated in field settings in LMICs (73, 74). However, interpreting sTfR can be challenging due to lack of assay standardization, common reference ranges, or cut-offs (75).

2.4.3 Iron depletion

Iron depletion in its earliest stages is often hard to detect. As mentioned, while bone marrow iron is the gold standard approach is it too difficult and expensive as a standardized biomarker. Serum ferritin, detailed below, is the commonly used biomarker of iron depletion.

2.4.3.1 Serum Ferritin

In the absence of inflammation, serum ferritin corresponds to iron stores by reflecting intracellular iron content (76). When iron stores are depleted (reflected by decreased serum ferritin levels), the first stage of ID is present, but there are not yet consequences for erythrocytes or the presence of anaemia (59). As with Hb, serum ferritin has relatively low sensitivity and the cut-off to define ID is 12-15 µg/L. However, different cut-offs for different populations may be more appropriate as ferritin is an acute phase protein, so it is expected to increase during infection and inflammation (4, 77). In infants and children, when iron metabolism greatly varies and in settings where infection and inflammation are present, defining ID through ferritin alone can be challenging (78, 79). As mentioned in Section 2.1, in children under five years of age, ID is defined as a serum ferritin concentration of less than 12 µg/L (27).

The Biomarkers Reflecting Inflammation and Nutritional Determinants of Anaemia (BRINDA) project assesses the relationship between ferritin concentrations and inflammation in children from six to 59 months of age and women of reproductive age (65). It provides adjustment algorithms to account for the effects from inflammation and malaria in these populations (65). C-reactive protein (CRP) and α_1 -acid glycoprotein (AGP) are measured to adjust for inflammation to better estimate the prevalence of depleted iron stores (65).

2.4.4 Other biomarkers of iron deficiency and anaemia

Other biomarkers of ID and IDA, detailed in Table 3, are more readily coming into use.

Table 3. *Other biomarkers of iron deficiency and anaemia.*

Biomarker	Indicator of	Important factors
Serum transferrin receptor (log ratio) and serum ferritin	Total body iron	Useful and practical as it can be analysed as a continuous variable
Mean corpuscular volume (MCV)	Can help distinguish the following types of anaemia (often seen in the presence of inflammation): - Microcytic: smaller than average erythrocyte, indicating iron deficiency anaemia - Normocytic: average erythrocyte - Macrocytic: larger than average erythrocyte, subcategorizes as: ➔ Megaloblastic: impaired DNA synthesis ➔ Non-megaloblastic: normal DNA synthesis	Measure the average size and volume of red blood cells; useful in the presence of inflammation
Mean corpuscular haemoglobin (MCH)	May be a more superior biomarker of iron deficiency and anaemia than MCV	
Erythropoietin (EPO)	In high levels may indicate anaemia	Hormone produced by the kidneys which controls erythrocyte production; increases the synthesis of erythroferrone
Erythroferrone (ERFE)	May play an important role in anaemia in the presence of high inflammation	Main erythroid suppressor of hepcidin; found in bone marrow erythroblasts

2.4.5 Measuring iron status in early infancy

There are many indicators of iron status, all of which can be used in infants and children, but certain considerations must be made for iron levels during early infancy, iron requirements during growth, as well as inflammation and/or infection (20). In LMICs anaemia is more common in infants and children because generally iron demands are higher, iron intakes are lower, and these infants are more at risk of inflammation and infection. Cut-offs in children cannot always be used in infants as the physiological and inflammatory processes of early infancy may make the cut-offs less appropriate for this life stage (80).

2.5 IRON AND PHYSIOLOGY

Iron is crucial for many physiological processes, especially during pregnancy, infancy, and childhood. These processes include oxygen supply to other parts of the body, neurodevelopment, growth, gut microbiome function, and immune and infection response. Erythropoiesis is sensitive to low iron levels (81). Growth related blood volume expansion continues in infancy and childhood; however, iron restricted erythropoiesis is often seen in LMICs (4). This is a result of either a nutritional ID or limited iron recycling and absorption, often seen in the presence of infection and inflammation (4).

However, conversely, iron has also been associated with an increased risk of infection and some evidence of adverse impact on growth, and can be toxic when in excess (82). Therefore, benefits and risks of iron therapy must be carefully considered when developing iron intervention regimens. This risk-benefit ratio is especially important to consider in contexts such as LMICs where rates of ID, anaemia, and infection are higher. Global guidelines may need to be adapted with population specific policies. WHO guidelines recognise context specific needs with iron supplementation in infants and children and that it should be aimed at those who are anaemic or at a high risk of ID, with supplementation being accompanied by strategies to prevent and treat malaria, hookworm, schistosomiasis and other nutrient deficiencies such as vitamin B12 and A, and folate (27).

The following sections outline the physiological importance of iron as well as some considerations for supplementation.

2.5.1.1 *Oxygen supply*

Anaemia is a condition in which the oxygen carrying capacity of blood (Hb levels) is too low to support normal physiological functions (4). Compared to other life stages, total body iron is most concentrated just after birth in healthy newborn infants, with about 70% of total body iron contained within Hb. However, due to the change in environment post-partum, Hb levels then notably drop in the first few days following birth, often referred to as “physiological anaemia of infancy” (83). A Hb concentration of 15g/dL is optimal for preterm and full-term infants at birth but may be as low as 11g/dL (84). This then decreases by approximately 1g/dL/week for the first five to six weeks of life while there is a decreased oxygen affinity (84). From six to eight weeks of age, as erythropoiesis increases, Hb levels should too, back up to a non-anaemic range (>11g/dL) (83). In this crucial period for growth and development, most iron is supplied for erythropoiesis through iron recycling from cells (4).

2.5.1.2 *Neurodevelopment*

Iron is crucial for neurodevelopment. ID in infancy is associated with impaired cognitive, behavioural, and motor function development, therefore having detrimental long-term effects (17-19, 22). Iron is

especially crucial for brain development in the first three years of life (85). The developing brain is spatially and temporally sensitive with suggestions that each developmental stage depends on the previous one having been completed (86-88). A large body of evidence from both humans and animal models indicates that ID during this crucial phase of growth and development can alter the brain and nervous system (89-94). Additionally, when iron is restricted, iron re-circulation for erythropoiesis may be prioritised, leading to decreased brain iron levels (95, 96).

Neurological development requires a wide array of iron and haem dependent proteins (4). Studies in rodent models have shown ID linked to compromised neurodevelopment such as lowered axon myelination, impaired monoamine, and gamma-aminobutyric acid neurotransmitter system development, reduced hippocampal function, and compromised central nervous system energy metabolism (19, 61, 97). The specific region of the brain affected will most likely depend on the timing of ID, with those areas associated with the most rapid changes in growth and development being most sensitive to ID (97).

The effect of iron supplementation on cognitive development in young infants under six months of age is not fully understood, due a weak evidence base (reviewed in full in Chapter 3 of this Thesis). In infants above six months of age, reported effects are inconsistent (4, 98). This could be due to a number of factors such as heterogeneity in study design or difficulty in assessing cognitive development in young children (99). A benefit from iron supplementation (10mg/day of ferrous sulphate for 12 months) was seen in a study of pre-school aged children's motor and language development in Zanzibar (100). However, one study in Chile found infants given high doses of iron supplements had reduced cognitive function at ten years of age (89). A systematic review looking at iron supplementation in varying stages from pregnancy to four years of age found that evidence for the impact of ID or iron supplementation on early childhood development is inconsistent (101).

2.5.1.3 Growth

The physiology of growth in infants and children is important to consider in the context of ID. Within the context of a resource-constrained environment, poor maternal nutritional status, leading to poor breastmilk micronutrient composition may be one of a number of contributing factors leading to poor growth in infants and young children. More rapid growth across infancy and early childhood has been correlated with a faster decline in iron status (6, 102-104). A sharper decline in serum iron is often seen in males than in females, due to greater requirements for growth in males (6).

There is conflicting evidence on if iron supplementation in infants and children may promote or deter growth. In Chapter 3 of this Thesis, the effect of iron and/or multiple micronutrient (MMN) supplements in infants under six months of age is systematically reviewed, with one of the outcomes

assessed being growth (105). There is conflicting evidence of the impact of iron supplementation on growth for infants over six months of age; some studies on infant populations over six months of age with high rates of IDA have shown a beneficial impact on growth (106-108), other studies found no effect, and some even report adverse effects (107, 109-111). Iron supplementation for more than six months in replete children has been shown to impair growth and must be carefully considered (112). However, effects of iron supplementation on growth of children under five years of age was inconclusive in a meta-analysis (113). A two-site randomised controlled trial (RCT) in both Sweden and Honduras, found that in Sweden, where infants tended to be iron replete, higher doses of iron increased the rate of gastrointestinal side effects and decreased linear growth in infants four to nine months of age, but this effect was not seen in Honduras (80). A proposed mechanism for negative impacts of iron supplementation on growth include iron's inhibition of zinc absorption (114) or that supplementation of iron replete children could cause oxidative stress and/or gut inflammation (further discussed in Section 2.5.1.5.2) (115-117).

2.5.1.4 Gut microbiome

The gut microbiome undergoes crucial developmental changes in the first few years of life (118). Unabsorbed dietary iron from fortification or supplementation can modify the gut microbiota to favour the growth of pathogenic strains over commensal strains (119). Beneficial host strains of bacteria have little to no requirement for iron, whereas iron is essential in the virulence and colonization of most pathogenic strains (120). In human adults, three hours after consumption of an iron tablet, enhanced rates of replication of pathogenic bacteria were observed in blood (121). Studies in children have shown detrimental effects of iron supplementation on the gut microbiome, with increased biomarkers of intestinal inflammation also observed (115, 119, 120). This dysbiosis leads to reduced resistance to infection, higher prevalence of diarrhoea and faecal calprotectin (a biomarker of gut inflammation) and an increased ratio of lactulose to mannitol, which leads to a further increase in gut permeability (115, 122, 123).

There is evidence to show that iron's effect on the microbiome could be setting specific. In LMICs, iron supplementation has been associated with decreased immunity, increased infection and diarrhoea, gut inflammation, and detrimental dysbiosis (119, 124-127). Some studies in LMICs are now providing prebiotic fibres, such as galacto-oligosaccharides, and ascorbic acid alongside iron supplementation to mitigate its adverse effects on the microbiome and increase bioavailability (117).

However, iron supplements on their own, versus iron within the food matrix (such as in iron containing formula milk) may have different rates of bioavailability and gastrointestinal side effect.

In Swedish infants from six to eight months of age, no increase in pathogenic bacterial strains or changes in faecal calprotectin were seen with use of high iron containing formulas (128).

2.5.1.4.1 Gastrointestinal side effects

Iron supplementation is commonly noted to cause adverse gastrointestinal side effects in children such as diarrhoea, nausea, constipation, and vomiting, therefore limiting adherence in supplementation trials (129, 130). During pregnancy, IFA supplementation often has low adherence due to adverse gastrointestinal side effects. The largest comprehensive systematic review of intermittent iron supplementation in pregnancy found that intermittent supplementation produced similar maternal and infant outcomes as daily supplementation but was associated with fewer side effects (131). A systematic review (see Chapter 4) found side effects to be one of the leading barriers to acceptability in nutrition intervention trials in infants under 24 months of age (132). Recent studies have shown iron containing micronutrient powders (MNPs) to cause increased risk of diarrhoea in young children (120, 125, 133, 134). However, in South African school aged children, where water supply, sanitation, and hygiene were better, there was no detrimental impact on the microbiome from therapeutic high dose iron (130).

2.5.1.5 Immunity and infection

In 2019, an estimated 5.2 million children under five years of age died, with about 82% of these deaths being in LMICs (135). Among the leading causes were acute respiratory infections (14.2%), diarrhoea (10.6%), and malaria (7.6%) (135). The human immune system undergoes dynamic changes in early life and is impacted by a range of nutritional exposures, such as iron (136, 137). Effects of iron on immunity may be direct, such as increased hepcidin levels, or indirect such as through modification of the microbiome, influencing systematic immunity (118, 136-145). There is a growing body of evidence that low iron may be protective against infection and impact immunity during the early years of life (146-148).

2.5.1.5.1 The role of iron on the immune system

Altered cellular iron has been shown to influence innate immune responses (149). For example, neutrophils (the most abundant type of white blood cell) are iron dependent and cellular iron influences macrophage polarization, response to certain cytokines, and antimicrobial peptide function (150, 151). The adaptive immune response is heavily nutrient dependent, undergoing metabolic changes to rapidly create antigen specific T and B cells (152). Additionally, amino acids play an important role in lymphocyte immune response, with adaptive immunity being compromised when lymphocytes cannot access iron (153, 154). While cellular proliferation and metabolic activity are iron dependent, the direct link of supplementation to antigen-specific immunity is still unclear (155). Iron

restriction inhibits the proliferation of lymphocytes (156-158). Additionally, ID has been shown to negatively impact T cell numbers, but supplementation in children shows restoration (159, 160).

As discussed in Section 2.3.1.1 hepcidin upregulation is an acute phase response, seen during chronic infection and inflammation, thereby blocking the release of iron (through down regulation of ferroportin) into the blood stream (45, 46). Hepcidin upregulation is seen during infections such as malaria, human immunodeficiency virus, tuberculosis, and salmonella, as well as childhood infections, such as respiratory infections, and in fever (161-168). This may explain the increase in hepcidin during neonatal hypoferrremia in the first few days of life following birth, therefore protecting infants from early infections (169, 170).

While supplementation with iron in LMICs may increase risk of infection, inflammation, and malaria in iron replete children, host iron homeostasis may play a role through hepcidin (81, 171). Overall, targeting and treating infections is vital to limit inflammation and enhance the efficacy of administered iron. When administering therapeutic iron doses in settings with high infection rates, underlying infections should be treated first, which may increase erythropoiesis and Hb levels even before iron administration (34, 172, 173). After which, iron status should be re-evaluated before treatment. Below, three crucial factors for consideration with iron supplementation, especially in LMICs are discussed.

2.5.1.5.2 Inflammation (oxidative stress)

Chronic infection, leading to persistent inflammation, likely contributes to the burden of IDA through raised hepcidin levels and therefore further inhibition of iron absorption (72, 168). The reactive properties of iron are vital for cellular biochemistry; however, unchaperoned iron can cause the generation of free radicals (4). Iron transport and regulation are crucial to mitigate this risk, given that iron homeostasis is regulated through its absorption (174). Iron absorption mechanisms must be adequate when non-regulated losses, such as blood loss or infection, occur (4). Homeostasis must be carefully controlled because loss of absorption control can either cause deficiencies or over accumulation, with systemic iron homeostasis being dependent on hepcidin (38, 175). Likewise, children in LMICs have a higher risk of infection from parasites, which can cause intestinal blood loss if untreated, and therefore further iron depletion (176, 177).

2.5.1.5.3 Environmental enteric dysfunction

Environmental enteric dysfunction (EED) is characterised by changes in intestinal permeability and inflammation, microbial translocation, and systemic inflammation (178). It is more common among children living in areas with poor water, hygiene, and sanitation as it may arise from continuous exposure to environmental pathogens and toxins (179, 180). Additionally, EDD may contribute to

micronutrient deficiencies such as ID, and limit response to nutrition interventions (178). A study in six month old infants in Uganda showed that EED biomarkers were significantly negatively correlated with Hb concentrations (178). Similarly, increased EED biomarkers were significantly associated with low ferritin levels in a study of Bangladeshi children under two years of age (181).

2.5.1.5.4 Malaria

Mild ID may be an adaptive protective mechanism against malaria and has been linked to reduced ID risk in many malaria endemic regions of sub-Saharan Africa (6, 182-185). As recommended by the WHO, malaria should be treated and managed alongside iron supplementation in infants and children (32). The Pemba trial, a large trial in Tanzanian children giving iron-containing supplements had to stop the iron containing arms early due to increased hospital admissions and deaths in children within those arms (186). However, a secondary analysis showed that this was among infants and children who were iron replete at entry into the trial (186). Other trials in malaria-endemic areas have also seen an increased risk of malaria in deficient children supplemented with iron (187, 188).

2.5.2 Iron interactions with other micronutrients

Potential interactions of iron with other trace minerals needs to be considered with iron supplementation (189). Most notably zinc, as iron and zinc use the same absorptive pathways: DMT-1 and ferroportin (30). Iron has also been found to interact with lead, copper, vitamin A, calcium, and vitamin C (36). It is suggested that in areas with high lead exposure, lead poisoning is associated with ID in children, due to the upregulation of DMT-1, which increases lead absorption (190). In iron deficient infants exposed to lead, iron supplementation reduced high serum lead levels (190). Additionally, ID has been linked to negative copper metabolism and vice versa as many of the cells that mediate dietary assimilation, storage, and distribution (enterocytes and hepatocytes) as well as the users and recyclers of iron (erythroid cells and reticuloendothelial macrophages) are shared (191). DMT-1 is also a copper transporter, so iron and copper may compete with each other for uptake into enterocytes (192). Vitamin A interacts with iron metabolism and may contribute to ID due to vitamin A deficiency's upregulation of hepcidin (114, 193). Until 12 months of age it is recommended to avoid calcium rich food such as cow's milk while consuming iron-rich foods due to calcium's ability to limit iron absorption (194). Vitamin C on the other hand is a promoter of dietary iron absorption and can counteract phytate and tannins inhibitory effect of dietary iron absorption (195). Phytates (found in grains, seeds, nuts, and legumes) and tannins (in tea, coffee, red wine, chocolate, and legumes) may interfere with and decrease the absorption of iron (196, 197). In a study of North-Eastern Chinese children with mild IDA, daily vitamin C intake with as little as 50mg/day for six weeks showed a dose response to various biomarkers of iron status (Hb, serum ferritin, free erythrocyte, and haematocrit)

(198, 199). Locally available vitamin C rich foods are encouraged in settings with low dietary iron intakes to help increase absorption.

2.6 IRON REQUIREMENTS IN WOMEN, INFANTS, AND CHILDREN

IDA is highly prevalent among women of reproductive age and highest in women from LMICs (33). An estimated one third of women of reproductive age worldwide are anaemic (33). The major causes of anaemia for these women is inadequate iron intake, increased chronic blood loss from menstruation, and increased iron requirements during pregnancy and growth (200). Low levels of iron during pregnancy then puts infants and children at risk of starting their life with lower than required iron levels. This section discusses the iron requirements of women of reproductive age, infants, and children.

2.6.1 Women of reproductive age and in pregnancy

Prevention of IDA in women of reproductive age is essential to improve health status and enhance health for any future pregnancies (3). During pregnancy, maternal ID does not appear to lower iron stores of their newborn; however, maternal IDA does (201, 202). Infants born to moderately and severely anaemic mothers have low iron stores at birth (203, 204). While daily iron requirements range by life stage and setting, non-pregnant women of reproductive age need about 15-20 mg of iron daily, while in pregnancy this increases to about 27mg/day. (205-207). Table 4 outlines recommended daily dietary requirements of iron for women across varying life stages.

Table 4. *Iron requirements in women.*

Age group (years)	Daily iron requirements
9-13	8mg
14-18	15mg
14-18 (pregnancy)	27mg
14-18 (lactation)	10mg
19-50	18mg
19-50 (pregnancy)	27mg
19-50 (lactation)	9mg
51+	8mg

Data from: NIH. Iron: Fact Sheet for Health Professionals. National Institutes of Health (NIH) Office of Dietary Supplements. 2021. (208).

2.6.2 Infants and children

As discussed in Section 2.5, iron is crucial in infancy and childhood, an important period of growth and development. Iron requirements of infants and children are summarised in Table 5.

Table 5. Iron requirements in infants and children.

Age group	Iron requirements	Recommended interventions
Low birth weight infants (until 6 months) (defined as: <2,500; very < 1500; extremely < 1000 grams)	1.0-2.0 mg/kg [‡] /day and as much as 3.0-4.0mg/kg/day 6-8 weeks after birth for extremely low birth weight infants	Prophylactic enteral iron (ESPGHAN*) (209); delayed cord clamping
Pre-term infants (until 6 months) (defined as: moderate to late = 32-37; very = 28-32; extremely = less than 28 weeks gestation)	2.0-3.0 mg/kg/day	2mg/kg/day from 1-12 months of age (American Academy of Paediatrics) (210); Prophylactic enteral iron at 2.0-3.0mg/kg/day from 2-6 weeks of age (ESPGHAN) (209); delayed cord clamping
Healthy infants under 6 months	Liver iron stores (75mg/kg of iron) should provide enough iron for growth and development until four to six months of life in exclusively breastfed infants	Delayed cord clamping; exclusive breastfeeding; fortified formulas
6-12 months	0.9-1.3 mg/kg/day	Fortified follow on formulas; iron rich complementary food; avoidance of cow's milk when consuming iron rich foods
1-3 years	0.5-0.8 mg/kg/day	Iron rich complementary foods
4-8 years	0.3-0.5 mg/kg/day	Foods rich in iron: beef, pork, poultry, and seafood; tofu; dried beans and peas; dried fruits; leafy dark green vegetables; iron-fortified breakfast cereals and breads

*ESPGHN: European Society for Paediatric Gastroenterology Hepatology and Nutrition

[‡]Kg: Kilogram

Data from: Domellöf M, Braegger C, Campoy C, Colomb V, Decsi T, Fewtrell M, et al. Iron requirements of infants and toddlers. *J Pediatr Gastroenterol Nutr.* 2014. (20)

2.6.3 Early infancy

2.6.3.1 Iron endowment

Healthy full-term infants born to iron replete mothers should be born with an iron endowment to support their growth and development for the first four to six months of life (211). While breastmilk contains highly bioavailable iron, it is in small quantities (0.3mg/L), so most of the iron required for the first few months of infant growth and development are obtained from the iron endowment acquired in utero (211, 212). Infants are born with approximately 75mg/kilogram (kg) of iron, with about 70% as Hb in the bloodstream, 25% stored in the liver, and the remainder is in myoglobin and iron containing enzymes, mainly acquired in the final weeks of gestation (95, 211). Hb levels at birth are usually higher than at any point in life in order to ensure ample oxygen supply be maintained in

the hypoxic environment in utero (211). While Hb levels drop from about 17.0 g/dL to 12.0 g/dL in healthy, full term infants in the first six weeks of life, liver iron stores should provide enough iron for growth and development until four to six months of age (20). However, this is impacted by maternal iron status, gestational age, birthweight, and time to cord clamping (211). Delayed cord clamping, through increased blood volume to the neonate, has been shown to improve iron status in newborn infants (211). As mentioned, utility rate of the iron endowment varies; with lower endowments being used up faster, as well as faster growing infants, often males, showing sharper declines in iron stores in the first few months of life (6, 102-104).

2.6.3.2 Low birth weight and preterm infants

LBW (< 2500 grams) and PTB (< 37 weeks gestation) infants must be carefully considered when it comes to iron needs. Both LBW and PTB infants are born with lower total serum iron, ferritin and Hb levels due to a lower blood volume for LBW infants and less time in utero for PTB infants. The total amount of iron required for LBW and PTB infants is estimated by weight, as outlined in Table 5.

Due to the timing of the iron endowment being late in pregnancy, PTB infants have additional iron requirements. The iron requirements of growth have been estimated at approximately 0.29 mg/kg/day at 32 weeks, increasing to 0.37 mg/kg/day when approaching term, and declining gradually after this (213). Iron absorption is about 30%, so the necessary intakes are about 1.0-1.3 mg/kg/day between 32 weeks gestation and term (189), with other recommendations as high as 2.0-3.0 mg/kg/day for the first six months of life when weighing less than 1800 grams at birth (214).

LBW infants need at least 1 mg/kg/day between six weeks and six months of life (20). A meta-analysis has shown that prophylactic iron given after birth, greatly reduces anaemia rates at six months of age, with most studies recommending 2 mg/kg/day of enteral iron starting from two to six weeks of age (215).

2.6.4 Early childhood

Iron is required to support normal physiology with iron requirements per kg of body weight being far greater in early childhood than adulthood (4, 17-20). Growing tissue requires an increase in iron to maintain the continuous repletion for expanding blood volume, increasing muscle and tissue mass, and neurodevelopment, especially brain growth (4). It is estimated that total iron body needs double from about 300 mg/day at six months of age, to 600 mg/day by 24 months of age (20).

2.7 IRON INTERVENTIONS IN WOMEN, INFANTS, AND CHILDREN

A range of current international iron supplementation recommendations are highlighted in Table 6 with a further discussion in section 2.7.1-2.7.3. The WHO international recommendations have been chosen as the international guidelines. Three high income regions of the UK, Europe, and United States (US) have been detailed, as well as two LMIC regions: The Gambia, where the data collection for this PhD took place, as well as India, a setting in Southeast Asia, a global region of concern for ID.

2.7.1 Population level – World Health Organisation

At the population level, the WHO has various recommendations for iron supplementation at all life stages, except for infants under six months of age, when EBF is one of the only recommended practices to prevent ID (Table 6). For women of reproductive age and children between six and 23 months of age, when IDA levels are greater than 40%, daily iron supplementation for three consecutive months per year is recommended at age appropriate doses (24, 216). In pregnancy daily IFA supplementation is recommended, with intermittent supplementation being acceptable in areas with lower rates of IDA (< 20%) if unwanted side effects are experienced from supplementation (23). LBW and PTB infants have a daily iron requirement based on weight (217). In childhood, in areas with IDA prevalence of greater than 20%, point-of-use fortification of complementary foods (CFs) with iron-containing MNPs is recommended (218). Additionally, it is cautioned that iron supplementation in children should be accompanied by prevention and/or treatment of malaria, hookworm, schistosomiasis and other nutrient deficiencies (e.g. vitamin B12 and A, and folate) (27). Likewise, introduction of CFs at six months of age, as well as sustaining breastfeeding up to two years of age, is recommended (24).

2.7.2 High income regions - United Kingdom, Europe, and the United States

2.7.2.1 *The United Kingdom*

In the UK, the Department of Health, National Health Service and Royal College of Paediatrics provide the recommendations around iron supplementation at varying life stages for women of reproductive age, infants, and children (Table 6). Routine iron supplementation (enteral) is only recommended for LBW and PTB infants from 28 days postnatally until six to 12 months of age (219). For infants under six months of age, EBF or formula if the mother chooses, are the only recommendations to ensure adequate iron intakes (220). After six months of age, CFs and sustaining breastfeeding (or fortified formula) for up to one year of age is recommended (220).

2.7.2.2 *Europe*

In Europe, the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) provides the recommendations around iron supplementation at varying life stages for women of

reproductive age, infants, and children (Table 6). In pregnancy, ESPGHAN recommends following country specific guidelines, but states that there is no evidence that iron supplementation in pregnancy will improve infant iron status in a European setting (20). For LBW and PTB infants, routine iron supplementation from two to six weeks until six months of age is recommended (20). For infants under six months of age, no routine supplementation for normal birth weight, healthy infants and toddlers is recommended, however, EBF is recommended until six months of age and infant formulas should be fortified with 4-8mg/L of iron (20). After six months of age, iron rich CFs or follow on fortified formulas, as well as avoiding over 500mL/day of cow's milk until 12 months of age, is recommended (20).

2.7.2.3 The United States

In the US, the CDC provides the recommendations around iron supplementation at varying life stages for American women of reproductive age, infants, and children (Table 6). Screening of all non-pregnant women every five to ten years during child bearing years at routine health examinations, as well as oral iron when anaemia is present, is recommended (221). While the CDC says that conclusive evidence of benefits of oral iron in pregnancy for all women is lacking, routine daily oral iron supplementation is recommended in pregnancy (221). Postpartum, women at risk of anaemia should be screened four to six weeks postpartum, with treatment the same as during pregnancy, however, if there are no risk factors for anaemia, iron supplementation can stop at delivery (221). For breastfed PTB infants, either iron fortified formula or daily iron supplementation based on weight is recommended from one month after birth until 12 months of age (221). The introduction of iron rich CFs at four to six months of age, as well as the avoidance of low iron containing milks (e.g. cows, goats and soy milks) until after 12 months of age is recommended (221).

2.7.3 Low- and middle- income regions – The Gambia and India

2.7.3.1 The Gambia

In The Gambia, where the field research for this PhD took place, the Ministry of Health and Welfare make recommendations for iron supplementation for women of reproductive age, infants, and children (Table 6). During pregnancy, daily oral IFA alongside malaria prophylaxis and deworming is recommended (222). Outside of pregnancy the only other recommendations are for infants under six months of age and from six to 23 months of age as per the WHO guidelines. EBF until six months of age is recommended (8). From six to 23 months of age, depending on prevalence of IDA, daily oral iron or MNP supplementation along with prevention and/or treatment of malaria, hookworm, schistosomiasis and other nutrient deficiencies, alongside CFs from six months of age and sustained breastfeeding up until two years of age, are recommended (24, 27, 218)

2.7.3.2 *India*

The Indian Ministry of Health and Family Welfare provides recommendations on iron supplementation for women of reproductive age, infants, and children (Table 6). For women of reproductive age, a weekly dose of IFA and biannual deworming is recommended (223). For 100 days of pregnancy, and 100 days post-partum, daily oral IFA is recommended (223). EBF is encouraged until six months of age (223). Lastly, for children from six months of age, 100 days of daily oral IFA and deworming, as well as introduction of CFs and continued breastfeeding is recommended (223).

Table 6. *Iron supplementation recommendations.*

Iron Supplementation Recommendations							
<i>Maternal/Reproductive and Infant/Child Life Stage</i>							
Setting	Country/ Policy Level	Reproductive Age	Pregnancy	Lactation	Low-birth-weight (LBW)/ Pre-term birth (PTB)	Early Infancy	Childhood
Inter- national	World Health Organisation (WHO) at the population level	When iron deficiency anaemia (IDA) prevalence > 40%: daily iron (30-60 mg of elemental iron) for 3 consecutive months/year (216)	Daily oral iron and folic acid (IFA) (30-60mg of elemental iron + 0.4mg of folic acid). If daily IFA is unacceptable due to side effects + anaemia prevalence < 20%: intermittent oral IFA (120mg of elemental iron + 2.8mg of folic acid) 1x/week (23). In contexts with rigorous research, if multiple micronutrient (MMN) deficiencies are common, IFA containing MMN supplements are recommended (224)	See 'Reproductive Age'	Very LBW (VLBW) breastfed infants: 2-4 mg/kg/day iron from 2 weeks - 6 months of age (217)	Under 6 months of age: exclusive breastfeeding (EBF) (8)	Between the age of 6-23 months, in areas with an IDA prevalence > 40%: daily iron (10-12.5mg elemental iron) for 3 consecutive months/year; In areas with an IDA prevalence >20% point-of-use fortification of complementary foods (CFs) with iron-containing micronutrient powders (MNPs) is recommended (218); CFs at 6 months and sustain breastfeeding (BF) up to 2 years of age (24); Supplementation should be accompanied by prevention/treatment of malaria, hookworm, schistosomiasis and other nutrient deficiencies (e.g. vitamin B12/A and folate) (27)

High Income	UK (United Kingdom) Department of Health/ National Health Service/ Royal College of Paediatrics	Eating a varied and balanced diet; If taking iron supplements do not take too much as this could be harmful (17mg/day or less is unlikely to cause any harm); Continue taking a higher dose if advised by a GP (207)	Routine iron supplementation for all women in pregnancy is not recommended (225)	See ' <i>Reproductive Age</i> '	Enteral iron (5.5mg/day elemental iron) for all infants under 1500g/34 weeks gestation from 28 days post-natal age to 6-12 months of age (219)	Under 6 months of age: EBF (or formula if the mother chooses) (220)	Encourage CF at 6 months of age and sustain BF up to at least 1 year (or formula if the mother chooses) (220)
	Europe (European Society for Paediatric Gastroenterology Hepatology and Nutrition [ESPGHAN]) (20)		Country specific guidelines, however, ESPGHAN states: there is no evidence that iron supplementation of pregnant women improves iron status in their offspring in a European setting		Marginally LBW infants (2000–2500 g) should receive 1–2 mg/kg/day of iron starting at 2-6 weeks until 6 months of age (regardless of term/ preterm); LBW infants with birth weights <2000 g should receive iron at a dose of 2-3 mg/kg/day	Encourage EBF for 6 months; if formula fed, use iron fortified formulas (4-8mg/L); no need for general iron supplementation of healthy European infants and toddlers of normal birth weight	Introduce CF at 6 months of age with iron rich foods; If using formula use follow on formulas; Avoid >500mL/day of unmodified (non-formula) cow's milk until 12 months of age because of effects on iron
	United States of America (USA) Centers for Disease Control and	Starting in adolescence, screen all nonpregnant women for anaemia every 5-10 years throughout their childbearing years	Iron for pregnant women (30mg/day) because women have difficulty maintaining iron stores during pregnancy and IDA	Women at risk for anaemia should be screened at 4-6 weeks postpartum;	BF PTB infants: 2-4mg/kg/day (to a maximum of 15 mg/day) elemental iron starting at 1 month after birth	EBF for 4-6 months; BF infants: 1mg/kg/day elemental iron starting at 4 months of age	Introduce iron-rich CF between 4-6 months of age; Iron sufficient infants: needs provided by two servings/day iron-fortified infant cereal or pureed meats

	Prevention (CDC) (221)	during routine health examinations; Treat adolescent girls and women who have anaemia by prescribing an oral dose of 60-120 mg/day of iron	in pregnancy is associated with adverse outcomes (Lacking conclusive evidence of benefits of universal iron for all women)	treatment and follow-up are the same as for nonpregnant women; if no risk factors for anaemia are present, iron supplements should stop at delivery	and continuing until 12 months; If using infant formula ensure iron fortified (> 6.7mg/L iron)	until sufficient iron is provided through CF; If using infant formula ensure iron fortified (> 6.7mg/L iron)	are also good sources of iron; Discourage use of low-iron milks (e.g., cow's milk, goat's milk, and soy milk) until age 12 months
Low Income	The Gambia, Ministry of Health and Welfare (222)		Daily oral IFA with 200mg ferrous sulphate and 0.5mg folic acid; intermittent preventive malaria treatment with sulfadoxine-pyrimethamine in the second trimester given at least one month apart, at least four doses received; deworming tablets during pregnancy			As per WHO guidelines: under six months of age EBF is recommended	As per WHO guidelines: , in areas with an IDA prevalence > 40%: daily iron (10-12.5mg elemental iron) for 3 consecutive months/year; in areas with an IDA prevalence >20% point-of-use fortification of CFs with iron-containing MNPs is recommended (218); CFs at 6 months and sustain BF up to 2 years of age (24); Supplementation should be accompanied by prevention/treatment of malaria, hookworm, schistosomiasis and other nutrient

							deficiencies (e.g. vitamin B12/A and folate) (27)
	India (Ministry of Health and Family Welfare) (223)	Weekly dose of 100 mg elemental iron and 500 mcg folic acid with biannual deworming	100 mg of elemental iron and 500 mcg of folic acid daily for 100 days during pregnancy	100 mg of elemental iron, 500 mcg of folic acid daily for 100 days postpartum		EBF is encouraged until 6 months of age	CF from 6 months of age, with continued BF; 20mg/ml elemental iron, 100 mcg/ml folic acid (liquid formulation) and age appropriate deworming for 100 days

Strategies to prevent and rectify ID are detailed below with a summary of the strategies found in Table 7. Iron supplementation is multifaceted as different iron compounds have different bioavailability, effectiveness, and cost implications. Oral iron is widely available in tablet form. However, in pregnancy, oral iron has been associated with negative gastrointestinal side effects, and in young infants, liquid oral iron may be required, which is often more expensive and has a limited shelf life (26).

Table 7. *Strategies to prevent and rectify iron deficiency in women of reproductive age, infancy, and childhood.*

Life stage	Strategies to prevent/rectify iron deficiency
Women of reproductive age	• Daily oral iron supplementation
Pregnancy	• Daily or intermittent (due to side effects from daily) oral iron folic acid (IFA) supplementation • Multiple micronutrient supplementation including IFA in areas of rigorous research
Early infancy (0-6 months of age)	• Exclusive breastfeeding • Delayed umbilical cord clamping • Iron fortified formula
Childhood (6 months to 5 years of age)	• Weaning diets with high iron content • Daily oral iron supplementation
Other strategies (at varying life stages but important for consideration for women of reproductive age and children)	• Intermittent iron supplementation (when daily supplementation is not acceptable due to side effects) • Food fortification • Alternative oral irons • Intravenous iron • Increasing dietary iron (specifically haem iron) ○ Increasing dietary enhancers of iron absorption such as vitamin C rich foods ○ Limiting inhibitors of iron absorption such as phytates and tannins

Strategies to prevent and rectify ID and IDA are now summarised below with further details by age group of intervention: women of reproductive age, pregnancy, early infancy, and childhood; followed by general strategies to improve iron status, not specific to any age group, but important to consider for these age groups of interest.

2.7.4 Women of reproductive age

For all women of reproductive age, in areas with an IDA prevalence over 40%, the WHO recommends daily iron supplementation (30 – 60 mg of elemental iron) for three consecutive months per year (216). Treatment of IDA has been shown to improve aerobic and endurance capacity, cognitive performance, productivity, symptomatic fatigue, and mood in women (26). A 2016 Cochrane review found that daily supplementation of women of reproductive age reduced the prevalence of anaemia and ID, raised Hb

and iron stores, improved exercise performance, and reduced fatigue, however it also increased gastrointestinal side effects such as constipation and abdominal pain (200).

2.7.5 Pregnancy

During pregnancy the WHO recommends the use of daily oral IFA supplementation (30 - 60 mg of elemental iron and 0.4 mg of folic acid, daily) to prevent maternal anaemia, puerperal sepsis, LBW, and PTB (16, 23). However, the evidence for anaemia reduction in women is much stronger than it is for LBW and PTB outcomes. Due to side effects, pregnant women may have difficulties with compliance to daily supplementation, so if the anaemia prevalence is less than 20% among this population, intermittent (once weekly) oral IFA supplementation (120 mg of elemental iron and 2.8 mg of folic acid) is recommended (23). Additionally, in contexts with rigorous research, where MMN deficiencies are common, antenatal MMN supplements that include IFA are recommended in place of IFA alone during pregnancy (224). Recent RCTs in pregnancy have shown that iron supplementation, increased birth length and weight of infants, length of gestation, and increased infant iron status at birth, and this was more notable in women who were deficient at baseline (16, 226). Additionally, antenatal iron has been found to significantly reduce early neonatal mortality (227).

2.7.6 Early infancy (0-6 months of age)

The following section focuses on policy and recommendations for infants under six months of age to control and prevent ID. Iron and/or MMN interventions in infants under six months of age are reviewed in detail Chapter 3 of this Thesis (105).

2.7.6.1 *Exclusive breastfeeding*

WHO policy states that EBF, should be sufficient to meet the iron requirements of infants under six months of age because of both breastmilk iron being highly bioavailable and the iron endowment at birth (8, 20). According to the WHO, absorption of iron from breastmilk is as high as 50% (114).

2.7.6.2 *Delayed umbilical cord clamping*

Delayed umbilical cord clamping, the process of delayed cutting of the umbilical cord, is another method to reduce ID in neonates. Delaying clamping can allow the transfer of approximately an extra 100 mL of placenta blood to the infant within three minutes (26). Delaying clamping for two minutes can increase body iron content by approximately 33% (75 mg), and results in greater iron stores at six months of age (211). A potential risk of delayed cord clamping is polycythaemia (high concentration of red blood cells) and jaundice. However, an RCT reviewing delaying cord clamping by three minutes found no adverse effects (228), further supported by a Cochrane review showing no risk of post-partum haemorrhage from delayed cord clamping and for the neonate the mean birthweight and Hb

levels were higher in the late cord clamping group, with ID rates much higher at three to six months of age in the early cord clamping group (229).

2.7.6.3 Fortified formulas

Infant formula milks are commonly iron fortified, with a minimum iron content of 4 mg/L (189). In the US, infant formulas are recommended to contain at least 6.7 mg/L of iron and in Europe, 4-8 mg/L (20, 230). Much higher concentrations of iron are found in fortified formulas than in breastmilk due to the comparatively low bioavailability, with only about 10% of iron in infant formula being absorbed (20, 114).

RCTs in HICs where infants had a normal iron status at birth found evidence that high versus low iron fortified formula in infants under six months of age showed no significant difference in Hb or ferritin levels at six months of age (231-234). However, a study of infants with low iron levels in a low income area of Canada receiving iron fortified formula (12.8 mg/L) found evidence for increased Hb and serum ferritin and decreased anaemia at six months of age when compared to those receiving non-iron fortified formula (1.1 mg/L) (235).

2.7.7 Six months to five years of age

Between six and 24 months of age, infants and children require additional iron to support rapid growth and development (20). During this critical window of growth, weaning diets with high iron content are encouraged. Additionally, and as already highlighted, in areas with an IDA prevalence over 40%, the WHO recommends daily iron supplementation (10 – 12.5 mg elemental iron) for three consecutive months per year in children between six and 23 months of age (24, 236). While this is strongly recommended, there is low quality of evidence for this recommendation (24, 236).

An area of increasing interest to address ID, alongside the deficiency of other micronutrients is the use of MNPs. In children aged six to 23 months, where the prevalence of anaemia is 20% or higher, home fortification with MNPs is recommended by the WHO (218). A well-known MNP for home fortification are 'Sprinkles'. Sprinkles contain iron (alongside other micronutrients, typically zinc and vitamin A), in a lipid layer, preventing them from altering the appearance and taste of foods. RCTs have shown MNPs to be as effective as IFA in controlling anaemia and improving iron and micronutrient status in children in LMICs (237, 238). A Cochrane review looking at use of home fortification with MNPs vs placebo in children under two years of age found MNPs reduced anaemia by 31% and ID by 51% (239). However, MNPs are often more expensive than iron alone, and this study did not have sufficient data to compare iron status for MNPs vs iron supplements (239).

2.7.8 Interventions to improve iron status

Daily oral iron supplementation in women of reproductive age and children is widely available and used. Interventions, besides daily oral supplementation, to improve iron status are discussed below. While not all interventions are specific to women of reproductive age, infants, and children, the interventions are worth consideration for these age groups of interest.

2.7.8.1 *Intermittent iron supplementation*

Given that adverse effects of iron supplementation may be dose dependent, intermittent dosing may have higher bioavailability and cause fewer adverse effects (71, 240, 241).

A Cochrane systematic review in women of reproductive age found that iron administered intermittently (once, twice or three times a week on alternating days) improved Hb and ferritin while reducing anaemia risk by 27% (242). This review also found that daily iron increased women's risk of anaemia by 26% versus intermittent, but that endline mean Hb was similar (242). However, no impacts were seen on incidence of adverse events (242).

A further Cochrane review assessing daily vs intermittent iron (one to three times a week) in pregnant women found no difference in infant outcomes (birthweight or gestation) (131). However, the evidence also showed that while mothers receiving intermittent iron had fewer adverse effects, they also had lower Hb and an increased risk of ID and anaemia at term versus daily administration (131).

In children under 12 years of age, intermittent iron supplementation (one to three times a week) versus placebo reduced the risk of anaemia by 49% and ID by 76% while improving Hb and ferritin concentrations (129). This review also looked at daily versus intermittent iron supplementation and found that daily iron decreased the risk of anaemia by 23%, but Hb and ferritin concentrations were similar (129).

2.7.8.2 *Food fortification*

For infants and children many CFs are low in bioavailable iron so food fortification with low doses of highly bioavailable iron may be a beneficial approach to meeting iron requirements (243, 244). Targeted foods for iron fortification are wheat, maize, and rice, as well as spices and sauces such as salt, curry powder, and fish and soy sauces, and candy (245). Ferrous sulphate and fumarate are often used thanks to their high bioavailability (245). However, while ferrous sulphate is inexpensive and widely used, it has a short lifespan. Insoluble compounds have also been used due to a lower reactivity with foods, but they are also less bioavailable (245). Elemental iron is known to have poor bioavailability and also affect the taste of food (245). The use of ethylenediaminetetraacetic acid (EDTA) has been effective for grains, salts, and sauces and has high absorption rates (245). Lastly,

ferrous salts encapsulated in an oil layer have high bioavailability and minimal reactivity with food but are expensive (245). The iron compounds recommended by the WHO to fortify cereals are ferrous sulphate, ferrous fumarate, ferric pyrophosphate, and electrolytic iron (246). However, there are issues with iron fortification such as ensuring quality control (26). Additionally, cooking or industrial thermal processing may decrease iron bioavailability (247).

A clinical trial in women with iron fortified juices found an increase in iron status, Hb, haematocrit, serum ferritin, and TSAT (248). However, when fortifying a dairy product for women, no effect was seen (249). Likewise, encapsulated iron pyrophosphate was shown to be bioavailable when added to a meat paste (250).

Another approach, known as biofortification, is the modification of crops to obtain a final product with a higher micronutrient content and bioavailability. Genetic biofortification uses sequencing and gene technology, offering a sustainable approach to the global health burden of ID (251). An alternative form of biofortification is agronomic biofortification which uses the application of mineral micronutrient fertilizers to soils and plants, thereby increasing the micronutrient content of the edible part of the crop (252). While agronomic biofortification is more cost effective in the short term, genetic biofortification may be more cost effective in the long run (252). Lastly, transgenic plant breeding aims to genetically modify a crop that naturally does not contain that nutrient, by inserting genes from another species. A review and meta-analysis looking at biofortified rice, pearl millet, and beans for improving iron status in at risk populations found that iron-biofortified interventions significantly increased serum ferritin concentrations and total body iron, with effects highest among individuals who were iron deficient at baseline (253). A specific biofortification initiative, HarvestPlus, has developed iron beans and pearl millet providing up to 80% of daily iron needs for women of reproductive age and children, with over 63 countries reached worldwide, mainly in sub-Saharan Africa, Asia, and Latin America and the Caribbean.

2.7.8.3 Alternative oral irons

Oral iron salts such as ferrous sulphate, ferrous gluconate, and ferrous fumarate are most used to treat ID due to their high bioavailability. However, other forms of iron are being explored as issues with gastrointestinal side effects are common (254). Ferric iron has low solubility and must be reduced to ferrous iron before cellular uptake, therefore having poor bioavailability (255). Carbonyl iron can be effective at high doses but absorption is generally poor and therefore its use is not recommended (256). Ferric iron-polysaccharides are often marketed for their improved taste and lower incidence of side effects, but the evidence does not support such claims (257). Polysaccharide iron complex and haem iron polypeptide offer improved absorption and tolerability over traditional iron salts but are

significantly more expensive (254). In a systemic review of tolerability of varying forms of iron supplementation in the general population, it was found that incidence rates of overall adverse events were 7.3% for iron protein succinylate, 23.5% for ferrous glycine sulphate, 30.9% for ferrous gluconate, 32.3% for ferrous sulphate without mucoprotease, and 47.0% for ferrous fumarate (258).

Use of prebiotics, and compounds such as galacto-oligosaccharides, alongside oral iron supplementation has been shown to mitigate most adverse effects of the supplements (117, 259).

2.7.8.3.1 IHAT: iron hydroxide adipate tartrate

Iron delivered naturally through foods may be a safer alternative, so there is growing interest in developing novel nano iron compounds or delivery systems for use in fortification and oral supplementation (21, 260-263). A proposed strategy is a slow-release nano-molecular formulation, iron hydroxide adipate tartrate (IHAT), that mimics the natural food matrix and may provide a safer option by reducing the adverse effects of unabsorbed iron on the gut microbiota (264). A study in rural Gambia supplementing infants from six to 35 months of age for three months with either daily oral IHAT, ferrous sulphate, or placebo found evidence that IHAT showed non inferiority compared to the standard of care: ferrous sulphate; and had a lower incidence of moderate-severe diarrhoea versus ferrous sulphate, with no increased adverse events in comparison to placebo (265).

2.7.8.4 Intravenous iron

An alternative approach to oral iron is intravenous (IV) iron. IV iron is recommended in clinical practice for the treatment of anaemia associated with a range of conditions such as chronic kidney disease, heart failure, and inflammatory bowel disease (266). However, more recently there has been interest in expanding IV iron to other settings where oral iron is less well absorbed, such as in the presence of chronic inflammation (266). This is also due in part to newer preparations of IV iron, reported to have higher safety and ease of administration than older forms (266). Additionally, IV iron is indicated for patient's intolerant to oral iron or for those for whom oral iron has been ineffective (267).

A systemic review and metanalysis of data from trials in pregnant women found that mean Hb at delivery was significantly higher after IV iron, as well as being associated with a higher infant birthweight (268). Studies have shown IV iron restores iron reserves and raises Hb more rapidly than oral therapy (268-271). A recent study by Pasricha and colleagues in iron deficient but otherwise healthy Australians found that a newer form of IV iron caused no serious adverse events and produced an increase in Hb levels within two weeks, but also caused elevations in hepcidin sustained to four weeks post injection (267). However, a systematic review of 154 trials in the general population found that IV iron was associated with an increased risk of infection, so it must be carefully balanced with the benefits for treating anaemia (266). Contrastingly, another recent study by Pasricha and

colleagues of anaemic pregnant Malawian women found evidence that in this malaria-endemic setting, treatment with IV iron was safe but did not reduce maternal anaemia prevalence at 36 weeks' gestation or increase infant birthweight (272). An RCT of short IV infusions to infants, children, and adolescents with IDA who have poor responses to oral iron found evidence that short infusions with IV iron caused no adverse effects (273).

2.7.8.5 Increasing dietary iron

In adults, dietary iron only supplies about 5% of daily iron needs, with the remainder coming from recycling of iron stored in cells (274). In contrast, infants and children require 30% of their daily iron to come from diet due to their rapid growth and development (274). While dietary iron interventions are more sustainable than universal supplementation, such dietary interventions are often not feasible in resource limited settings.

Two types of dietary iron exist: haem (organic) and non-haem (inorganic) iron (195). Haem iron is found in animal based foods and is highly bioavailable, with about 20-50% of ingested iron being absorbed (195). This includes animal sources such as meat, liver, fish, and eggs (275). Non-haem iron has lower bioavailability, but makes up a higher proportion of dietary intake (195). This includes sources such as cereals, legumes, dark leafy greens, nuts, and dried fruits (275). Non-haem iron's absorption is regulated by other dietary enhancers, such as ascorbic acid and muscle protein, or inhibitors such as phytates and tannins (276). Such enhancers include citrus fruits while inhibitors include unrefined grains and pulses, alcohol, coffee, and black tea (275). In LMICs, weaning diets in infancy tend to contain low absolute iron content in addition to higher ratios of iron inhibitors to enhancers (4). Increasing consumption of vitamin C rich foods has been shown to improve iron status (248).

2.8 THE OUTSTANDING ISSUE

Current iron supplementation regimens do not seem to be working as the prevalence of anaemia among women, infants, and children remains high. While supplementation to pregnant women has been shown to benefit maternal and infant birth outcomes, the impact of maternal supplementation on infant iron status in the long-term is less clear. To date, the WHO often only recommends EBF and delayed cord clamping in healthy, full-term, normal birth weight infants under six months of age in order to prevent anaemia (8). However, recent evidence suggests that the foetal iron endowment may not always be sufficient to meet infant's needs across the period of EBF, especially in contexts where rates of maternal anaemia are high, as breastmilk alone cannot support iron requirements during this time. Additionally, the best route and timing of iron interventions is still debated, and the benefits and

risks of supplementation must be carefully considered, especially in countries with high rates of infection and inflammation.

2.9 AIMS AND OBJECTIVES

On this backdrop, the overall aim of this Thesis is to examine if iron supplementation before six months of age in healthy, term, normal birthweight infants, with a specific focus on LMICs, improves infant outcomes, specifically the health impacts for the infant (breastfeeding and growth) as well as the barriers and associated practicalities of implementing an early intervention.

The research objectives of this Thesis are to:

1. Systematically review iron and/or MMN supplementation in healthy, term, normal birthweight infants under six months of age to summarise the evidence and identify any gaps in the literature (Chapter 3).
2. Systematically review caregiver acceptability of nutrition interventions in their infants and children (under 24 months of age) to inform the feasibility of future proposed nutrition interventions in infants (Chapter 4).
3. Conduct a double blind, placebo controlled, randomised pilot trial with daily oral iron in Gambian infants under six months of age, known as *Iron Babies* (Chapter 6).
4. Analyse the impacts on nutrition specific outcomes from *Iron Babies* such as the link between supplementation, breastfeeding rates, and anthropometry (Chapter 7).
5. Assess the acceptability of early administration of iron, through qualitative research with local stakeholders and mothers from *Iron Babies*, to inform future scale up and implementation of nutrition interventions in young infants (Chapter 8).
6. Summarise the overall research findings and contextualise any research gaps to inform future clinical trials and policy recommendations for iron supplementation, with a specific focus on contexts most at risk of ID and the associated pathologies (Chapter 9).

3 ACKNOWLEDGING THE GAP: A SYSTEMATIC REVIEW OF MICRONUTRIENT SUPPLEMENTATION IN INFANTS UNDER SIX MONTHS OF AGE

3.1 INTRODUCTION

This Chapter incorporates an original, published systematic review (Stelle, et al., 2020) published in *Wellcome Open Research* in 2020. This systematic review was undertaken at the start of this PhD to review the evidence on micronutrient intervention trials in young infants and address any gaps in the literature, especially in relation to iron interventions in infants under six months of age. Interventions in LBW and PTB infants were later excluded due to extensive reviewing elsewhere (277). The Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) guidelines were used (see Appendix 1 for the PRISMA Checklist). The review protocol was registered on PROSPERO (available at: https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=165641). As part of this systematic review, I co-supervised an iBSc medical student (SV). This consisted of teaching SV how to create, run, and perform a systematic search of interest. SV's primary outcome was the biochemical outcomes of this systemic review, for which she extracted, analysed, and assessed the bias for this data, while acting as a second reviewer for the systematic review. Additionally, later, I co-supervised a second iBSc medical student (TE) in undertaking a meta-analysis of the biochemical outcomes.

This systematic review is the first comprehensive review of iron and/or MMN supplementation in infants under six months of age. As mentioned, there are currently IFA supplementation guidelines for pregnant women, and more recently MMNs (including IFA) are recommended during pregnancy in contexts of rigorous research. However, it is still unclear if prenatal iron and/or MMN supplementation impacts on infant iron status. The only practices recommended by the WHO to prevent anaemia in young infants are delayed cord clamping and EBF until six months of age, a time at which exclusively breastfed infants have been shown to have serum iron levels below the clinical reference range. This may leave infants under six months of age at risk, during a window of critical growth and development. Iron supplementation is additionally complicated because the safety and timings of interventions are still debated.

Therefore, the aim of the review and this Chapter is to examine the effects of iron, iron containing MMNs and/or MMNs during the first six months of life on infant outcomes (biochemical, growth, morbidity and/or mortality, and neuro-behavioural development) in hopes to summarise this gap in policy and inform future interventions at this young age. Appendix 1 presents additional files associated with this publication.

3.2 AUTHOR ROLES

Conceptualization: Isabella Stelle, Karen Edmond, Sophie E Moore

Funding Acquisition: Sophie E Moore

Methodology: Isabella Stelle, Karen Edmond, Sophie E Moore

Analysis plan: Isabella Stelle

Paper specific data management and main analysis: Isabella Stelle, Sruthi Venkatesan

Supervision: Isabella Stelle, Karen Edmond, Sophie E Moore

Original Draft Preparation: Isabella Stelle

Reviewing and Editing: All authors

Approval of final manuscript: All authors

3.3 PUBLISHED PAPER 1: ACKNOWLEDGING THE GAP: A SYSTEMATIC REVIEW OF

MICRONUTRIENT SUPPLEMENTATION IN INFANTS UNDER SIX MONTHS OF AGE

Stelle I, Venkatesan S, Edmond K and Moore SE. Acknowledging the gap: a systematic review of micronutrient supplementation in infants under six months of age. *Wellcome Open Res.* 2020, 5:238

<https://doi.org/10.12688/wellcomeopenres.16282.1>

The full paper can be found below and additional files in **Appendix 1** or accessed here:

<https://doi.org/10.12688/wellcomeopenres.16282.1>



Check for updates

SYSTEMATIC REVIEW

REVISED Acknowledging the gap: a systematic review of micronutrient supplementation in infants under six months of age [version 2; peer review: 2 approved]

Isabella Stelle¹ , Sruthi Venkatesan¹, Karen Edmond¹, Sophie E. Moore^{1,2}

¹Department of Women and Children's Health, King's College Hospital, London, Westminster Bridge Road, London, SE1 7EH, UK

²Nutrition Unit, MRC Unit The Gambia at London School of Hygiene and Tropical Medicine, Banjul, The Gambia

v2 First published: 12 Oct 2020, 5:238

<https://doi.org/10.12688/wellcomeopenres.16282.1> Latest published: 20 Feb 2023, 5:238

<https://doi.org/10.12688/wellcomeopenres.16282.2>

Abstract

Background: Micronutrient deficiencies remain common worldwide, but the consequences to growth and development in early infancy (under six months of age) are not fully understood. We present a systematic review of micronutrient interventions in term infants under six months of age, with a specific focus on iron supplementation. **Methods:** We searched the Cochrane Central Register of Controlled

Trials (CENTRAL), MEDLINE (Ovid) and Embase (Ovid) from January 1980 through December 2019. Interventions included iron or multiple micronutrients (MMNs).

Results: Of 11,109 records identified, 33 publications from 24 trials were included (19 iron and five MMN supplementation trials). All but one trial (evaluating only morbidity and mortality) evaluated the effect of supplementation on biochemical outcomes, ten reported on growth, 15 on morbidity and/or mortality and six on neurobehavioural development. Low- and middle- income countries made up 88% (22/25) of the total trial locations. Meta-analysis was not possible due to extensive heterogeneity in both exposure and outcome measures. However, these trials indicated that infants less than six months of age benefit biochemically from early supplementation with iron, but the effect of additional nutrients or MMNs, along with the impacts on growth, morbidity and/or mortality, and neuro-behavioural outcomes remain unclear.

Conclusions: Infants less than six months of age appear to benefit biochemically from micronutrient supplementation. However, well powered randomised controlled trials are required to determine whether routine supplementation with iron or MMNs containing iron should commence before six months of life in exclusively breast-fed infants in low-resource settings.

Keywords

Iron, Iron Deficiency Anaemia, Anaemia, Multiple Micronutrients, Micronutrients, Infants, Supplementation

Open Peer Review**Approval Status**

1

2

version2

(revision)

20 Feb 2023

**version1**

12 Oct 2020

[view](#)[view](#)

1. **Filomena Gomes** , The New York Academy of Sciences, New York City, USA

2. **Peter Cooper**, University of the Witwatersrand, Johannesburg, South Africa

Any reports and responses or comments on the article can be found at the end of the article.

Corresponding author: Isabella Stelle (isabella.stelle@kcl.ac.uk)

Author roles: **Stelle I:** Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Resources, Software, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Venkatesan S:** Investigation, Writing – Review & Editing; **Edmond K:** Conceptualization, Supervision, Validation, Writing – Review & Editing; **Moore SE:** Conceptualization, Funding Acquisition, Methodology, Resources, Supervision, Validation, Visualization, Writing – Review & Editing **Competing interests:** No competing interests were disclosed.

Grant information: This work was supported by funding to SEM from The Wellcome Trust [220225] and the Medical Research Council (UK) [MR/P012019/1]. *The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*

Copyright: © 2023 Stelle I *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Stelle I, Venkatesan S, Edmond K and Moore SE. **Acknowledging the gap: a systematic review of micronutrient supplementation in infants under six months of age [version 2; peer review: 2 approved]** Wellcome Open Research 2023, 5:238 <https://doi.org/10.12688/wellcomeopenres.16282.2>

First published: 12 Oct 2020, 5:238 <https://doi.org/10.12688/wellcomeopenres.16282.1>

REVISED Amendments from Version 1

One paper (Sazawal *et al.* 2006) was omitted by error due to the title indicating the study took place in preschool aged children. Our updated manuscript now includes this study. This study was an RCT in 1–36 month old Tanzanian infants supplemented daily with IFA, IFA + zinc, zinc alone, or placebo. Due to the style in which outcomes were reported, it was not possible to determine what the biochemical outcomes were in infants under six months of age, but only on morbidity and mortality in this age group. Results on adverse events (85/2135), morbidity (17/295), and hospital admissions (68/1840) were reported for infants under six months of age, but no conclusions could be drawn from this data due to enrolment numbers not being divided by age group. However, it is worth noting the iron and folic acid-containing groups of the trial were stopped early on the recommendation of the data and safety monitoring board. Due to missing enrolment data, no conclusions on the impact in infants under six months could be drawn. All figures as well as the extended datafiles have been updated to reflect this additional study. The inclusion of this paper does not change our overall findings and analysis, rather it is numerical in terms of the number of papers reported.

Any further responses from the reviewers can be found at the end of the article.

Introduction

Iron deficiency anaemia (IDA) is estimated to affect 1.2 billion people worldwide¹. Overt IDA is associated with increased risk of impaired immunity, serious morbidity, poor pregnancy outcomes and poor motor function and mental development in children². It remains the leading cause of years lived with disability in low- and middle-income countries (LMICs) and is responsible for more than 120,000 maternal deaths per year³. The prevalence of anaemia is five times higher in LMICs than high-income countries (HICs) and globally about 43% of children between the ages of six to 59 months are reported to be anaemic^{3,4}. As such, IDA is the largest nutritional deficiency disorder worldwide and one of the five leading contributors to the global disease burden³.

To date there are global and national level policies and guidelines around iron supplementation in women of reproductive age and young children. For all women of reproductive age, in areas with an IDA prevalence over 40%, the World Health Organisation (WHO) recommends daily iron supplementation (30 – 60 mg of elemental iron) for three consecutive months per year⁵. During pregnancy, regardless of IDA rates, the addition of folic acid is recommended through the use of daily oral iron folic acid (IFA) supplementation (30 - 60 mg of elemental iron and 0.4 mg of folic acid) to prevent maternal anaemia, puerperal sepsis, low birth weight (LBW), and preterm (PT) birth⁶. If pregnant women find daily IFA unacceptable due to side effects, and when the anaemia prevalence is less than 20% among pregnant women, intermittent oral IFA supplementation (120 mg of elemental iron and 2.8 mg of folic acid) once weekly is recommended⁶. For young children between the age of six and 23 months, in areas with an IDA prevalence over 40%, the WHO recommends daily iron supplementation (10 – 12.5 mg elemental iron) for three consecutive months per year⁷. In infants under six months of age, exclusive breast feeding (EBF) is the only practice recommended to prevent anaemia⁸. However, despite several widespread supplementation schemes, IDA prevalence has remained in the top five leading causes of death worldwide since 1990³.

This lack of change in IDA prevalence is significant, as micronutrient deficiencies during pregnancy and lactation have implications for infant nutritional status⁹. When maternal diets are habitually low in micronutrients, infants are at risk of poor status in early life by consequence of a reduced endowment during foetal life coupled with – for some micronutrients – low levels in human milk^{10–12}. Such undernutrition during the first 1000 days from conception until two years of age can cause irreversible damage to growth and cognitive development¹³. This is especially relevant in LMICs, where one third of children fail to reach their developmental milestones by school age¹⁴.

Sufficient iron is essential in pregnancy as iron stores for the first few months of life are passed from mother to infant in utero¹⁵. In healthy, full-term infants the iron endowment accumulates in the foetal liver, stored as ferritin¹⁶. The iron received in utero is intended to support growth and development in the first six months of life, acting as a buffer for the needs of new tissue formation. This is especially pertinent in countries where breast feeding is an infant's main nutrition source, as human milk is very low in iron¹⁷. Iron supplementation for deficient mothers during gestation has only been shown to improve maternal anaemia and increases neonatal birthweight, while reducing the risk of PT birth¹⁸. However, the long-term efficacy of antenatal iron to benefit infant iron status has not been proven¹⁸.

A recent analysis of 317 rural Gambian infants, from an area where EBF rates are high, found infants were born with a reasonable endowment of iron despite being born to mothers with high levels of deficiency^{19,20}. However, following birth there was a rapid deterioration of both haemoglobin (Hb) and ferritin¹⁹. By five months of age, about 95% of infants had serum iron levels below the clinical reference range and this continued beyond the first year of life¹⁹. These data suggest that exclusively breast-fed infants, born in low-resource settings, are at risk of iron deficiency (ID) in the first six months. This leaves these infants vulnerable, as this age group is not addressed by policy recommendations beyond EBF⁸.

Adding to the burden of IDA is the confusion around the best iron therapy regarding safety and effectiveness²¹. Oral iron may not be the best route and the timing of interventions, if not in pregnancy and early infancy, may be too late to impact on early brain development²².

It is known that undernutrition significantly contributes to chronic inflammation by weakening immune function and increasing susceptibility to recurrent and persistent infections (e.g. diarrhoea and upper respiratory tract infections)²³. Likewise, environmental enteric dysfunction (EED), a syndrome caused by frequent bacteria transmission through the faecal-oral route due to poor sanitation, is characterised by chronic inflammation and morphological changes in the small intestine (i.e. villous blunting), further contributing to malabsorption²⁴. However, unabsorbed dietary iron through fortification or supplementation has been shown to negatively impact the gut microbiota²⁵. It has also been shown that four hours after consumption of an iron tablet by adults (2 mg/kg ferrous sulphate), human blood greatly supports enhanced rates of replication of pathogenic bacteria²⁶. This leads to reduced resistance to infection, higher prevalence of diarrhoea and increased faecal

calprotectin, a marker of gut inflammation²⁷. However, there is a lack of sufficient data on side effects from iron supplementation in infants and children to negate their benefits in low-resource settings²⁸.

Intermittent oral iron supplementation in children under 12 years of age has been systematically reviewed, but none of the included trials were conducted in infants under six months of age²⁸. The authors concluded that intermittent iron supplementation “improve[d] haemoglobin concentrations and reduce[d] the risk of having anaemia or iron deficiency in children younger than 12 years of age when compared with a placebo or no intervention”²⁸. It was noted, however, that information on mortality, morbidity, developmental outcomes and side effects are still lacking²⁹.

Further, maternal acquired immunity to malaria begins to decline in infants around three months of age and, in malaria endemic settings, iron supplementation in children has been shown to cause harm^{30,31}. However, a 2016 Cochrane review by Neuberger and colleagues assessed oral iron supplements for children in malaria-endemic areas and found that if malaria prevention and management services are offered, there was no increased risk to clinical malaria from iron supplementation²⁹.

The WHO now may recommend the use of multiple micronutrients (MMNs) containing iron over IFA alone in regions where the benefits outweigh the disadvantages⁶. There is strong evidence that maternal supplementation with MMNs during pregnancy has positive impacts on several birth outcomes, but a positive impact on infant iron status has not been shown³². Likewise, there was insufficient data of MMN supplementation during lactation to report any impact on infant iron status³³. In pre-school age children, point of use fortification with micronutrient powders (MNP) has been shown to reduce anaemia and ID³⁴. Recently published data also concluded that, in infants under two years of age, MNPs are better than no intervention and placebo, and may be as effective as daily iron supplementation³⁵. However, none of the included trials were in infants under six months of age and further research is needed to determine developmental outcomes³⁵.

Given the lack of policy addressing additional iron needs in infants under six months of age, in order to understand both the short and longer-term impacts of supplementation with iron or MMNs in this age group, we present here a systematic review of MMN supplementation in infants under six months of age, with a specific focus on iron.

Objectives

Primary objective

To examine the effects of iron, iron containing MMNs and/or MMNs during the first six months of life on infant outcomes (biochemical, growth, morbidity and/or mortality, and neuro-behavioural development).

Methods

The Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) guidelines were used³⁶. The PRISMA Checklist is presented in *Extended data* file 1³⁷. The review protocol was

registered on PROSPERO (registration number [CRD42020165641](#), 11th February 2020).

Search methods

On January 21st, 2020, the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE (Ovid) and Embase (Ovid) were searched without geographical limitations for resources from 1980 through 2019.

Clinical trials registries for ongoing or recently completed trials were also searched ([clinicaltrials.gov](#); [controlled-trials.com](#); and [who.int/ictpr](#)).

MeSH search terms included: Iron; Iron, Dietary; Anaemia, Iron-Deficiency; Folic Acid; Dietary Supplements; Trace Elements; Ferric Compounds; Ferrous Compounds; Micronutrients; Drug Administration Schedule; Dose-Response Relationship, Drug; Time Factors; Infancy. The full search strategy for this systematic review can be found in *Extended data* file 2³⁷.

Exclusion & inclusion criteria

Trials were included if they supplemented infants with iron, MMNs (> two micronutrients) containing iron or MMNs not containing iron - noting that iron could be a control arm; were in infants under six months of age; or were randomised controlled trials (RCTs).

Trials were excluded if supplementation was initiated in all infants after six months of age; fortified or complementary formulas and/or foods were outlined in the protocol for use before six months of age; or they were trial protocols, quasi experimental trials, observational and exploratory trials, case trials, economic evaluations, programme reports, clinical charting, conference proceedings, letters to the editor, opinion papers or editorials.

Trials conducted in infants over six months of age or in other languages other than English were later excluded. Trials of LBW or PT infants were later excluded due to extensive reviewing elsewhere³⁸.

Selection of trials

Search results were uploaded to EndNote X9, where duplicates were removed. Titles and abstracts of publications retrieved using the search strategies were screened to identify trials that potentially met the inclusion criteria. Full texts of these publications were then reviewed.

In addition, previous reviews (including cross references) were searched, as well as checking reference lists of identified trials for further relevant trials.

Trial selection was carried out by two reviewers independently (IS and SV) and then cross-checked. Any disagreements were discussed and resolved. To assess the entire scope of research conducted, no country was excluded.

Data extraction, management & assessment of bias

Data was extracted into Microsoft Excel to aid extraction of relevant information from each included trial. A list of *a priori*

variables to categorise and extract the data were used. Data extracted are included in *Extended data file 5*³⁷.

The Cochrane Risk of Bias 2 (RoB 2) Tool³⁹, along with Review Manager 5 (RevMan 2011)⁴⁰, were used to assess bias and create associated tables and figures. No trials were excluded from the narrative synthesis based on their quality assessment.

Results

The search identified 11,109 publications for possible inclusion, 2,505 of which were duplicate references (*Figure 1*). 210 full text articles were reviewed for eligibility. Of these, 108 articles were excluded because they assessed LBW or PT infants, 28 because the full texts were not in English, 19 because further reading identified the age groups as over six months, 12 because they either used formula or complementary foods before six months of age as part of trial protocol, eight because they were reviews, one because it was a commentary and one because it was a conference abstract with full data published elsewhere. 33 publications, consisting of 24 trials, were retained for qualitative analysis by outcome. A further 13 references (trial protocols and registered and on-going trials) were identified through additional resources, but do not make up the 24 remaining trials for analysis (*Extended data file 3*³⁷).

For risk of bias analysis, of the 33 publications, six publications showed a low risk, 25 unclear risk and nine high risk of bias (*Extended data file 4*³⁷). Those showing unclear risk of bias were found to have small sample sizes ($N < 100$), unclear specifications on randomisation and/or mother's supplementing the infants themselves. The trials with a high risk of bias had a lack of specifications on randomisation, a high dropout/non-compliance rate and/or lack of data reporting⁴¹.

Trial characteristics and outcomes

Nineteen iron and five MMN supplementation trials published between 1997 and 2019 were included in this review, with findings

published across 33 separate papers. Sample sizes varied widely across the trials, from 70 to 94,359 infants and children, with data from 133,391 infants and children for analysis in this review.

Below, the results are reported by outcome: 1. biochemical, 2. growth, 3. morbidity and/or mortality, and 4. neuro-behavioural development. They are then further sub-divided by iron supplementation trials and MMN trials (*Figure 2*). The iron supplementation trials are further subdivided into trials of supplementation in pregnancy and infancy, generic two-arm iron versus placebo trials, comparing formulations, comparing timings and doses, comparing age of initiation, with added zinc or with added malaria prophylaxis. The MMN supplementation trials are further subdivided into those with and those without iron.

Three of the five MMN trials gave MMNs also containing iron. All but one trial (evaluating only morbidity and mortality) evaluated the effect of supplementation on biochemical outcomes, ten additionally reported on growth, 15 on morbidity and/or mortality and six on neuro-behavioural development. Further trial characteristics and results, grouped by outcome, are provided as *Extended data file 5*³⁷.

Outcome 1: Biochemical

All but one trial⁴² reported on biochemical outcomes for infants. Eighteen of these assessed supplementations with iron and five with MMNs. One of the iron trials additionally randomised mothers to iron or placebo in pregnancy⁴³. Of the trials assessing iron supplementation, four also included malaria prophylaxis in their interventions. Three of the five MMN trials included iron.

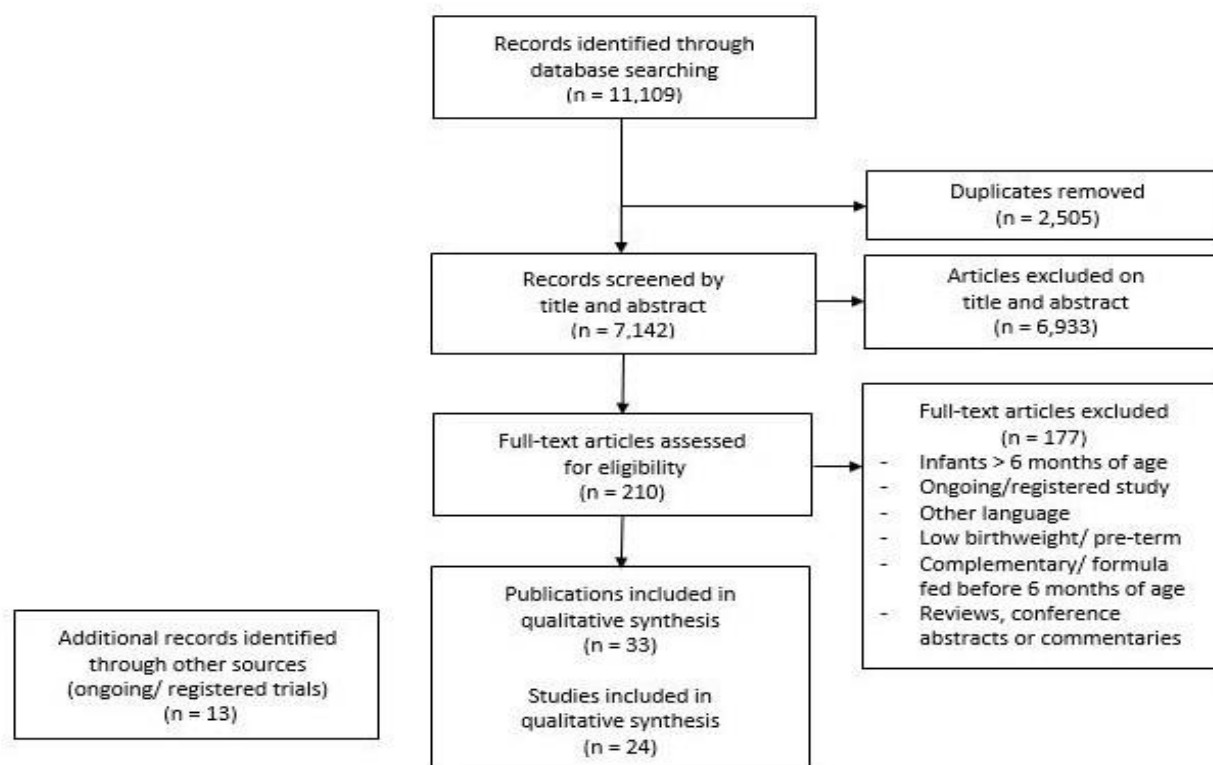


Figure 1. Selection of trials using the PRISMA flow chart.

Iron supplementation – in pregnancy and infancy. An RCT in rural China (N = 1,276) supplemented mother-infant pairs in pregnancy from 16 weeks gestation with daily ferrous sulphate or placebo, and infants from six weeks until nine months of age with daily iron proteinsuccinylate or placebo⁴³. Iron supplementation in infancy, but not pregnancy, reduced ID risk in infants at nine months of age, but more than 60% of infants still had ID at this age⁴³. However, effects of iron supplementation in pregnancy were observed when higher amounts of iron were distributed in infancy⁴³.

Iron supplementation – two arm trials. A trial conducted in Northeast India randomised 200 infants (1/2 born to anaemic mothers) at 36 hours after birth until six months of age to daily ferrous ascorbate drops or standard of care⁴⁴. Infants born to anaemic mothers had significantly higher rates of ID at birth⁴⁴. Further, significantly higher Hb and serum ferritin (SF) in the supplemented versus placebo group were seen at six months of age,

with comparable beneficial effects of supplementation in infants born to anaemic and non-anaemic mothers⁴⁴.

Another trial in rural India randomising 100 infants from four to six months of age for eight weeks to daily ferric ammonium citrate or placebo found significantly higher Hb and SF in the iron versus placebo group after eight weeks of supplementation⁴¹. Likewise, the adjusted rise in Hb was higher in initially anaemic infants at the second follow-up⁴¹.

A smaller (N = 77) two-arm trial in a low-income Canadian population found that infants randomised at one until six months of age to daily ferrous sulphate syrup or placebo had significantly higher Hb and mean corpuscular value (MCV) at six months of age versus the placebo group, but the significance was no longer seen at 12 months of age, six months following cessation of the intervention⁴⁵. There was a decline in SF over time; however, the decline was less in the iron supplemented group⁴⁵. At six months of

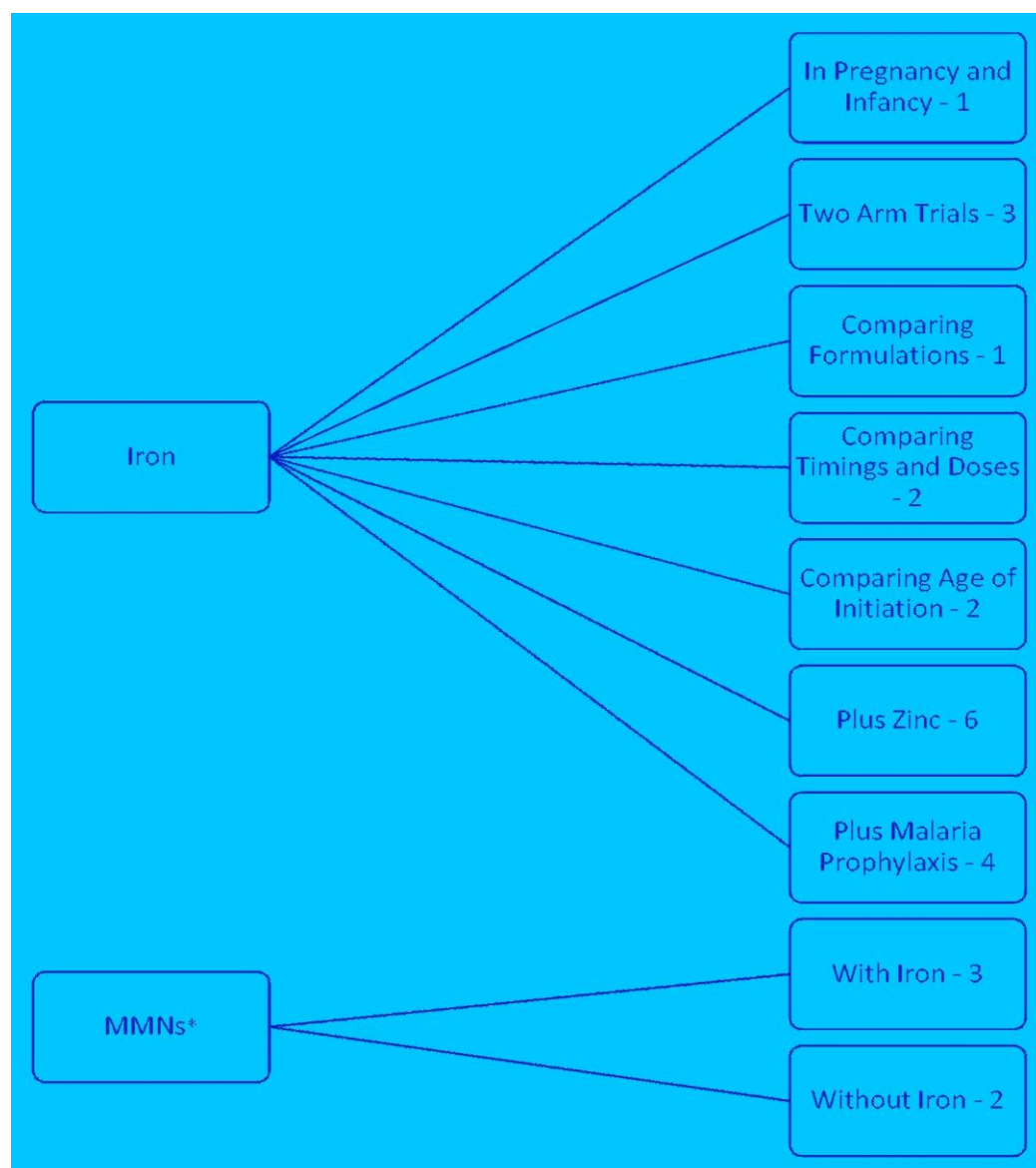


Figure 2. Flowchart of results grouped by trial design with the number of applicable trials (24 included in this review).

*MMNs = multiple micronutrients.

age, infants in the iron group had significantly lower rates of IDA and ID⁴⁵.

Iron supplementation – comparing formulations. An RCT testing ferrous sulphate drops versus ferric polymaltose drops in 112 Turkish infants from four until nine months of age found a significant increase in iron markers at nine months of age for both groups, but with the ferrous sulphate group being significantly higher than the ferric polymaltose group⁴⁶. ID and IDA rates were lower in infants receiving ferrous sulphate versus ferric polymaltose⁴⁶.

Iron supplementation – comparing timings and doses. Another RCT in Turkey tested ferrous sulphate drops daily or weekly versus a standard of care (control) group in 70 infants at four until seven months of age⁴⁷. Infants supplemented weekly had the lowest rates of ID and IDA⁴⁷. Infants whose mothers had ID or IDA during the trial period were more likely to develop ID or IDA⁴⁷. Likewise, SF levels decreased between four and six months of age in the control and daily iron group, while the weekly groups showed no such decrease⁴⁷. However, in all groups, the mean levels of SF were significantly increased during the weaning period⁴⁷.

A third RCT in Turkey tested ferrous sulphate drops at different time intervals and quantities by giving 113 five month old infants iron at either 1 mg/kg/d; 2 mg/kg/d; or 2 mg/kg/48hours versus placebo until nine months of age⁴⁸. No ID was observed in all three iron groups at nine months of age and there were significant increases compared to baseline values for Hb, MCV and SF for all three iron groups⁴⁸. Further, MCV, SF and Hb were significantly higher in all three iron groups versus placebo, but MCV was higher in the 2 mg/kg/d group versus the other iron groups, and SF was significantly higher at nine versus five months of age in this group also⁴⁸.

Iron supplementation – comparing age of initiation. A trial in the Republic of Benin supplemented 612 infants twice daily with ferrous fumarate powder at four (intervention group) or six to 18 months of age (control group), both for two months⁴⁹. After supplementation, there was a significant increase in mean Hb and mean corpuscular Hb, but no significant change in values of MCV in the intervention and control groups, respectively⁴⁹. A decreased anaemia prevalence from 42.6 to 33.8 % in the intervention and from 62 to 30.2 % in the control groups were also seen⁴⁹. In infants who were anaemic at the start of the trial, the Hb increase was higher than that of the whole population⁴⁹.

One trial, conducted across two contrasting settings (Sweden (N = 101) and Honduras (N = 131)) compared three trial arms in infants from four until nine months of age, supplemented daily with either ferrous sulphate from four to nine months of age (early initiation group); placebo from four to six months of age and then ferrous sulphate from six to nine months of age (later initiation group); or only placebo from four to nine months of age⁵⁰. A statistically significant increase in Hb and SF was observed in the early initiation group in both countries versus both other groups at six months of age⁵⁰. A statistically significant increase was also seen in SF in both countries' intervention groups versus placebo at nine months of age⁵⁰. However, only in infants from Honduras was there

a statistically significant increase in Hb in supplemented groups versus placebo, along with a statistically significant lower IDA prevalence in the intervention groups, both at nine months of age⁵⁰. In Sweden, iron supplements caused no reduction in the already low prevalence of IDA at nine months of age⁵⁰.

Iron supplementation – plus zinc. A trial supplemented 478 Indonesian infants at four until ten months of age with either iron, zinc, iron + zinc, or placebo syrups⁵¹. Iron alone was more effective than iron + zinc in increasing Hb and SF and in reducing the prevalence of anaemia⁵¹. Further, IDA prevalence was significantly lower in the iron and iron + zinc groups versus the zinc only and placebo groups⁵¹.

A further trial supplemented 609 Thai infants at four to six months of age for six months with either ferrous sulphate, zinc sulphate, iron + zinc; or placebo syrups⁵². Infants in the two groups not receiving iron had a decrease in Hb concentration between baseline and six months, while the overall effect of iron supplementation on end point Hb concentrations was an increase of 10.8 g/L⁵². However, the effect of zinc supplementation with or without iron was a decrease in Hb concentrations⁵². Likewise, after six months of supplementation, the two groups receiving iron supplementation (iron or iron + zinc) had significantly higher SF than those receiving only zinc or placebo⁵². It was also observed that anaemia prevalence was significantly lower in infants receiving only iron than in infants receiving iron + zinc, only zinc or placebo⁵². ID and IDA prevalence were lower in infants receiving iron or iron + zinc versus zinc only and placebo⁵².

A third trial supplemented 915 Vietnamese infants at four until seven months of age, daily, with either iron, zinc, iron + zinc (doses as previous) or placebo syrups⁵³. Hb and SF were higher in both iron and iron + zinc groups compared to zinc and placebo groups⁵³.

A very large RCT tested daily IFA, IFA + zinc, or placebos supplements in 26,250 Nepalese infants at one to 35 until 36 months of age⁵⁴. The IFA containing groups of the trial were stopped early on the recommendation of the data and safety monitoring board⁵⁴. However, in a subsample, 12 months after the start of supplementation, Hb was highest in the IFA group and median SF was significantly higher in the IFA group than both other groups and significantly higher in the IFA + zinc containing group versus placebo⁵⁴. The prevalence of IDA was lowest in the IFA only group⁵⁴.

Finally, another very large RCT conducted in a low-middle socioeconomic neighbourhood of India randomising 94,359 infants at one to 23 months of age for 12 months, daily, to IFA or IFA + zinc found that % SF < 20 µg/L was marginally lower in the IFA group versus the IFA + zinc group⁵⁵.

Iron supplementation – plus malaria prophylaxis. An RCT in Tanzania supplemented 832 infants from eight weeks until six months of age with iron and from eight weeks until 12 months of age with malaria prophylaxis. At the trial end point, combined iron supplementation and malaria prophylaxis was found to have a protective effect on severe anaemia when compared to infants that

did not receive iron⁵⁶. A second publication from the above trial also found that iron supplemented infants had a significantly lower prevalence of ID at five, eight and 12 months of age, but ID did not differ between those who did and those who did not receive malaria prophylaxis at any time point^{56,57}.

A second RCT in Tanzania supplemented 291 infants from 12 – 16 weeks of age for six months with either daily ferric ammonium citrate mixture; amodiaquine; daily iron; or double placebo⁵⁸. Enrolment took place during the time of year that infants were most vulnerable to malaria⁵⁸. Infants receiving malaria prophylaxis and malaria prophylaxis + iron were protected against anaemia, but those receiving only iron were only partly protected against anaemia⁵⁸.

A further trial in Tanzania compared 14 days of daily ferrous sulphate tablets + one dose of sulfadoxine-pyrimethamine (SP) on day one versus three months of daily ferrous sulphate + three SP doses at the start of each month in 311 infants from two months up to five years of age⁵⁹. Two weeks after completing treatment, the prevalence of PCV < 33% was higher in the 14-day versus three-month intervention group, with mean PCV significantly higher in the three-month versus 14-day treatment groups⁵⁹. However, there was no difference in the prevalence of PCV < 25% two weeks after supplementation and the benefits of the extended therapy were only apparent six months after recruitment⁵⁹.

A four-arm RCT randomised 546 Western Kenyan infants with mild anaemia, at two to 36 months of age, for 12 weeks, to either intermittent preventive treatment (IPT) with SP at four and eight weeks + ferrous sulphate drops; placebo IPT SP + iron; IPT SP + placebo iron; or double placebo⁶⁰. Mean Hb at 12 weeks was higher for all three supplemented groups versus placebo and daily iron in conjunction with malaria prophylaxis was most effective in treating mild anaemia, while the prevalence of severe anaemia was lowest for the IPT + iron group⁶⁰.

MMNs supplementation – with iron. An RCT conducted in Indonesia randomised 387 infants from four months of age for six months to either ferrous sulphate, zinc sulphate, iron + zinc, beta-carotene, zinc + beta-carotene, or placebo syrups⁶¹. Hb was higher in infants receiving iron only versus the zinc + iron group but significantly higher compared to those receiving only zinc, only beta-carotene, zinc + beta-carotene or placebo⁶¹. Likewise, SF was highest in the group receiving iron and significantly higher in the iron + zinc versus placebo groups⁶¹.

Another RCT supplemented Indonesian infants (N = 800) from three to six months of age for six months, daily, with either zinc sulphate, zinc + ferrous sulphate, zinc + iron + vitamin A, or placebo syrups⁶². Only the MMN group was found to have an increase in Hb, whereas the zinc only and placebo groups had a significant decrease in Hb. Hb in the MMN and zinc + iron groups were significantly higher than the zinc only and placebo groups after six months of supplementation⁶². Likewise, anaemia prevalence significantly increased in the zinc only and placebo groups, with the MMN arm being the only group to see a decrease after six months of supplementation⁶².

One further RCT supplemented 75 American infants at one until 5.5 months of age, daily, with MMNs with or without iron⁶³.

Infants were followed until 18 months of age. Infants receiving MMN with iron had significantly improved SF levels at four and 5.5 months of age compared to the non-iron group. However, SF decreased continuously throughout the trial in both groups and Hb concentration showed no difference at any age, although it was higher for the group receiving iron than those not during the supplementation period, but not afterwards⁶³. At 5.5 months of age, but not at other ages, plasma soluble transferrin receptor (sTfR) was significantly lower for the iron versus the non-iron group and MCV was significantly higher in the iron than the non-iron group at 7.5 and nine months of age⁶³.

MMNs supplementation – without iron. 2,387 Tanzanian infants, born to HIV positive mothers, were part of a large RCT in which they were supplemented from six weeks of age for 24 months, daily, with a multivitamin or placebo^{64,65}. Hb concentrations were significantly higher in the treatment versus placebo group at 12, 18, and 24 months⁶⁵. Compared to those in the placebo group, infants in the treatment group had a lower risk of anaemia⁶⁵. The treatment was associated with a reduced risk of severe anaemia among infants born to women without anaemia, but not among those born to women with anaemia⁶⁵.

One trial analysed results from 2,006 Tanzanians who were part of a RCT supplementing infants daily from six weeks of age for 18 months, with either zinc sulphate; multivitamins; zinc + multivitamins; or placebo^{66,67}. Infants given multivitamins had higher mean Hb than those given placebo or zinc alone at 18 months⁶⁷. Multivitamins were also associated with lower odds of ID and a reduction in risk of severe anaemia through 18 months, whereas zinc was associated with greater odds of ID at six months of age and had no positive effect on severe anaemia through 18 months⁶⁷.

All trial designs have now been described and will not be repeated in the remaining three outcome sections below; trials will be described by country with the relevant references provided.

Outcome 2: Growth

Ten trials assessed growth, seven of which supplemented with iron and three with MMNs. One of the iron trials also randomised mothers to iron or placebo in pregnancy⁴³. Two of the three MMN trials included iron.

Iron supplementation – in pregnancy and infancy. The RCT in China supplementing mother-infant pairs across pregnancy and infancy found no statistically significant effects of supplementation on anthropometry at nine months of age and no differences were observed when comparing infants who were iron sufficient versus those who were deficient at birth⁴³. Between groups, weight gain was significantly lower in infants whose mothers received placebo in pregnancy and there were no group differences in length gain at nine months of age⁴³.

Iron supplementation – two arm trials. The trial in rural India randomising infants from four to six months of age to iron or

placebo found no significant difference in anthropometry after eight weeks of supplementation⁴¹.

The trial in a low-income Canadian population with infants at one until six months of age, followed until 12 months of age, found no effect of iron therapy on anthropometry when iron and placebo were compared at any clinic visit⁴⁵.

Iron supplementation – comparing formulations. An RCT in Turkey testing ferrous sulphate drops versus ferric polymaltose drops in infants from four until nine months of age, found similar increases for both groups for weight, length and head circumference at nine months of age⁴⁶.

Iron supplementation – plus zinc. The trial in Indonesia supplementing infants at four until ten months of age with either iron, zinc, iron + zinc syrup, or placebo syrups found that during the trial z-scores decreased significantly in all groups, with no differences among the groups at the end of supplementation⁵¹.

The trial in Northeast Thailand supplementing infants at four to six months of age, daily, with either iron, zinc, iron + zinc, or placebo syrups found that after six months of supplementation, group length did not differentiate, but that when controlled by gender and birth weight, iron supplementation improved weight-for-length z-score (WLZ) whereas zinc did not⁵².

A similar trial design, but conducted in Vietnam and recruiting infants from four to seven months of age found that weight gain was higher in the zinc versus iron + zinc, placebo, and iron only groups at nine months of age⁵³. No significant effects of the different interventions on length at nine months of age were seen⁵³.

MMNs supplementation – with iron. The RCT with infants from Indonesia who were supplemented from three to six months of age daily with either zinc, zinc + iron, zinc + iron + vitamin-A, or placebo syrups found no beneficial effect on the prevalence of stunting, wasting and underweight across all four groups after six months of supplementation. Additionally, stunting prevalence doubled between end of supplementation and six months later for all groups⁶². However, in initially stunted infants their mean height-for-age z-scores (HAZ) decreased during the six months of supplementation and this inter-group difference became significantly larger in the zinc + iron and MMN groups but not the placebo and zinc only groups, after four months of supplementation⁶².

The RCT supplementing infants in The United States at one month of age with daily MMNs with (intervention) or without (control) iron found that iron in addition to the MMNs had no significant effect on growth across the groups at 5.5 months of age⁶³.

MMNs supplementation – without iron. The MMN trial in Tanzania supplementing infants at six weeks of age for 18 months daily with either zinc; multivitamins; zinc + multivitamins or placebo found that there were no significant differences in any of the growth biomarkers of infants who received zinc compared with those who did not, or of infants who received multivitamins compared to those who did not at any of the assessed time points

(six and 12 months of age)⁶⁸. A further publication from the same trial highlighted that infants in all groups experienced growth faltering and that supplementation did not have a significant effect on mean change in HAZ or on rates of stunting, wasting, or underweight⁶⁹. Changes in weight-for-age z-score (WAZ) and WHZs were significantly different across the four groups, where the mean decline in the WAZ from baseline to the end of follow-up in the zinc + multivitamin group was significantly less than in the placebo group and the decline in the WHZ was significantly greater in the zinc-only group than in the placebo group⁶⁹.

Outcome 3: and/or mortality

Fifteen RCTs assessed infant and childhood morbidity and/or mortality as an outcome. Thirteen of these trials supplemented with iron and two with MMNs. One of the iron trials also randomised mothers to iron or placebo in pregnancy⁴³. Of the trials assessing iron supplementation, four also included malaria prophylaxis in their randomisation. None of the MMN trials included iron.

Iron supplementation – in pregnancy and infancy. The large four-arm RCT supplementing mother-infant pairs in pregnancy and infancy in China found no significant group differences in hospitalization or illnesses by nine months of age⁴³.

Iron supplementation – two arm trials. Indian infants randomised from four to six months of age for eight weeks to daily iron or placebo drops had no statistical difference in reported interval morbidity in the two groups, but black colouring of the stools was significantly higher in the iron-supplemented versus placebo group⁴¹.

A second trial, from Northeast India, randomising infants (100 born to anaemic and 100 born to non-anaemic mothers) at 36 hours after birth until six months of age to daily iron drops or standard of care found no differences in the parental report of morbidities between groups⁴⁴.

Iron supplementation – comparing formulations. When testing ferrous sulphate drops versus ferric polymaltose drops in Turkish infants from four until nine months of age, the prevalence of reported adverse effects was 34.8%, with no statistical difference between either group and with the most common side effects being gastrointestinal complaints such as diarrhoea, constipation, and vomiting⁴⁶.

Iron supplementation – comparing timings and doses. The RCT testing daily or weekly iron drops compared to no treatment (control) in infants from Turkey at four until seven months of age found that side effects of iron supplementation occurred in 44.4% of the infants, with no significant difference between groups, but higher percentages in the weekly group⁴⁷.

Iron supplementation – plus zinc. The trial conducted in Vietnam supplementing infants daily at four until nine months of age for six months with either iron, zinc, iron + zinc, or placebo syrup found no significant effects of supplementations on morbidity⁵³.

The very large RCT randomising infants from India at one to 23 months of age for 12 months, daily, to IFA or IFA + zinc found the overall death rates did not differ significantly between the two groups when adjusted for cluster randomisation and the addition of zinc to IFA did not affect adjusted hospitalizations rates overall or specific rate ratios for diarrhoea and pneumonia⁵⁵.

In a similar trial design, in Nepal, with the addition of placebo, in infants at one to 35 until 36 months of age, Tielsch and colleagues found that although the IFA containing arms had to be stopped early, the all-cause mortality between treatment groups did not differ⁵⁴. Likewise, there were no significant differences in the reported morbidities between groups⁵⁴.

The one RCT that did not have biochemical results for infants under six months of age supplemented infants from 1–36 months with IFA, IFA + zinc, zinc alone, or placebo. Results on adverse events (85/2135), morbidity (17/295), and hospital admissions (68/1840) were reported for infants under six months of age, but no conclusions could be drawn from this data due to enrolment numbers not being divided by age group⁴². However, it is worth noting the iron and folic acid-containing groups of the trial were stopped early on Aug 19, 2003, on the recommendation of the data and safety monitoring board⁴².

Iron supplementation – plus malaria prophylaxis. The RCT conducted in 832 Tanzanian infants supplemented at eight weeks until six months of age with iron and until 12 months of age with malaria prophylaxis found that during the main trial period, iron supplementation had no effect on the frequency of malaria and groups that received malaria prophylaxis had lower frequencies of malaria⁵⁶. However, during the follow up period, after the intervention, from 12 to 24 months of age, infants who received malaria prophylaxis had higher rates of malaria than those not receiving malaria prophylaxis during their first year of life⁵⁶.

Another RCT in Tanzania supplementing infants vulnerable to malaria from 12–16 weeks of age for six months with either daily iron; amodiaquine; iron + amodiaquine; or double placebo found a protective effect of malaria prophylaxis in prevention of malaria fevers at the end of the intervention, and at the four month follow up, they did not show rebound morbidity⁵⁸.

The third trial in Tanzania comparing either 14 days of daily iron + one dose of SP versus three months of daily iron + three SP doses at the start of each month in infants from two months up to five years of age found no differences in morbidities between the two groups⁵⁹. The prevalence of *P. falciparum* parasitaemia was also similar in the two groups in both cross-sectional surveys⁵⁹.

The RCT in West Kenya randomising infants with mild anaemia at two to 36 months of age, for 12 weeks, to IPT SP at four and eight weeks + daily iron; placebo IPT SP + iron; IPT SP + placebo iron; or double placebo found no significant interaction between iron supplementation and malaria prophylaxis on the risk of malaria or non-malaria morbidity. Further, between four and 12 weeks after enrolment, IPT versus iron was associated with significant

reductions in malaria parasitaemia and clinic visits and a nonsignificant reduction in clinical malaria⁶⁰.

MMNs supplementation – without iron. The trial supplementing Tanzanian infants at six weeks of age for 18 months, daily, with either zinc; multivitamins; zinc + multivitamins; or placebo found a nonsignificant increase in all-cause mortality among infants who received zinc compared with those who did not⁶⁴. Further, multivitamins did not affect the occurrence of any of the morbidity symptoms⁶⁶. Likewise, there was no significant treatment effects of zinc or multivitamins on EED or systemic inflammation at six months of age^{66,68}.

Another trial assessed Tanzanian infants, born to HIV positive mothers, who were supplemented at six weeks of age for 24 months with daily multivitamin or placebo⁶⁴. They found that morbidities were not significantly different between the groups, except for fever and vomiting which were significantly lower in the multivitamin group⁶⁴. Among 429 children whose mothers received antiretroviral therapy, multivitamin use had no effect on mortality but was associated with a significant reduction in morbidities⁶⁴.

A sub-analysis of infants from the previous RCT identified new cases of HIV infection during follow-up in 3.9% children in the placebo group and 4.7% in the multivitamin group with no effect of multivitamins on mother to child transmission^{64,65}.

Outcome 4: Neuro-behavioural development

Six RCTs assessed the impact of supplementation on neurobehavioural developmental outcomes. Four of these supplemented with iron and two with MMNs. One of the iron trials also randomised mothers to iron or placebo in pregnancy⁷⁰. Neither of the MMN trials included iron. Only two of the trials looked at longer term developmental outcomes, one at six to eight and the other at nine years of age^{71,72}. The remaining trials assessed outcomes between six and fifteen months of age.

Iron supplementation – in pregnancy and infancy. The large RCT in China supplementing mother-infant pairs in pregnancy and infancy with daily iron or placebo followed 1,196 infants until nine months of age for developmental scores^{43,70}. Iron supplementation in infancy but not pregnancy improved gross motor scores overall, but there were no group differences in overall neurologic integrity^{43,70}.

Iron supplementation – two arm trials. Northeast Indian infants (100 born to anaemic and 100 born to non-anaemic mothers) were randomised to daily iron or standard of care at 36 hours after birth until six months of age⁴⁴. Motor development was closer to age appropriate norms and their active and passive tone milestones were significantly higher in the iron supplemented group at six months of age⁴⁴.

The other trial, conducted in a low-income Canadian population, randomised infants at one until six months of age to daily iron or placebo⁴⁵. The iron supplementation group scored significantly higher in Bayley psychomotor developmental indices and tended

toward improved visual acuity at 13 months of age, which became significant when the data were analysed excluding noncompliers⁴⁵.

Iron supplementation – plus zinc. North-eastern Thai infants from a trial who were supplemented at four to six months of age for six months, daily, with either iron, zinc, iron + zinc, or placebo syrups were followed up with at nine years of age (N = 560)^{52,72}. No significant differences in any of the neurodevelopmental outcomes between the four groups were seen^{52,72}. Further, school performance scores were not different between groups^{52,72}.

MMNs supplementation – without iron. Tanzanian infants (N = 365), from a trial, were followed up with at six and eight years of age to assess three developmental domains^{66,71}. In the intervention trial, infants were randomised at six weeks of age for 18 months, to either daily, zinc; multivitamins; zinc + multivitamins; or placebo^{66,71}. There was no effect of either zinc or multivitamin supplementation on any of the three developmental domains at six to eight years of age following the supplementation in infancy^{66,71}.

A second MMN trial assessed 192 HIV negative Tanzanian infants at 15 months of age, who were born to HIV positive mothers and from a trial with supplementation at six weeks of age for 24 months with daily multivitamin or placebo^{64,71}. No effect of MMN supplementation on any of the developmental domains at 15 months of age were seen, but there was a trend toward improved fine motor skills among infants in the MMN group⁷³.

Countries represented in trials

Of the 24 trials, 13 countries were represented (Figure 3); three HICs and ten LMICs. The majority of the trials took place in a single location, but one trial recruited half of its participants in a LMIC (Honduras) and the other half in a HIC (Sweden)⁵⁰. The two other HICs represented were Canada and the United States of America, but the Canadian trial was conducted in a low-income population^{63,74}. LMICs made up 88% of the total trial locations (22/25). Five of the middle-income countries were middle-upper, constituting China, Thailand, and Turkey. Ten of the countries were low-middle, constituting Honduras, Kenya, India, Indonesia, and Vietnam. Seven were low-income, constituting Tanzania and Republic of Benin. 72% of the trials took place in Asia and sub-Saharan Africa (18/25), with 8/25 of the trials being in sub-Saharan Africa and 10/25 in Asia.

Discussion

Summary of main results

Infants living in settings with poor dietary diversity may be at risk of micronutrient deficiencies in early infancy (under six months of age); a period largely missed by policy guidelines, except for recommendations on EBF. This systematic review aimed to examine the effects of iron, iron containing MMNs and/or MMNs during the first six months of life on infant outcomes (biochemical, growth, morbidity and/or mortality, and neuro-behavioural development). We observed that infants less than six months of age benefit biochemically from early supplementation with iron, but the impacts of additional nutrients or MMNs, along with the impacts on growth, morbidity and/or mortality, and neuro-behavioural outcomes are less clear.

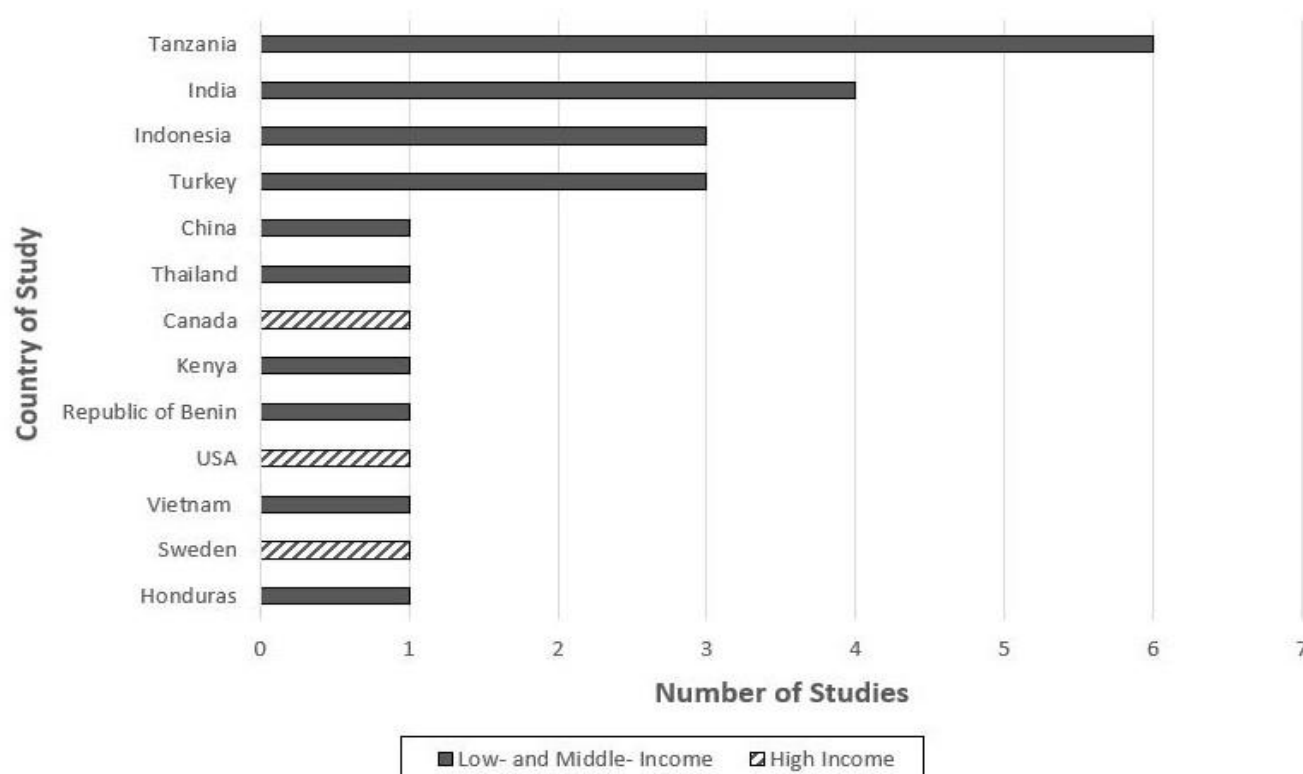


Figure 3. Distribution of countries included in this review. USA = United States of America.

In infants less than six months of age, iron (alone or in combination with other nutrients) is effective in increasing Hb and SF concentrations and reducing the prevalence of anaemia. Positive impacts of supplementation were seen in infants from low resource settings in a HIC and in LMICs; however, in resource secure HICs the impact was not as significant. Responses may differ based on iron formulation and timing of doses, but data is scarce and therefore no clear conclusions can be drawn. Likewise, while biochemical status was improved with MMNs not containing iron, the effect is greater when MMNs contain iron. In comparison to iron and MMN supplementation, infants receiving zinc only were more likely to develop anaemia.

The impact of supplementation on growth, morbidity and/or mortality and longer-term neuro-behavioural development was found to be mixed. For growth, no consistent beneficial effects of supplementation in early infancy were observed. In areas with a high prevalence of malaria, iron supplementation was effective in preventing severe anaemia without increasing susceptibility to malaria while treatment was on-going. While there were no significant increases in morbidity and/or mortality, gastrointestinal side effects were reported for infants receiving iron. One study did stop the iron and folic acid- containing groups on the recommendation of the data and safety monitoring board⁴². However, while this study did report adverse events, mortality, and hospital admissions in infants under six months of age, enrolment data for this age group was missing, so no conclusions could be drawn⁴². Some benefits were seen directly after supplementation with MMNs (without iron) and with iron only, but longer-term impacts were less clear after supplementation with MMNs (without iron) and iron and/or zinc. However, the evidence on neuro-behavioural outcomes is limited and, therefore, no firm conclusions can be drawn.

Finally, it was consistently observed that supplementation must continue past six months of age, because any beneficial impacts seen across all outcomes and supplementations disappear when supplementation ceases. A further detailed review of the results, by outcome, follows.

Effects of supplementation on infant biochemical outcomes

Of the nine trials assessing biochemical outcomes and supplementing with iron only versus placebo (or none), all nine showed a beneficial effect for infant biochemical outcomes^{41,43-50}. From these trials, the following further themes emerged. First, supplements administered directly to the infants outperformed supplementation administered to mothers during pregnancy, although, this observation was limited to a single trial⁴³. Second, the impact of supplementation was greater among infants who were anaemic at the start of the trial^{49,50}. Third, iron formulation may impact on efficacy, with ferrous sulphate performing better than ferric polymaltose, although again, this observation was limited to a single trial⁴⁶. Fourth, continued supplementation during the weaning period is beneficial^{45,48}. Lastly, while increasing dose (e.g. 2 versus 1 mg/kg) improved outcomes⁴⁸, the impact of frequency of dosing was less clear; in one trial daily supplementation outperformed alternate day supplementation⁴⁸, but in another trial weekly supplementation was found to outperform daily

supplementation⁴⁸. It must be noted that trials considering dose and timing had small sample sizes (N = 70 - 113)⁴⁵⁻⁴⁸.

A further finding was that supplementation with additional micronutrients did not enhance infant iron status. For example, trials that included zinc or beta carotene (either alone or in combination with iron) showed no added benefit over iron alone^{51-55,61,62}. Likewise, MMNs without iron are not as beneficial as those with iron⁶³, but are more beneficial than a placebo⁶⁴ or zinc alone⁶⁶ on infant iron status. Another emerging theme was that infants born to mothers with ID or IDA were more likely to benefit from supplementation⁶⁴.

In comparison, all trials of iron and/or malaria prophylaxis found iron supplementation in combination with malaria prophylaxis to be more protective against anaemia than iron alone^{56-58,60}. In addition, long-term iron supplementation and malaria prophylaxes (three months) is more beneficial than short-term (14 days), although, this observation was limited to a single trial⁵⁹.

Effects of supplementation on infant growth outcomes

The beneficial effects of supplementation in the first six months of life for infant growth were less evident. From four trials supplementing with iron only versus placebo (or none) and assessing growth outcomes (N = 1,565), no beneficial effects were observed for infant growth outcomes^{41,43,45,46}. Further, supplementation with additional micronutrients had an equivocal impact on infant growth. In all trials with additional micronutrients, there was no benefit to infant growth of the additional micronutrients on growth markers^{51,52,62,68,69}, except for one trial reporting zinc supplementation as more beneficial for weight gain only⁵³. Additionally, in all trials assessing growth, all infants experienced growth faltering^{51-53,62,68,69}. Further themes emerged from the trials reporting growth outcomes. First, stunting prevalence increased more than two-fold between the end of MMN supplementation (including iron) and a six-month follow up, with initially stunted infants at even greater risk⁶². Second, when controlled for gender and birth weight, iron only may improve WLZ over zinc only supplementation⁵². Last, one trial found a lower decrease in WHZ and WAZ in multivitamins (without iron) versus placebo supplementation⁶⁹. However, all three of these findings were limited to single trials^{52,62,69}. Further, with only a single trial (N = 112) comparing iron formulations, no conclusions can be drawn⁴⁶.

Effects of supplementation on infant morbidity and/or mortality outcomes

The beneficial effects of supplementation in the first six months of life on morbidity and/or mortality were equivocal in their findings. Of the five trials assessing these outcomes and supplementing with iron only versus placebo (or none), all showed no significant differences for infant outcomes^{41,43,44,46,47}. In view of the concerns regarding the potential negative consequences of iron supplementation²³⁻²⁷ it is relevant that no differences were observed with respect to iron formulation⁴⁶, but weekly supplementation did result in fewer side effects⁴⁷. However, these observations were limited to single trials, with small sample sizes (N = 112 and 70)^{46,47}.

With respect to combining iron with other micronutrients, supplementation with additional zinc did not affect infant morbidity and/or mortality outcomes⁵³⁻⁵⁵. One trial had to stop the iron and

folic-acid containing arms of the trial early, on recommendation of the data and safety monitoring board, however conclusions could not be drawn from this trial due to lack of missing recruitment data for infants under six months of age⁴². However, except for EED and systemic inflammation for which there were no differences⁶⁸, MMNs (without iron) may be more beneficial than zinc alone or placebo supplementation on other morbidity and/or mortality outcomes such as hospitalizations or unscheduled outpatient visits^{65–67}.

Trials of iron supplementation in combination with malaria prophylaxis found, firstly, that malaria prophylaxis does not negatively interact with iron supplementation⁶⁰. Secondly, long-term iron supplementation and malaria prophylaxes (three months) may not be more beneficial than short-term (14 days) for morbidity and/or mortality⁵⁹, but when supplementation stops, infants may be at higher risk of malaria than previously⁵⁶. However, both observations were limited to single trials.

Effects of supplementation on neuro-behavioural outcomes

All three trials assessing neuro-behavioural outcomes and supplementing with iron only versus placebo showed significant differences for some of the short-term infant outcomes assessed^{44,45,70}, suggesting that iron, in the short-term, may improve neuro development in early infancy^{44,46,70}. Supplementation with MMNs (without iron) was suggested to improve fine motor skills in the short term (single trial, N = 192 infants assessed at 24 months of age)⁷³, but little evidence was found to support longer term effects at six to eight years of age⁷¹.

Explanatory factors

Given the multi-factorial nature of supplementation in infants and children, various factors may influence the heterogeneity in the results of some outcomes. For biochemical outcomes, it is apparent that iron supplementation in infants under six months of age has a beneficial effect on infant iron status^{41,43–50}. However, the impact on growth, morbidity and/or mortality, and neuro-behavioural development is less conclusive. For growth, initially stunted infants are at even greater risk of later stunting⁶², so prior undernutrition may be an important predictor of response to supplementation. Further, in areas of malaria endemicity, iron supplementation in conjunction with malaria prophylaxis is more beneficial on infant iron status, indicating a potential interaction between infection control and iron supplementation^{56–58,60}. Lastly, while EBF up to six months of age was encouraged in all trials, it was an inclusion criteria for only two trials^{47,48}. Likewise, only five of the trials reported on mean age of EBF^{43,51,52,65,67}. Therefore, while heterogeneity in rates of EBF could also be an explanatory factor, it precludes a sensitivity analysis by mode of feeding.

Comparison to the literature

To our knowledge, this is the first systematic review to look at iron or MMN supplementation in infants under six months of age. A Cochrane protocol is registered to analyse daily iron supplementation for prevention or treatment of IDA in infants, children, and adolescents; however, it does not specify if this will include infants under six months of age⁷⁵. A handful of published systematic reviews, discussed below, of iron and/or MMN supplementation in infants and children have been conducted;

however, none focused specifically on infants under six months of age^{29,30,35,36,76,77}.

Intermittent iron supplementation (versus placebo or standard of care) improved biochemical markers of iron status in a study of children under 12 years of age²⁸, but the evidence to support the relative benefit of intermittent versus daily supplementation was less clear in our results^{41,43–50}. In the children, type of regimen, dose or composition had no biochemical impact²⁸, whereas one trial from our results found ferrous sulphate to be more effective than ferric polymaltose⁴⁶ and another found iron supplementation with 2 versus 1 mg/kg as more effective⁴⁸. Second, zinc supplementation in children aged six months to 12 years of age had no effect on Hb or iron status⁷⁶, which was consistent with our results^{51–55}. However, in the children, the effects on growth showed a very small improvement in height and a small increased risk of death due to diarrhoea, lower respiratory tract infection or malaria⁷⁶, which is not consistent with our results^{51–55,76}. Third, in children under 18 years of age in areas at risk of malaria, iron and malaria prophylaxis were more protective against anaemia than iron alone and decreased the number of clinical admissions due to malaria²⁹, which was consistent with our results in young infants^{56–58,60}.

Strengths and limitations

There were a limited number of trials available, with much heterogeneity in the exposure and outcome measures, to provide a comprehensive assessment of the impact of iron interventions early in life on growth, morbidity and/or mortality and neurobehavioural development. Only six of the 24 trials were efficacy trials, in which infants were supplemented directly by health care workers rather than by caregivers. Further, eight of the 11 MMNs trials included did not include iron in their supplementation, but still reported on biochemical markers in relation to iron. Data for longer term neuro-behavioural development was limited, with only two trials assessing infants at either six to eight or nine years of age. Only one trial included supplementation to both mothers during pregnancy and then infants directly. Lastly, almost all trials took place in LMICs with only one low socio-economic group in a HIC.

The main strength of this systematic review is that it is the first comprehensive review of iron and/or MMN supplementation in infants under six months of age. In addition, trials were not excluded based on supplementation regimen or outcomes assessed, allowing the authors to understand a wide scope of the literature in the field.

We also acknowledge several limitations to our review. We purposefully only included RCTs, but a review of the full scope of literature, including observational trials, may have added insight. We excluded trials that used fortified or complementary formulas and/or supplementary foods before six months of age within the trial protocol to ensure a level of homogeneity across the data, as volumes and quantities consumed by subjects could vary widely. A further limitation is that most trials were not limited to young infancy, but included a wide age range from infants under six months of age and beyond. Also, only two studies specifically excluded non EBF infants. While heterogeneity in the data was a strength in terms of assessing the full scope of the literature, it made comparison of trials via a quantitative meta-analysis difficult.

Conclusions

Growing evidence indicates that infants less than six months of age may be vulnerable to micronutrient deficiencies. This review shows that daily oral iron supplementation has beneficial effects on short-term biochemical outcomes such as iron status. Likewise, MMN formulations containing iron are more beneficial for iron status than those without. However, overall, short- and long-term evidence for this age group is limited. It is unclear whether iron supplementation in the first few months of life to exclusively breast-fed infants is beneficial across various outcomes and the longer-term growth and neuro-behavioural developmental outcomes are less clear. Evidence is lacking as to whether MMN formulations containing iron are more beneficial than iron alone and longer-term health and developmental impacts are also less clear. Well-powered randomised controlled trials are required to determine whether routine supplementation with iron or MMNs containing iron should commence before six months of life for exclusively breast-fed infants in low-resource settings.

Data availability

Underlying data

All data underlying the results are available as part of the article and no additional source data are required.

Extended data

Figshare: Acknowledging the gap: a systematic review of micronutrient supplementation in infants under 6 months of age_Extended Data File_Updated.docx. <https://doi.org/10.6084/m9.figshare.22015367>³⁷.

This project contains the following extended data:

- Extended datafile 1: PRISMA checklist.
- Extended datafile 2: Search terms.
- Extended datafile 3: Registered and ongoing trials.
- Extended datafile 4: Risk of bias analysis.
- Extended datafile 5: Table: Results by outcome.

Reporting guidelines

Figshare: PRIMSA checklist for 'Acknowledging the gap: a systematic review of micronutrient supplementation in infants under six months'. <https://doi.org/10.6084/m9.figshare.22015367>³⁷.

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

References

1. GBD Mortality and Causes of Death Collaborators: **Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015**. *Lancet*. 2016; **388**(10053): 1459–544. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
2. Prentice AM, Mendoza YA, Pereira D, *et al.*: **Dietary strategies for improving iron status: balancing safety and efficacy**. *Nutr Rev*. 2017; **75**(1): 49–60. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
3. GBD 2016 Disease and Injury Incidence and Prevalence Collaborators: **Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016**. *Lancet*. 2017; **390**(10100): 1211–59. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
4. Miller JL: **Iron deficiency anemia: a common and curable disease**. *Cold Spring Harb Perspect Med*. 2013; **3**(7): a011866. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
5. World Health Organization: **Guideline: Daily iron supplementation in adult women and adolescent girls**. Geneva, Switzerland, 2016. [Reference Source](#)
6. World Health Organization: **WHO recommendations on antenatal care for a positive pregnancy experience**. Geneva, Switzerland, 2016. [Reference Source](#)
7. World Health Organization: **Guideline: Daily iron supplementation in infants and children**. Geneva, Switzerland, 2016. [Reference Source](#)
8. World Health Organization: **NUTRITIONAL ANAEMIAS: TOOLS FOR EFFECTIVE PREVENTION AND CONTROL**. In: *Development NfHa*. ed. G Geneva, Switzerland, 2017. [Reference Source](#)
9. Christian P, Mullany LC, Hurley KM, *et al.*: **Nutrition and maternal, neonatal, and child health**. *Semin Perinatol*. 2015; **39**(5): 361–72. [PubMed Abstract](#) | [Publisher Full Text](#)
10. Barker DJ, Clark PM: **Fetal undernutrition and disease in later life**. *Rev Reprod*. 1997; **2**(2): 105–12. [PubMed Abstract](#) | [Publisher Full Text](#)
11. Barker DJ: **In utero programming of chronic disease**. *Clin Sci (Lond)*. 1998; **95**(2): 115–28. [PubMed Abstract](#)
12. Merialdi M, Carroli G, Villar J, *et al.*: **Nutritional interventions during pregnancy for the prevention or treatment of impaired fetal growth: an overview of randomized controlled trials**. *J Nutr*. 2003; **133**(5 Suppl 2): 1626S–1631S. [PubMed Abstract](#) | [Publisher Full Text](#)
13. Martorell R: **Improved nutrition in the first 1000 days and adult human capital and health**. *Am J Hum Biol*. 2017; **29**(2). [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
14. McCoy DC, Peet ED, Ezzati M, *et al.*: **Early Childhood Developmental Status in Low- and Middle-income Countries: National, Regional, and Global Prevalence Estimates Using Predictive Modeling**. *PLoS Med*. 2016; **13**(6): e1002034. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
15. Dror DK, Allen LH: **Overview of Nutrients in Human Milk**. *Adv Nutr*. 2018; **9**(suppl_1): 278S–94S. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
16. Armitage AE, Moretti D: **The Importance of Iron Status for Young Children in Low- and Middle-income Countries: A Narrative Review**. *Pharmaceuticals (Basel)*. 2019; **12**(2): 59. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
17. Widdowson EM: **Trace elements in foetal and early postnatal development**. *Proc Nutr Soc*. 1974; **33**(3): 275–84. [PubMed Abstract](#) | [Publisher Full Text](#)
18. Peña-Rosas JP, De-Regil LM, Garcia-Casal MN, *et al.*: **Daily oral iron supplementation during pregnancy**. *Cochrane Database Syst Rev*. 2015; **2015**(7): CD004736. [PubMed Abstract](#) | [Publisher Full Text](#)
19. Armitage AE, Agbla SC, Betts M, *et al.*: **Rapid growth is a dominant predictor of hepcidin suppression and declining ferritin in Gambian infants**. *Haematologica*. 2019; **104**(8): 1542–53. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
20. Eriksen KG, Johnson W, Sonko B, *et al.*: **Following the World Health Organization's Recommendation of Exclusive Breastfeeding to 6 Months of Age Does Not Impact the Growth of Rural Gambian Infants**. *J Nutr*. 2017; **147**(2): 248–55. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
21. Lönnerdal B: **Excess iron intake as a factor in growth, infections, and development of infants and young children**. *Am J Clin Nutr*. 2017; **106**(Suppl 6): 1681S–87S. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
22. Prado EL, Larson LM, Cox K, *et al.*: **Do effects of early life interventions on linear growth correspond to effects on neurobehavioural development? A systematic review and meta-analysis**. *Lancet Glob Health*. 2019; **7**(10): e1398–e413. [PubMed Abstract](#) | [Publisher Full Text](#)
23. Calder PC, Jackson AA: **Undernutrition, infection and immune function**. *Nutr Res Rev*. 2000; **13**(1): 3–29. [PubMed Abstract](#) | [Publisher Full Text](#)
24. Tickell KD, Atlas HE, Walson JL: **Environmental enteric dysfunction: a review of potential mechanisms, consequences and management strategies**. *BMC Med*. 2019; **17**(1): 181. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
25. Zimmermann MB, Chassard C, Rohner F, *et al.*: **The effects of iron fortification on the gut microbiota in African children: a randomized controlled trial in Cote d'Ivoire**. *Am J Clin Nutr*. 2010; **92**(6): 1406–15. [PubMed Abstract](#) | [Publisher Full Text](#)

26. Cross JH, Bradbury RS, Fulford AJ, *et al.*: Oral iron acutely elevates bacterial growth in human serum. *Sci Rep.* 2015; 5: 16670. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
27. Jaeggi T, Kortman GA, Moretti D, *et al.*: Iron fortification adversely affects the gut microbiome, increases pathogen abundance and induces intestinal inflammation in Kenyan infants. *Gut.* 2015; 64(5): 731–42. [PubMed Abstract](#) | [Publisher Full Text](#)
28. De-Régil LM, Jefferds MED, Sylvestry AC, *et al.*: Intermittent iron supplementation for improving nutrition and development in children under 12 years of age. *Cochrane Database Syst Rev.* 2011; 2011(12): CD009085. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
29. Neuberger A, Okebe J, Yahav D, *et al.*: Oral iron supplements for children in malaria-endemic areas. *Cochrane Database Syst Rev.* 2016; 2(2): CD006589. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
30. Goheen MM, Wegmuller R, Bah A, *et al.*: Anemia Offers Stronger Protection Than Sick Cell Trait Against the Erythrocytic Stage of Falciparum Malaria and This Protection Is Reversed by Iron Supplementation. *EBioMedicine.* 2016; 14: 123–30. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
31. World Health Organization: World malaria report 2019. Geneva, Switzerland, 2019. [Reference Source](#)
32. Keats EC, Haider BA, Tam E, *et al.*: Multiple-micronutrient supplementation for women during pregnancy. *Cochrane Database Syst Rev.* 2019; 3(3): CD004905. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
33. Abe SK, Balogun OO, Ota E, *et al.*: Supplementation with multiple micronutrients for breastfeeding women for improving outcomes for the mother and baby. *Cochrane Database Syst Rev.* 2016; 2(2): CD010647. [PubMed Abstract](#) | [Publisher Full Text](#)
34. De-Régil LM, Jefferds MED, Pena-Rosas JP: Point-of-use fortification of foods with micronutrient powders containing iron in children of preschool and school-age. *Cochrane Database Syst Rev.* 2017; 11(11): CD009666. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
35. De-Régil LM, Suchdev PS, Vist GE, *et al.*: Home fortification of foods with multiple micronutrient powders for health and nutrition in children under two years of age. *Cochrane Database Syst Rev.* 2011; 9(9): CD008959. [PubMed Abstract](#) | [Publisher Full Text](#)
36. Moher D, Liberati A, Tetzlaff J, *et al.*: Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med.* 2009; 151(4): 264–9, w64. [PubMed Abstract](#) | [Publisher Full Text](#)
37. Stelle I: Acknowledging the gap: a systematic review of micronutrient supplementation in infants under 6 months of age_Extended Data File_Updated.docx. *figshare.* Dataset. 2023. <http://www.doi.org/10.6084/m9.figshare.22015367>
38. Mills RJ, Davies MW: Enteral iron supplementation in preterm and low birth weight infants. *Cochrane Database Syst Rev.* 2012; 3(3): CD005095. [PubMed Abstract](#) | [Publisher Full Text](#)
39. Sterne JAC, Savović J, Page MJ, *et al.*: RoB 2: a revised tool for assessing risk of bias in randomised trials. *BMJ.* 2019; 366: l4898. [PubMed Abstract](#) | [Publisher Full Text](#)
40. Review ManagerWeb (RevMan Web). [program]: The Cochrane Collaboration, 2019. [Reference Source](#)
41. Nagpal J, Sachdev HPS, Singh T, *et al.*: A randomized placebo-controlled trial of iron supplementation in breastfed young infants initiated on complementary feeding: effect on haematological status. *J Health Popul Nutr.* 2004; 22(2): 203–11. [PubMed Abstract](#)
42. Sazawal S, Black RE, Ramsan M, *et al.*: Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial. *Lancet.* 2006; 367(9505): 133–43. [PubMed Abstract](#) | [Publisher Full Text](#)
43. Lozoff B, Jiang Y, Li X, *et al.*: Low-Dose Iron Supplementation in Infancy Modestly Increases Infant Iron Status at 9 Mo without Decreasing Growth or Increasing Illness in a Randomized Clinical Trial in Rural China. *J Nutr.* 2016; 146(3): 612–21. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
44. Bora R, Ramasamy S, Brown B, *et al.*: Effect of iron supplementation from neonatal period on the iron status of 6-month-old infants at-risk for early iron deficiency: a randomized interventional trial. *J Matern Fetal Neonatal Med.* 2021; 34(9): 1421–1429. [PubMed Abstract](#) | [Publisher Full Text](#)
45. Friel JK, Aziz K, Andrews WL, *et al.*: A double-masked, randomized control trial of iron supplementation in early infancy in healthy term breast-fed infants. *J Pediatr.* 2003; 143(5): 582–6. [PubMed Abstract](#) | [Publisher Full Text](#)
46. Aydın A, Gur E, Erener-Ercan T, *et al.*: Comparison of Different Iron Preparations in the Prophylaxis of Iron-deficiency Anemia. *J Pediatr Hematol Oncol.* 2017; 39(7): 495–99. [PubMed Abstract](#) | [Publisher Full Text](#)
47. Yurdakök K, Temiz F, Yalçın SS, *et al.*: Efficacy of daily and weekly iron supplementation on iron status in exclusively breast-fed infants. *J Pediatr Hematol Oncol.* 2004; 26(5): 284–8.
- [PubMed Abstract](#) | [Publisher Full Text](#)
48. Ermiş B, Demirel F, Demircan N, *et al.*: Effects of three different iron supplementations in term healthy infants after 5 months of life. *J Trop Pediatr.* 2002; 48(5): 280–4. [PubMed Abstract](#) | [Publisher Full Text](#)
49. Rahimy MC, Fanou L, Somasse YE, *et al.*: When to start supplementary iron to prevent iron deficiency in early childhood in sub-Saharan Africa setting. *Pediatr Blood Cancer.* 2007; 48(5): 544–9. [PubMed Abstract](#) | [Publisher Full Text](#)
50. Domellöf M, Cohen RJ, Dewey KG, *et al.*: Iron supplementation of breast-fed Honduran and Swedish infants from 4 to 9 months of age. *J Pediatr.* 2001; 138(5): 679–87. [PubMed Abstract](#) | [Publisher Full Text](#)
51. Dijkhuizen MA, Wieringa FT, West CE, *et al.*: Effects of iron and zinc supplementation in Indonesian infants on micronutrient status and growth. *J Nutr.* 2001; 131(11): 2860–5. [PubMed Abstract](#) | [Publisher Full Text](#)
52. Wasantwisut E, Winichagoon P, Chitchumroonchokchai C, *et al.*: Iron and zinc supplementation improved iron and zinc status, but not physical growth, of apparently healthy, breast-fed infants in rural communities of northeast Thailand. *J Nutr.* 2006; 136(9): 2405–11. [PubMed Abstract](#) | [Publisher Full Text](#)
53. Berger J, Ninh NX, Khan NC, *et al.*: Efficacy of combined iron and zinc supplementation on micronutrient status and growth in Vietnamese infants. *Eur J Clin Nutr.* 2006; 60(4): 443–54. [PubMed Abstract](#) | [Publisher Full Text](#)
54. Tielsch JM, Khatri SK, Stoltzfus RJ, *et al.*: Effect of routine prophylactic supplementation with iron and folic acid on preschool child mortality in southern Nepal: community-based, cluster-randomised, placebo-controlled trial. *Lancet.* 2006; 367(9505): 144–52. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
55. Bhandari N, Taneja S, Mazumder S, *et al.*: Adding zinc to supplemental iron and folic acid does not affect mortality and severe morbidity in young children. *J Nutr.* 2007; 137(1): 112–7. [PubMed Abstract](#) | [Publisher Full Text](#)
56. Menendez C, Kahigwa E, Hirt R, *et al.*: Randomised placebo-controlled trial of iron supplementation and malaria chemoprophylaxis for prevention of severe anaemia and malaria in Tanzanian infants. *Lancet.* 1997; 350(9081): 844–50. [PubMed Abstract](#) | [Publisher Full Text](#)
57. Menendez C, Schellenberg D, Quinto L, *et al.*: The effects of short-term iron supplementation on iron status in infants in malaria-endemic areas. *Am J Trop Med Hyg.* 2004; 71(4): 434–40. [PubMed Abstract](#) | [Publisher Full Text](#)
58. Massaga JJ, Kitua AY, Lemnge MM, *et al.*: Effect of intermittent treatment with amodiaquine on anaemia and malarial fevers in infants in Tanzania: a randomised placebo-controlled trial. *Lancet.* 2003; 361(9372): 1853–60. [PubMed Abstract](#) | [Publisher Full Text](#)
59. Schellenberg D, Kahigwa E, Sanz S, *et al.*: A randomized comparison of two anemia treatment regimens in Tanzanian children. *Am J Trop Med Hyg.* 2004; 71(4): 428–33. [PubMed Abstract](#) | [Publisher Full Text](#)
60. Desai MR, Mei JV, Kariuki SK, *et al.*: Randomized, controlled trial of daily iron supplementation and intermittent sulfadoxine-pyrimethamine for the treatment of mild childhood anemia in western Kenya. *J Infect Dis.* 2003; 187(4): 658–66. [PubMed Abstract](#) | [Publisher Full Text](#)
61. Wieringa FT, Dijkhuizen MA, West CE, *et al.*: Redistribution of vitamin A after iron supplementation in Indonesian infants. *Am J Clin Nutr.* 2003; 77(3): 651–7. [PubMed Abstract](#) | [Publisher Full Text](#)
62. Fahmida U, Rumawas JS, Utomo B, *et al.*: Zinc-iron, but not zinc-alone supplementation, increased linear growth of stunted infants with low haemoglobin. *Asia Pac J Clin Nutr.* 2007; 16(2): 301–9. [PubMed Abstract](#)
63. Ziegler EE, Nelson SE, Jeter JM: Iron supplementation of breastfed infants from an early age. *Am J Clin Nutr.* 2009; 89(2): 525–32. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
64. Duggan C, Manji KP, Kupka R, *et al.*: Multiple micronutrient supplementation in Tanzanian infants born to HIV-infected mothers: a randomized, double-blind, placebo-controlled clinical trial. *Am J Clin Nutr.* 2012; 96(6): 1437–46. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
65. Liu E, Duggan C, Manji KP, *et al.*: Multivitamin supplementation improves haematologic status in children born to HIV-positive women in Tanzania. *J Int AIDS Soc.* 2013; 16(1): 18022. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
66. McDonald CM, Manji KP, Kisenge R, *et al.*: Daily Zinc but Not Multivitamin Supplementation Reduces Diarrhea and Upper Respiratory Infections in Tanzanian Infants: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial. *J Nutr.* 2015; 145(9): 2153–60. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
67. Carter RC, Kupka R, Manji K, *et al.*: Zinc and multivitamin supplementation have contrasting effects on infant iron status: a randomized, double-blind, placebo-controlled clinical trial. *Eur J Clin Nutr.* 2018; 72(1): 130–35. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
68. Lauer JM, McDonald CM, Kisenge R, *et al.*: Markers of Systemic Inflammation and Environmental Enteric Dysfunction Are Not Reduced by Zinc or Multivitamins in Tanzanian Infants: A Randomized, Placebo-Controlled Trial. *J Pediatr.* 2019; 210: 34–40.e1.

[PubMed](#) [Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

69. Locks LM, Manji KP, McDonald CM, *et al.*: Effect of zinc and multivitamin supplementation on the growth of Tanzanian children aged 6-84 wk: a randomized, placebo-controlled, double-blind trial. *Am J Clin Nutr.* 2016; **103**(3): 910–8.

[PubMed](#) [Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

70. Angulo-Barroso RM, Li M, Santos DC, *et al.*: Iron Supplementation in Pregnancy or Infancy and Motor Development: A Randomized Controlled Trial. *Pediatrics.* 2016; **137**(4): e20153547.

[PubMed](#) [Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

71. Sudfeld CR, Manji KP, Darling AM, *et al.*: Effect of antenatal and infant micronutrient supplementation on middle childhood and early adolescent development outcomes in Tanzania. *Eur J Clin Nutr.* 2019; **73**(9): 1283–90. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

72. Pongcharoen T, DiGirolamo AM, Ramakrishnan U, *et al.*: Long-term effects of iron and zinc supplementation during infancy on cognitive function at 9 y of age in northeast Thai children: a follow-up study. *Am J Clin Nutr.* 2011; **93**(3): 636–43.

[PubMed](#) [Abstract](#) | [Publisher Full Text](#)

73. Manji KP, McDonald CM, Kupka R, *et al.*: Effect of multivitamin supplementation on the neurodevelopment of HIV-exposed Tanzanian infants: a randomized, double-blind, placebo-controlled clinical trial. *J Trop Pediatr.* 2014; **60**(4): 279–86.

[PubMed](#) [Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

74. Friel JK, Moffatt M, Miller A: Iron supplementation of full-term healthy breast fed infants. *Trace Elements and Electrolytes.* 2010; **27**(3): 169.

75. Finkelstein JL, Herman HS, Guetterman HM, *et al.*: Daily iron supplementation for prevention or treatment of iron deficiency anaemia in infants, children, and adolescents. *Cochrane Database of Systematic Reviews.* 2018(12): CD013227. [Publisher Full Text](#) | [Free Full Text](#)

76. Mayo-Wilson E, Imdad A, Junior J, *et al.*: Preventive zinc supplementation for children, and the effect of additional iron: a systematic review and metaanalysis. *BMJ Open.* 2014; **4**(6): e004647. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

77. Das JK, Hoodbhoy Z, Salam RA, *et al.*: Lipid-based nutrient supplements for maternal, birth, and infant developmental outcomes. *Cochrane Database Syst Rev.* 2018; **8**(8): CD012610.

[PubMed](#) [Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Open Peer Review

Current Peer Review Status:



Version 2

Reviewer Report 23 February 2023

<https://doi.org/10.21956/wellcomeopenres.21080.r55058>

© 2023 Cooper P. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Peter Cooper

Department of Paediatrics, Faculty of Clinical Sciences, University of the Witwatersrand, Johannesburg, South Africa

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Paediatrics, Neonatology, Nutrition

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 02 December 2020 <https://doi.org/10.21956/wellcomeopenres.17890.r41324>

© 2020 Cooper P. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Peter Cooper

Department of Paediatrics, Faculty of Clinical Sciences, University of the Witwatersrand, Johannesburg, South Africa

Previous systematic reviews on micronutrient supplementation in infants and children have largely concentrated on those over six months of age. This would appear to be the first systematic review of micronutrient supplementation, with a specific focus on iron supplementation, in term infants under the age of six months and thus is an important contribution.

Standard guidelines for systematic reviews were followed and the methodology outlined is clearly described. Figure 1 illustrates that a total of 11,109 records were identified through database searching and 2,505 were removed as they were duplicates. It is not clear how 7,142 records then remained to be screened – *this needs to be clarified*.

Their review identified only 32 publications from 23 studies and they have reported the findings in detail. However although their paper provides some answers, due the wide heterogeneity of these studies, many questions have been raised.

Most of the studies were done in low and middle-income countries (LMIC) and not surprisingly showed a high incidence of iron deficiency in these infants, more particularly in those born to iron deficient mothers and thus clearly is a problem that requires to be addressed. Supplementation of iron fairly consistently in the studies reviewed resulted in improvement in iron status of the infants. There was little evidence from this systematic review that supplements with iron, zinc or other micronutrients resulted in improvement in growth of the infants or their morbidity and mortality.

There was some evidence that iron supplementation did contribute to improvements in measures of short-term neurodevelopment. Only two of the studies looked at longer term neurodevelopment up to six to eight and 11-14 years of age respectively. Neither of these studies showed any benefit but importantly iron supplementation was not included in their intervention arms so this remains an open question.

This review also highlights the difficulties in doing studies of this nature. They were all done in the context that exclusive breast feeding was encouraged up to six months of age. However, the researchers of these studies were unable to control to what extent exclusive breast feeding took place and what additional foods may have been given. Commercially available infant foods and formulas often have supplements of iron and micronutrients, which may confound the results.

The authors are to be congratulated for raising an important issue around which there has not been adequate research. Further large randomized studies are required and may need to be individualized to the country or countries in which they are done. For example, one of the studies in a malaria area that they analyzed showed that the addition of malaria prophylaxis to iron supplementation resulted in reduced prevalence of anaemia compared with iron supplementation alone. Studies done in other areas may need to take different local specific factors into account.

This review will hopefully lead to the initiation of large randomized trials of supplementation of iron during the first six months of life. These studies need to take into account whether the infants are exclusively breast-fed or otherwise during the first six months of life and full dietary histories during this period need to be documented. Most importantly longer term follow up of haematologic status, growth, morbidity and mortality and neurodevelopment should be built into the study protocols.

Are the rationale for, and objectives of, the Systematic Review clearly stated?

Yes

Are sufficient details of the methods and analysis provided to allow replication by others?

Yes

Is the statistical analysis and its interpretation appropriate? Not applicable

Are the conclusions drawn adequately supported by the results presented in the review?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Paediatrics, Neonatology, Nutrition

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 23 November 2020 <https://doi.org/10.21956/wellcomeopenres.17890.r41347>

© 2020 Gomes F. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Filomena Gomes 

The New York Academy of Sciences, New York City, NY, USA

The manuscript describes an important, well conducted systematic review on iron and multiple micronutrient supplementation of infants under six months of age. PRISMA guidelines and a previously published protocol were followed. The authors did not perform a meta-analysis but presented the results in a balanced and informative way.

A few minor comments:

• "Exclusion & inclusion criteria": the first sentence should have been more clear that the beginning of supplementation (not the whole supplementation or follow-up period) needs to happen in the first six months of life as an inclusion criteria. Instead of "Trials were included if (...) were in infants under six months of age", it could have been stated that "Trials were included if (...) supplementation was initiated in infants under six months of age"

• The method used to assess risk of bias is correct but the way the findings are described is imprecise. For example, instead of stating that 25 publications had an unclear risk of bias, it could have been stated that 25 publications had unclear risk of bias for the domain "blinding of participants and personnel" (which may be difficult to achieve in nutrition/diet interventions). In particular, authors are encouraged to report the overall judgment for the most critical domains, which usually include bias for sequence generation, allocation concealment and blinding of outcome assessors (particularly for subjective outcomes).

• Under "strengths and limitations", the second paragraph starts with "The main strength of this systematic review is that is it (...)" but should be "The main strength of this systematic review is that it is (...).

Are the rationale for, and objectives of, the Systematic Review clearly stated? Yes

Are sufficient details of the methods and analysis provided to allow replication by others?

Yes

Is the statistical analysis and its interpretation appropriate? Yes

Are the conclusions drawn adequately supported by the results presented in the review?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Clinical nutrition, malnutrition, systematic reviews

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

4 CAREGIVER PERCEPTIONS OF NUTRITION INTERVENTIONS IN INFANTS AND CHILDREN UNDER 24 MONTHS OF AGE: A SYSTEMATIC REVIEW

4.1 INTRODUCTION

This Chapter incorporates an original, published systematic review (Stelle, et al., 2023) published in *Public Health Nutrition*. This systematic review was undertaken during this PhD to review the evidence of caregiver perceptions of nutrition interventions in infants and children under 24 months of age and address any gaps in the literature. A review protocol detailing the research question, search strategy, inclusion and exclusion criteria, quality assessment, and strategy for data synthesis was developed to refine the scope of the review and registered to Prospero (available at: https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=238050). Study selection was managed using Covidence (278).

This review contributes to a growing literature suggesting that despite widespread supplementation schemes, far too many children under five years of age still die mostly from preventable and treatable causes, with nutrition-related factors contributing to approximately 45% of these deaths (1). This review supports the need for implementation focused interventions to improve the nutritional status of infants and young children, especially in settings with high rates of deficiencies.

While efficacy studies in infants have shown that early nutrition interventions improve infant nutritional status, to examine whether such interventions can be effectively taken to scale, a better understanding of caregiver acceptability is required. Caregiver perceptions will strengthen sustainability and implementation of future nutrition interventions for this vulnerable age group.

Therefore, the aim of this Chapter is to examine the caregiver perceptions of nutrition interventions within a global context in infants and children under 24 months of age, as well as reported side effects and impacts on infant feeding practices, in hopes to summarise the literature around this topic and inform future interventions at this young age. Appendix 2 presents additional files associated with this publication.

4.2 AUTHOR ROLES

Conceptualization: Isabella Stelle, Sophie E Moore

Funding Acquisition: Sophie E Moore

Methodology: Isabella Stelle, Mai-Lei Woo Kinshella, Sophie E Moore

Analysis plan: Isabella Stelle

Paper specific data management and main analysis: Isabella Stelle, Mai-Lei Woo Kinshella

Supervision: Sophie E Moore

Original Draft Preparation: Isabella Stelle

Reviewing and Editing: All authors

Approval of final manuscript: All authors

4.3 PUBLISHED PAPER 2: CAREGIVER PERCEPTIONS OF NUTRITION INTERVENTIONS IN INFANTS AND CHILDREN UNDER 24 MONTHS OF AGE: A SYSTEMATIC REVIEW

Stelle I, Kinshella MLW and Moore SE. Caregiver perceptions of nutrition interventions in infants and children under 24 months of age: a systematic review. *Public Health Nutrition*. 2023, 1-24.

<https://doi.org/10.1017/S1368980023001246>.

The full paper can be accessed below and additional files in Appendix 2 or accessed here:

<https://doi.org/10.1017/S1368980023001246>

Systematic Review

Caregiver perceptions of nutrition interventions in infants and children under 24 months of age: a systematic review

Isabella Stelle^{1,*}, Mai-Lei Woo Kinshella² and Sophie E Moore^{1,3}

¹Department of Women and Children's Health, King's College London, St Thomas' Hospital, Westminster Bridge Rd, London SE1 7EH, UK; ²Department of Obstetrics and Gynaecology and BC Children's Hospital Research Institute,

University of British Columbia, Vancouver BC, Canada; ³Medical Research Council Unit The Gambia at The London School of Hygiene and Tropical Medicine, Fajara, The Gambia

Submitted 21 January 2022; Final revision received 19 May 2023; Accepted 14 June 2023; First published online 23 June 2023

Abstract

Objective: Efficacy studies show early nutrition interventions improving infant nutrition status, but understanding caregiver acceptability is required for implementation of such interventions. This systematic review examines caregivers' perceptions of nutrition interventions in young children.

Design: We searched the Cochrane Central Register of Controlled Trials, MEDLINE, Embase, CINAHL and PsycINFO from date of online journal inception through December 2020. Interventions included oral (powder/liquid/tablet) and/or intravenous supplementation, food fortification and nutrition counselling. Inclusion criteria included primary research, data presented on caregiver perception and studies published in English. Quality assessment was performed using the Critical Appraisal Skills Programme tool. Studies underwent narrative synthesis using inductive thematic analysis.

Setting: No restriction.

Participants: Caregivers of children under 24 months of age.

Results: Of 11 798 records identified, thirty-seven publications were included. Interventions included oral supplementation, food fortification and nutrition counselling. Caregivers included mothers (83 %), fathers, grandparents and aunts. Perceptions were gathered through individual interviews, focus group discussions, questionnaires, surveys and ratings. Totally, 89 % of studies noted high acceptability (n 33 most notably increased appetite (n 17)). In total, 57 % of studies (n 21) cited low acceptability, commonly from side effects (n 13) such as gastrointestinal issues, appetite loss and stained teeth.

Conclusions: Positive perceptions and enthusiasm for interventions were frequently reported. Key to implementation was the increased appetite noted Keywords by caregivers. A substantial proportion of studies reported negative perceptions, mainly due to side effects. In future interventions, mitigation and education around Infants common side effects are crucial for acceptability. Understanding both positive and Caregivers negative caregiver perceptions is important for informing future nutrition Acceptability interventions and strengthening sustainability and implementation.

KEYWORDS: Qualitative. Nutrition interventions. Infants. Caregivers. Acceptability.

*Corresponding author: Email Isabella.stelle@kcl.ac.uk

©The Author(s), 2023. Published by Cambridge University Press on behalf of The Nutrition Society. This is an Open Access article, distributed under the terms of the Creative Commons Attribution licence (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted re-use, distribution and reproduction, provided the original article is properly cited.

In 2019, an estimated 5.2 million children under 5 years of age died mostly from preventable and treatable causes⁽¹⁾. Nutrition-related factors contributed to approximately 45% of these deaths⁽¹⁾. Further, undernutrition during the first 1000 d of life (from conception until 2 years of age) can have lifelong consequences for growth and cognitive development⁽²⁾.

The first 1000 d of life are an especially sensitive period due to rapid growth and development, increased nutritional needs, greater vulnerability to infection and full dependency for care⁽²⁾. Evidence has shown that early years are critical for cognitive, language and social emotional development with risks for poor development being linked to inadequate quality of caregiver to child interaction⁽³⁾. Caregiver dependency at this young age is therefore vital in determining nutritional adequacy. For example, between the age of 6–23 months, in areas with an Fe deficiency anaemia prevalence greater than 40 %, the WHO recommends daily Fe supplementation (10–12.5 mg elemental Fe) for three consecutive months per year⁽⁴⁾. However, Fe supplementation is commonly noted to cause adverse gastrointestinal side effects in children such as diarrhoea, nausea, constipation and vomiting, therefore limiting adherence in supplementation trials (170). More recent studies have shown Fe-containing micronutrient powders to cause increased risk of diarrhoea in young children (162, 167, 172, 173). Therefore, a proposed intervention must be perceived as acceptable through the caregiver's perspective for effective implementation, given care providers are the ones administering interventions to their infants and young children. Efficacy studies in infants have shown that early nutrition interventions improve infant nutrition status, but a better understanding of caregiver acceptability is required to examine whether such interventions can be effectively taken to scale^(5–7).

This review explores caregivers' perceptions of various nutrition interventions within a global context for infants and young children under 24 months of age as well as reported side effects and impacts on infant feeding practices.

Methods

The primary objective of this review is to examine caregiver perception of nutrition interventions (i.e. oral (powder, liquid, tablet) and/or intravenous supplementation, food fortification and nutrition counseling) in their infants under 24 months of age. Further, secondary objectives include a consideration of the effects of nutrition interventions in early infancy (the first 24 months of life) on choices of infant feeding practices and any adverse effects of nutrition interventions in early infancy (the first 24 months of life).

We searched the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE (Ovid), Embase (Ovid), CINAHL and PsychINFO from date of online journal inception through December 2020. Searches were supplemented by scanning reference lists of papers included for review. Based on the PICOS research framework (Table 1), search terms were developed (Table 2). A review protocol detailing the research question, search strategy, inclusion and exclusion criteria, quality assessment and strategy for data synthesis was developed to refine the scope of the review. The protocol was registered to Prospero (CRD42021238050). Study selection was managed using Covidence⁽⁸⁾. Titles and abstracts were manually screened according to the eligibility criteria (Table 3) by two independent reviewers (IS and MWK). Discrepancies were resolved by discussion and a third reviewer (Sem) adjudicated in the absence of consensus. Full texts were then reviewed by the two independent reviewers (IS, MWK). The third reviewer (Sem) provided an independent assessment in any disputes regarding eligibility.

Inclusion and exclusion criteria can be found in Table 3. The primary outcome was the caregivers' perceptions of the intervention, and secondary outcomes included the impact on exclusive breast

feeding, feeding practices and/ or adverse events. All caregiver perceptions were considered and considered of equal importance. Secondary outcomes were summarised in the narrative synthesis.

Quality assessment was performed for all included studies using the Critical Appraisal Skills Programme tool⁽⁹⁾. Critical Appraisal Skills Programme is the most used tool for quality appraisal in health-related qualitative evidence syntheses, with endorsement from the Cochrane Qualitative and Implementation Methods Group^(10,11). Quality assessment was reported for all identified studies to inform interpretation. Due to the exploratory nature of qualitative studies, no studies were excluded based on the quality assessment.

A data extraction sheet was developed and piloted by the research team. Details about the nutrition intervention, study methodology, sample size, study design and outcomes (perceptions) were extracted into Excel⁽¹²⁾. Two reviewers (IS, MWK) independently extracted data and conducted the quality assessment from a sample of eligible studies (10 %) until agreement was achieved, with the remainder extracted by one reviewer (IS). The data extraction sheet was imported into NVivo where inductive thematic analysis of caregiver perception was conducted^(13,14). Excerpts from included studies were extracted to generate key themes⁽¹⁴⁾. Themes are repeated patterned responses within data sets that are then separated into subthemes⁽¹⁴⁾. An inductive approach was used in which the data analysis is data driven so that the participant's views take precedence⁽¹⁴⁾.

The Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) guidelines are used⁽¹⁵⁾. The PRISMA Checklist is presented in online supplementary material, Supplemental Extended Datafile 1.

Table 1 PICOS research framework

Population: Caregivers of infants under 24 months of age (i.e. mothers, fathers, mothers-in-law, grandmothers and family members)

Intervention: Nutrition interventions (oral (powder, liquid, tablet) and/or intravenous supplementation, food fortification and nutrition counselling)

Comparison: NA, placebo, no intervention/therapy and additional nutrition interventions

Outcome: Perception of intervention (secondary: impact on exclusive breast feeding, feeding practices and adverse events)

Study design: Experimental studies (controlled trials) and observational studies (cohort, case controlled, cross-sectional and qualitative)

Table 2 Search terms

Searches	Search term
Population	Caregiver/ Mothers/ (mother* or maternal*).ab. Fathers/ (father* or paternal*).ab. Parents/ Family/ (grand?parent* or grand?mother* or grand?father*).mp.

Intervention	Infant/ or Infants/ or Infancy/ exp Infant, Newborn/ child/ (infant* or newborn* or new born* or neonat* or baby or babies or child* or young children).ti,ab. Micronutrients/ or Trace Elements/ Dietary Supplements/ (micronutrient* or multinutrient* or multimicronutrient* or multivitamin* or multimineral* or MMN* or MMS* or MNP* or sprinkles or LNS*).tw. (trace adj (element* or mineral* or nutrient*)).tw. (diet* adj3 supplement*).tw. Nutrition.mp. (diet* counselling or nutrit* counselling or lactation counselling or breast?feeding counselling).mp. (fortification or fortified foods).mp.
Outcome	Qualitative Research/ or Interviews as Topic/ (qualitative or group discussion? or focus group? or themes).ti,ab. Perception/ Implementation/ (acceptab* or feasib* or program evaluation).tw. (barrier* or facilitator* or like* or dislike*).mp.

Table 3 Eligibility criteria

Selection criteria	Inclusion	Exclusion
Population	Caregivers; average age of infants < 24 months of age	Average age of infants > 24 months of age
Intervention	Oral (powder, liquid and tablet) and/or intravenous supplementation, food fortification and nutrition counselling	No nutrition intervention
Comparison	Placebo, no intervention/therapy, oral (powder, liquid and tablet) and/or intravenous supplementation, food fortification, nutrition counselling	
Outcome	Caregiver perception of intervention	No caregiver perception of intervention
Study type	Published experimental and observational studies in English including randomised or non-randomised controlled trials, cohort, case controlled, cross-sectional survey, facility evaluations and qualitative studies	Studies without primary data collection, such as reviews and study protocols, studies not in English and those not demonstrating a clear research methodology (i.e. abstracts, conference proceedings, commentaries, letters and editorials)
Language	Studies published in the English language	Not in English
Other	From conception of the journal through 2020	Published after 2020

Results

Characteristics of studies included

Of 11 798 records identified, thirty-seven publications were included (Fig. 1). One hundred and five full-text articles were reviewed for eligibility. Of these, thirty-one were excluded because they used no nutrition intervention, twenty-two were not full texts (i.e. conference proceeding, abstract, etc.), nine had no caregiver perceptions reported, three were in the wrong age group, two were the wrong study design (i.e. unapplicable intervention) and one was a duplicate (see online supplementary material, Supplemental Extended 2).

Despite no restriction on publication start date, all thirty seven studies were published between 2009 and 2020 (see online supplementary material, Supplemental Extended Datafile [3a](#) for characteristics of included studies). Sample sizes varied widely from eleven up to 1916 caregivers with data from 13 765 caregivers in total across the included studies. The data collection methodology gathered perceptions through interviews, focus group discussions, questionnaires, surveys and ratings. The scope of caregivers included mothers (n 11 466), fathers (n 370), maternal and paternal grandparents (n 20), aunts (n 4) and unspecified (n 1905). The nutrition interventions included small quantity lipid based nutrient supplements, ready to use supplementary foods, micronutrient interventions in different formulations (Fe folic acid syrup, multiple micronutrient powders and micronutrient powders), lipid-based nutrient supplements, ready to use therapeutic foods, oral rehydration solutions, infant and young child feeding practices, social and behavioural change communications, nutrition counselling, complementary foods, fortified porridges and crops and food grinders. Of the geographical regions represented, twenty four of the studies were in sub-Saharan Africa (one was a dual country study, both in sub-Saharan Africa), nine in South-Asia, four in South America and one in the Caribbean (Fig. 2). Despite no limitations on setting, all studies took place in low resource settings.

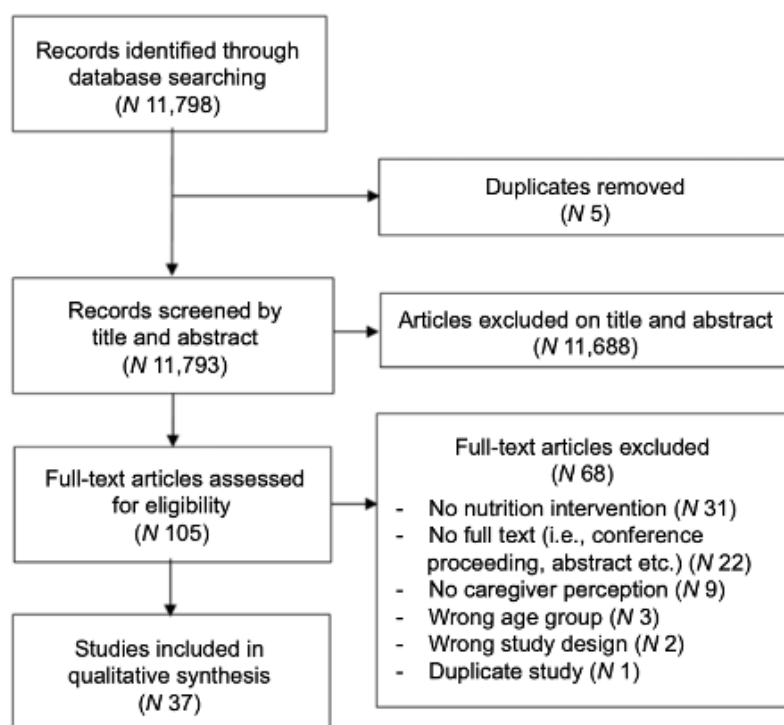


Fig. 1 PRISMA flowchart

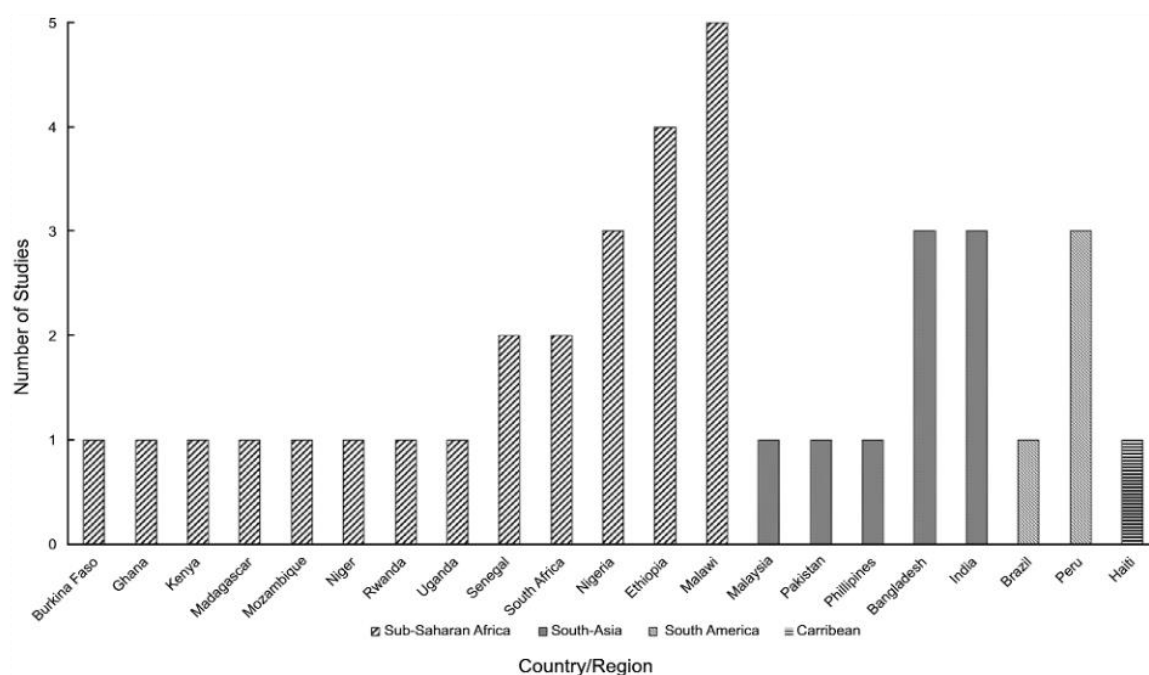


Fig. 2 Spread of countries included in this review

Quality assessment

For quality appraisal, of the thirty-seven publications, twenty four ranked 'good', twelve ranked 'fair' and one ranked 'poor'. The one study that ranked as 'poor' did not specify its recruitment strategy, the relationships between the researcher and the participants or how the data was analysed⁽¹⁶⁾. The full results from the Critical Appraisal Skills Programme tool can be found in online supplementary material, Supplemental Extended Datafile 4.

Themes and sub-themes

From the inductive thematic analysis of caregiver perception, two main themes emerged: high and low acceptability (Fig. 3). For high acceptability the following sub-themes emerged: reasons for use (n 29) and enthusiasm for continuation of the intervention (n 6). 'Reasons for use' of the intervention was further broken down, specifically into sub-themes around its use for 'perceived benefits' to the infant. For low acceptability, seven sub-themes of reasons for low acceptability emerged. These sub-themes included side effects (n 13), poor communication/ understanding (n 11), infant refusal (n 10), lack of caregiver self-efficacy/ forgetting (n 8), limited social support (n 7), availability/ accessibility (n 6) and other (n 3). Nineteen of the studies cited both low and high acceptability.

High acceptability

Eighty nine percent of studies noted high acceptability (n 33; detailed in online supplementary material, Supplemental Extended Datafile [3b](#)), with six mentioning enthusiasm for continuation of the intervention.

Perceived benefits

The most noted perceived infant benefits (n 25) were increased appetite (n 17), improved overall health and diet (n 16) and prevention of disease, illness or malnutrition (n 11). When noting improved overall diet and health, specifics of perceived increased blood health and digestion were highlighted^(17–21). In the context of prevention of disease, illness or malnutrition, the interventions were compared with a medicine in a beneficial sense^(22–25). Additionally, caregivers noted perceptions of improved growth (height and weight) and improved strength (n 10), as well as improved energy (n 10) and neurodevelopmental improvements (n 7)(17–21,24–32).

Other facilitators of high acceptability

Other facilitators associated with high acceptability: family and community support, ease of use, following nutrition guidance, palatability and others. A significant and important facilitator for high acceptability was family and community support (n 10)(18,21,25,29,31,33–37). Within the context of patriarchal households, when mothers felt supported by their husbands, they were more inclined to adhere to an intervention^(21,31,33,35–37). Ease in packing and storage, accessibility and affordability were keys factors in caregiver use of intervention (n 10)(20,24–26,29,31,35,38–40). Education around purpose and administration of the intervention were key to ensuring acceptability and continuation of use as caregivers wanted to follow nutrition guidance when it was given (n 7)(18,19,22,25,28,33,41). It was noted that giving the intervention to their infant gave caregivers a feeling of empowerment⁽³³⁾. Having an infant like the taste also increased the chances a caregiver continued the interventions use (n 4)^(24,26,31,42).

One study found that mothers preferred clinic administration of the supplements as it was perceived as 'more hygienic' and 'involving professional health workers'⁽⁴³⁾. However, other mothers, especially those living from the clinics, preferred house-to-house delivery of the supplements, which is the traditional method for a nutrition campaign in their region⁽⁴³⁾.

Side effects

Side effects that were noted, but not in the context of low acceptability were diarrhoea, vomiting, and constipation, nausea, lack of appetite and abdominal discomfort, respectively, from most to least cited^(21,23,27,32,36,37,44).

Low acceptability

Just over half of the studies (n 21) cited low acceptability (detailed in online supplementary material, Supplemental Extended Datafile 3b). This was most commonly due to side effects (n 13), poor communication and/or under- standing (n 11) and infant refusal (n 10).

Side effects

Side effects were noted in thirteen studies^(17,18,20,21,23,25,27,29,34,37,44–46). All thirteen studies caregivers reporting these side effects as negative; however, they were also noted as, when mild, a sign of the intervention being effective. For example, one mother noted her infants' change in stools as an indication that the intervention was working⁽³⁴⁾. Side effects that led to low acceptance were noted as the infant being hyperactive, diarrhoea, vomiting, constipation, abdominal discomfort, loose or black stools, infant rejection, appetite loss, stained teeth, the infant getting sick, a decrease in overall health, general negative effect and in one Peruvian peri-urban community, a mother cited impaired mental development as a concern⁽²¹⁾.

Other barriers to intervention acceptability

When there was lack of caregiver self-efficacy and forgetfulness (n 8), limited social support (n 7) and/or availability/accessibility to the interventions (n 6), these were significant barriers to caregiver lack of use. Other reasons for non-use (n 3) were simply that caregivers had no reason as to why they did not want to use it and because they were using another medication at the time instead so stopped the intervention^(17,26). One study noted a high prevalence of no impact from the intervention (20 %)⁽²⁰⁾.

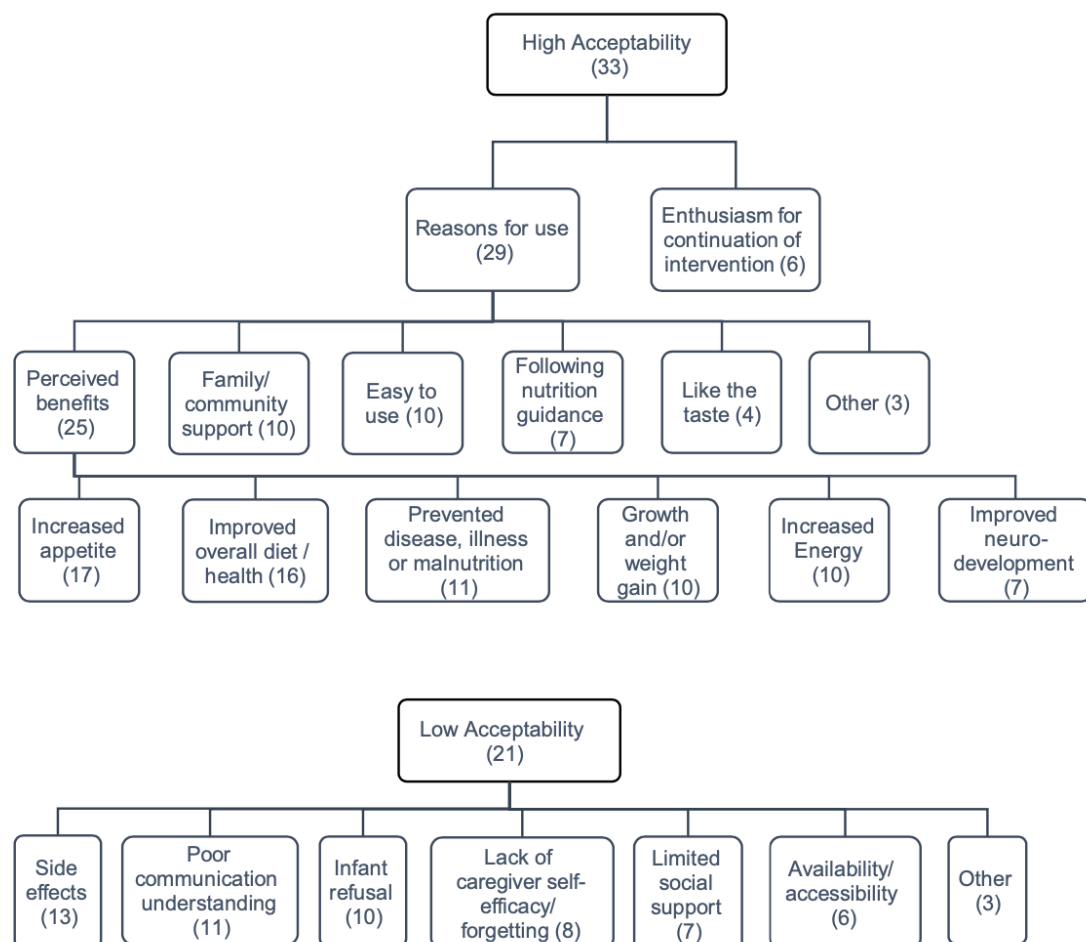


Fig. 3 Acceptability of interventions

Nutritional Intervention preferences

Among studies that compared acceptability of various supplements, the following preferences were found. Mothers noted that micronutrient powders v Fe syrups were better suited for older children who had 'become smart' and were able to eat on their own⁽³²⁾. However, they felt that the syrups were better in ensuring the infant received the full dose of the supplement because with powders the infant did not always finish their food⁽³²⁾. Overall, the enthusiasm for continued use of syrups v. powder was equal (38 % each) and 24 % of mothers said they would like to use both⁽³²⁾. Additionally, caregivers found it easier to give micronutrient powders in semi-solid foods such as purees and porridges rather than liquid preparations such as soups⁽²¹⁾. When looking at complementary foods fortified with or without Zn, those without Zn scored higher on a degree of liking scale⁽⁴⁷⁾. Novel rice lentil- and chickpea-based ready-to-use supplementary foods rating significantly better for 'overall liking' when compared with traditional Pushti packet⁽¹⁶⁾. In terms of frequency of supplementation, different studies found varying preferences for flexible verses daily verses every other day administration. For mothers giving flexible or daily administration, almost all mothers preferred flexible due to perceived benefits such as more time to give the Sprinkles, more autonomy and less anxiety around missing a dose⁽⁴⁸⁾. Alternatively, two studies found that caregivers preferred daily administration of micronutrient powders verses alternate days, so they were less likely to forget^(20,29). While not in a negative context, mothers also reported, organoleptic changes in food, such as colour, taste, smell and texture, when certain interventions, such as powders, were added to home foods^(29,37).

Misuse of supplements

Misuse of supplements was seen in seven studies through sharing with other infants or giving more to the infant when it was demanded^(18,23,24,26,29,30,49). Likewise, jealousy among other households or household members, as well as theft of supplements, posed an issue^(22,23,49).

Impact on mode of feeding

Secondary objectives included a consideration of the effects of nutrition interventions in early childhood (the first 24 months of life) on choices of infant feeding practices; however, this theme was not seen in any of the included studies.

Discussion

The purpose of this review was to understand caregiver perceptions around nutrition interventions for use in infants and young children. This is especially relevant in low resource settings where caregiver acceptability of nutrition interventions, alongside appropriate infant feeding practices, may be critical for both short- and long-term health. Interestingly, all the studies identified took place in low resource settings with the majority being in sub-Saharan Africa. Previous literature has found that low- and middle-income countries are unrepresented in clinical research⁽⁵⁰⁾, specifically with the number of clinical trials in

A significant emergent theme was the importance of family and community support^(18,21,25,29,31,33–37). Concurrent with the literature, mothers were by far the most cited caregiver⁽⁵²⁾, so in the context of patriarchal households, when the mothers felt supported by their husbands, they were more inclined to adhere to an intervention^(21,31,33,35–37). It has been previously found that to ensure acceptance, education played a significant role in caregivers' continuation of use^(53,54). Caregivers wanted to follow nutrition guidance when it was given but understanding of why the intervention was needed was pertinent. It was noted

that giving the intervention to their infant gave caregivers a feeling of empowerment as they knew they were doing something good for their infants⁽³³⁾. One of the most noted reasons for low acceptability was poor communication and/or understanding such as lack of caregiver self-efficacy and forgetfulness, limited social support and/or availability/accessibility to the intervention. Appropriate training of intervention use is essential; in one study, a participant reported stopping the intervention when the infant needed medication, counter to the intention of the study⁽²⁷⁾. One caregiver simply gave no reason, they just did not want to use the intervention, and another cited seeing no impact from the intervention^(17,20,26). Education around interventions not always having an immediate impact, but rather long-term benefits may have solved this issue. Lastly, ease of use of packaging and storing, as well accessibility and affordability, was indicated as crucial^(20,24–26,29,31,35,38–40).

Almost all the studies noted high acceptability. Instances of misuse were common due to the infants demanding more of the supplement^(18,23,24,26,29,30,49) or jealousy from other family or community members which lead to sharing^(22,23,49). In these communities, feeding is often done communally so sharing can be difficult to avoid, as has been seen in previous studies^(55,56). The most frequently cited reason for this high acceptability was an increase in the infant's appetite. While improved overall diet and health were almost equally as noted, interestingly a few caregivers specifically noted an increase in the infant's 'blood health'. Also highly cited was the interventions' ability to prevent disease, illness and malnutrition, with the intervention even being compared with medication^(22–25), as similarly reported in previous studies^(55,57). Interestingly, when noting improved growth, such as weight, height and strength, some caregivers also noted neurodevelopmental improvements^(17–21,24–32). Caregivers did report organoleptic changes in food (colour, taste, smell and texture) when micronutrient powders were added^(29,37). Having the infant like the taste was important in continuation of the intervention^(24,26,31,42). One of the most common reasons for low acceptability was when the infant refused the intervention.

Low acceptability was most commonly due to side effects, such as such as reports of infants' morbidity, including diarrhoea. However, side effects were not always noted in the context of low acceptability, but rather a result of the intervention and even sometimes as a sign that the intervention was working, in the case of darkening of stool⁽³⁴⁾. As previously noted in the literature, side effects are common with nutrition interventions at this young age, especially in those containing Fe⁽⁵⁸⁾. This difference in reporting side effects highlights the issue of heterogeneity in studies such as these. Given caregivers perceptions are being collated, this could simply reflect a difference in caregiver attention and sensitivity to their child's reaction.

It was evident that personal preferences varied, with nineteen of the studies citing both low and high acceptability. This is important to take into consideration with interventions, but heterogeneity in the studies makes it difficult to form conclusions for general recommendations. For example, some mothers preferred clinic administration because it was seen as more hygienic, whereas some mothers preferred home administration because it was more convenient⁽⁴³⁾. Additionally, age may impact what type of intervention should be given to children, with caregivers noting that syrups were better for infants to ensure the whole dose was received but that powders were better for older children who were able to eat on their own⁽³²⁾. Additionally, it was found that micronutrient powders were better suited for semi-solid foods such as purees and porridges⁽²¹⁾. Likewise, and as debated in previous literature, when it comes to frequency of the interventions, preferences were divided across daily or flexible administration⁽⁵⁹⁾. Some caregivers preferred daily as it became a routine and they were less likely to forget^(20,29), whereas other caregivers preferred flexible administration thanks to more autonomy and less worry about missing a dose⁽⁴⁸⁾. Additionally, misuse of supplements, such as using supplements for other family members, could be associated with variations in positive verses

negatives responses, which highlights the importance of appropriate counselling of family members around supplements and their use.

While negative perceptions were reported, overall positive perceptions and enthusiasm for continuation of the interventions were more frequent. Finally, though there were preferences expressed for type of supplementation, route, dosing, administration and packaging, these preferences were not frequent enough to draw conclusions. Caregiver perception is crucial for future acceptability, adherence to and effectiveness of interventions. As evident from the results, future interventions can best support this through education as well as family and caregiver support.

The main strength of this systematic review is that it is the first comprehensive review of caregiver perception of nutrition interventions in infants and young children. In addition, trials were not excluded based on supplementation regimen allowing the authors to understand a wide scope of the literature in the field. All studies, but one, were assessed to be good or fair quality, which supports the credibility of review findings.

We also acknowledge several limitations. While heterogeneity in the data was a strength in terms of assessing the full scope of the literature, it makes comparison of trials via a quantitative meta-analysis difficult. Most studies also cited both high and low acceptability, so it was difficult to categorise an intervention as acceptable or not. Likewise, conclusions for specific interventions were hard to draw due to the limited number of studies. While there was no start date limitation (journal induction), the first study included in this review was from 2009. Due to implementation research being a newer science, the research in this field is still limited. Some of the studies used focus group discussions and interviews, which gave useful insights into caregiver perceptions, but many studies simply using ratings, surveys and questionnaires. While still useful, these studies lacked as much depth as those using more personal research practices. Additionally, some studies did not specify who the caregivers were.

Conclusion

Positive perceptions and enthusiasm for continuation of interventions were frequently reported by caregivers. Key facilitators to implementation were increased appetite and overall general health of infants and children as noted by caregivers. A substantial proportion of studies reported negative perceptions, mainly due to side effects. In future interventions, mitigation and education around common side effects are crucial for acceptability. Caregivers wanted to follow nutrition guidance when it was given, but understanding of why the intervention was needed was pertinent as one of the most noted reasons for low acceptability was poor communication and/or understanding around the intervention. Likewise, cultural circumstances must be taken into consideration, for example, feeding is often done communally in low-resource communities so sharing can be difficult to avoid. Additionally, having the supplement be palatable is important as one of the most common reasons for low acceptability was when the infant refused the intervention. Continued research is needed in this area, especially in low-resource settings where nutrition deficiencies are common in infants and can have lifelong impacts. An understanding of the barriers and facilitators to implementing such interventions, especially through the perception of caregivers, is crucial for the success of future interventions.

Acknowledgments

Acknowledgements: NA. *Financial support:* S.E.M. is supported by a Wellcome Trust Senior Research Fellowship (220225/Z/20/Z). M.W.K. is supported by the Vanier Canada Graduate Scholarship funded by the Government of Canada through the Canadian Institutes of Health Research (CIHR). *Conflict of interest:* The authors have no conflicts to disclose. *Authorship:* The study was conceptualised by S.E.M. and I.S. I.S. formulated the search strategy and wrote the protocol. I.S. and M.W.K. carried out the study selection and data extraction. Any disputes were mediated by Sem. Quality and data synthesis were carried out by I.S. I.S. wrote the first draft of this manuscript. Final drafts were edited and approved by M.W.K. and Sem. *Ethics of human subject participation:* Ethical Standards Disclosure: NA.

Supplementary Material

For supplementary material accompanying this paper visit <https://doi.org/10.1017/S1368980023001246>

References

1. World Health Organization (2020) Children: Improving Survival and Well-Being. <https://www.who.int/news-room/fact-sheets/detail/children-reducing-mortality> (accessed January 2021).
2. Martorell R (2017) Improved nutrition in the first 1000 days and adult human capital and health. *Am J Hum Biol* 29, e22952.
3. Alderman H, Behrman JR, Glewwe P et al. (2017) Evidence of impact of interventions on growth and development during early and middle childhood. In *Child and Adolescent Health and Development*, Chapter 7 [Silva ND, Horton S, Jamison DT & Patton GC, editors]. Washington, DC: The International Bank for Reconstruction and Development/The World Bank.
4. WHO (2016) Guideline: Daily Iron Supplementation in Infants and Children. Geneva, Switzerland: World Health Organization.
5. Bhutta ZA, Ahmed T, Black RE et al. (2008) What works? Interventions for maternal and child undernutrition and survival. *Lancet* 371, 417–440.
6. Christian P, Mullany LC, Hurley KM et al. (2015) Nutrition and maternal, neonatal, and child health. *Semin Perinatol* 39, 361–372.
7. Goudet SM, Bogin BA, Madise NJ et al. (2019) Nutritional interventions for preventing stunting in children (birth to 59 months) living in urban slums in low- and middle-income countries (LMIC). *Cochrane Database Syst Rev* issue 6, CD011695.
8. Veritas Health Innovation (2014) Covidence Systematic Review Software (Internet). www.covidence.org (accessed December 2020).
9. Programme CAS (2018) CASP Qualitative Checklist (online). <https://casp-uk.net/wp-content/uploads/2018/01/CASPQualitative-Checklist-2018.pdf> (accessed December 2020).
10. Long HA, French DP & Brooks JM (2020) Optimising the value of the critical appraisal skills programme (CASP) tool for quality appraisal in qualitative evidence synthesis. *Res Meth Med. Health Sci* 1, 32–42.
11. Noyes J, Booth A, Cargo M et al. (2018) Cochrane qualitative and implementation methods group guidance series-paper 1: introduction. *J Clin Epidemiol* 97, 35–38.
12. Corporation M (2018) Microsoft Excel. <https://www.microsoft.com/en/microsoft-365/excel> (accessed December 2020).
13. QSR International (1999) NVivo Qualitative Data Analysis Software (Software). <https://lumivero.com/products/nvivo/> (accessed December 2020).
14. Braun V & Clarke V (2006) Using thematic analysis in psychology. *Qual Res Psychol* 3, 77–101.
15. Moher D, Liberati A, Tetzlaff J et al. (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med* 151, 264–269, w64.
16. Ahmed T, Choudhury N, Hossain MI et al. (2014) Development and acceptability testing of ready-to-use supplementary food made from locally available food ingredients in Bangladesh. *BMC Pediatr* 14, 164.

17. Adams KP, Okronipa H, Adu-Afarwuah S et al. (2018) Ghanaian parents' perceptions of pre and postnatal nutrient supplements and their effects. *Matern Child Nutr* 14, e12608.
18. Kwon HJ, Ramasamy R & Morgan A (2013) 'How often? How much? Where from?' Knowledge, attitudes, and practices of mothers and health workers to iron supplementation program for children under five in rural Tamil Nadu, South India. *Asia Pac J Public Health* 26, 378–389.
19. Loechl CU, Menon P, Arimond M et al. (2009) Using programme theory to assess the feasibility of delivering micronutrient Sprinkles through a food-assisted maternal and child health and nutrition programme in rural Haiti. *Matern Child Nutr* 5, 33–48.
20. Angdembe MR, Choudhury N, Haque MR et al. (2015) Adherence to multiple micronutrient powder among young children in rural Bangladesh: a cross-sectional study. *BMC Public Health* 15, 440.
21. Creed-Kanashiro H, Bartolini R, Abad M et al. (2016) Promoting multi-micronutrient powders (MNP) in Peru: acceptance by caregivers and role of health personnel. *Matern Child Nutr* 12, 152–163.
22. Cohuet S, Marquer C, Shepherd S et al. (2012) Intrahousehold use and acceptability of Ready-to-UseSupplementary-Foods distributed in Niger between July and December 2010. *Appetite* 59, 698–705.
23. Hess SY, Bado L, Aaron GJ et al. (2011) Acceptability of zincfortified, lipid-based nutrient supplements (LNS) prepared for young children in Burkina Faso. *Matern Child Nutr* 7, 357–367.
24. Phuka J, Ashorn U, Ashorn P et al. (2011) Acceptability of three novel lipid-based nutrient supplements among Malawian infants and their caregivers. *Matern Child Nutr* 7, 368–377.
25. Samuel A, Brouwer ID, Pamungkas NP et al. (2021) Determinants of adherence to micronutrient powder use among young children in Ethiopia. *Matern Child Nutr* 17, e13111.
26. Ashorn U, Alho L, Arimond M et al. (2015) Malawian mothers consider lipid-based nutrient supplements acceptable for children throughout a 1-year intervention, but deviation from user recommendations is common. *J Nutr* 145, 1588–1595.
27. Goyena EA, Barba CVC, Talavera MTM et al. (2019) Acceptance and compliance with micronutrient powder and complementary food blend use by Filipino mothers and their promotion by community workers. *Food Nutr Bull* 40, 202–220.
28. Kajjura RB, Veldman FJ & Kassier SM (2020) Maternal perceptions and barriers experienced during the management of moderately malnourished children in northern Uganda. *Matern Child Nutr* 16, e13022.
29. Roschnik N, Diarra H, Dicko Y et al. (2019) Adherence and acceptability of community-based distribution of micronutrient powders in Southern Mali. *Matern Child Nutr* 15, e12831.
30. Ruel-Bergeron J, Hurley K, Kapadia-Kundu N et al. (2018) Physical and sociocultural facilitators and barriers to access and utilization of a nutrition program in rural Malawi: a qualitative study. *Ecol Food Nutr* 57, 405–424.
31. Creed-Kanashiro H, Wasser HM, Bartolini R et al. (2018) Formative research to explore the acceptability and use of infant food grinders for the promotion of animal source foods and micronutrient powders in rural Peru. *Matern Child Nutr* 14, e12600.
32. Young MF, Girard AW, Mehta R et al. (2018) Acceptability of multiple micronutrient powders and iron syrup in Bihar, India. *Matern Child Nutr* 14, e12572.
33. Athavale PH, Hoeft K, Dalal RM et al. (2020) Qualitative assessment of barriers and facilitators to implementing recommended infant nutrition practices in Mumbai, India. *J Health Popul Nutr* 39, 1–12.
34. Brewer JD, Shinnick J, Román K et al. (2020) Behavioral insights into micronutrient powder use for childhood anemia in Arequipa, Peru. *Global Health: Sci Pract* 8, 721.
35. Jefferds MED, Ogange L, Owuor M et al. (2010) Formative research exploring acceptability, utilization, and promotion in order to develop a micronutrient powder (sprinkles) intervention among Luo families in Western Kenya. *Food Nutr Bull* 31, S179–S185.

36. McLean J, Northrup-Lyons M, Reid RJ et al. (2019) From evidence to national scale: an implementation framework for micronutrient powders in Rwanda. *Matern Child Nutr* 15, e12752.
37. Peltó GH, Tumilowicz A, Schnefke CH et al. (2019) Ethiopian mothers' experiences with micronutrient powders: perspectives from continuing and noncontinuing users. *Matern Child Nutr* 15, e12708.
38. Rothman M, Berti C, Smuts CM et al. (2015) Acceptability of novel small-quantity lipid-based nutrient supplements for complementary feeding in a Peri-Urban South African community. *Food Nutr Bull* 36, 455–466.
39. Shaari N, Mohd Shariff Z, Gan WY et al. (2019) A feasibility study of multiple micronutrient supplement for home fortification of foods among Orang Asli children in Negeri Sembilan, Malaysia. *Malays J Nutr* 25, 69–77.
40. Yeji F, Shibanuma A, Oduro A et al. (2015) Continuum of care in a maternal, newborn and child health program in Ghana: low completion rate and multiple obstacle factors. *PLoS One* 10, e0142849.
41. Kung'u JK, Owolabi O, Essien G et al. (2015) Promotion of zinc tablets with ORS through child health weeks improves caregiver knowledge, attitudes, and practice on treatment of diarrhoea in Nigeria. *J Health Popul Nutr* 33, 9–19.
42. Gunaratna NS, Bosha T, Belayneh D et al. (2016) Women's and children's acceptance of biofortified quality protein maize for complementary feeding in rural Ethiopia. *J Sci Food Agric* 96, 3439–3445.
43. Horton S, Blum LS, Diouf M et al. (2018) Delivering vitamin A supplements to children aged 6–59 months: comparing delivery through campaigns and through routine health services in Senegal. *Curr Dev Nutr* 2, nzy006.
44. Bashir A & Zaman S (2016) Effectiveness and acceptability of ready-to-use therapeutic foods among malnourished children in a tertiary care hospital. *J Ayub Med Coll Abbottabad* 28, 501–505.
45. Korenromp EL, Adeosun O, Adegoke F et al. (2016). Micronutrient powder distribution through maternal, neonatal and child health weeks in Nigeria: process evaluation of feasibility and use. *Public Health Nutr* 19, 1882–1892.
46. Tumilowicz A, Habicht J-P, Mbuya MNN et al. (2019) Bottlenecks and predictors of coverage and adherence outcomes for a micronutrient powder program in Ethiopia. *Matern Child Nutr* 15, e12807.
47. Aaron GJ, Lo NB, Hess SY et al. (2011) Acceptability of complementary foods and breads prepared from zinc-fortified cereal flours among young children and adults in Senegal. *J Food Sci* 76, S56–S62.
48. Ip H, Hyder SMZ, Haseen F et al. (2009) Improved adherence and anaemia cure rates with flexible administration of micronutrient sprinkles: a new public health approach to anaemia control. *Eur J Clin Nutr* 63, 165–172.
49. Kodish SR, Aburto NJ, Nseluke Hambayi M et al. (2017) Patterns and determinants of small-quantity LNS utilization in rural Malawi and Mozambique: considerations for interventions with specialized nutritious foods. *Matern Child Nutr* 13. doi: [10.1111/mcn.12234](https://doi.org/10.1111/mcn.12234).
50. Lang T & Siribaddana S (2012) Clinical trials have gone global: is this a good thing? *PLoS Med* 9, e1001228.
51. Isaakidis P, Swingle GH, Pienaar E et al. (2002) Relation between burden of disease and randomised evidence in sub-Saharan Africa: survey of research. *BMJ (Clin Res Ed)* 324, 702.
52. Sear R & Mace R (2008) Who keeps children alive? A review of the effects of kin on child survival. *Evol Hum Behav* 29, 1–18.
53. Hendricks MK, Roux ML, Fernandes M et al. (2003) Evaluation of a nutrition supplementation programme in the Northern Cape Province of South Africa. *Public Health Nutr* 6, 431–437.
54. Walsh CM, Dannhauser A & Joubert G (2002) The impact of a nutrition education programme on the anthropometric nutritional status of low-income children in South Africa. *Public Health Nutr* 5, 3–9.

55. Flax VL, Phuka J, Cheung YB et al. (2010) Feeding patterns and behaviors during home supplementation of underweight Malawian children with lipid-based nutrient supplements or corn-soy blend. *Appetite* 54, 504–511.
56. Sadler K, Myatt M, Feleke T et al. (2007) A comparison of the programme coverage of two therapeutic feeding interventions implemented in neighbouring districts of Malawi. *Public Health Nutr* 10, 907–913.
57. Galpin L, Thakwalakwa C, Phuka J et al. (2007) Breast milk intake is not reduced more by the introduction of energy dense complementary food than by typical infant porridge. *J Nutr* 137, 1828–1833.
58. Stelle I, Venkatesan S & Edmond K et al. (2020) Acknowledging the gap: a systematic review of micronutrient supplementation in infants under six months of age. *Wellcome Open Res* 5, 238.
59. De-Regil LM, Jefferds ME, Sylvetsky AC et al. (2011) Intermittent iron supplementation for improving nutrition and development in children under 12 years of age. *Cochrane Database Syst Rev* issue 12, CD009085.

5 SETTING

5.1 THE GAMBIA

Data used in Chapters 7 and 8 of this Thesis were collected from a randomised pilot trial conducted in rural Gambia; study methods are found in Chapter 6 – the published clinical trial protocol. Here, the study setting, and context is described.

5.1.1 Demographics

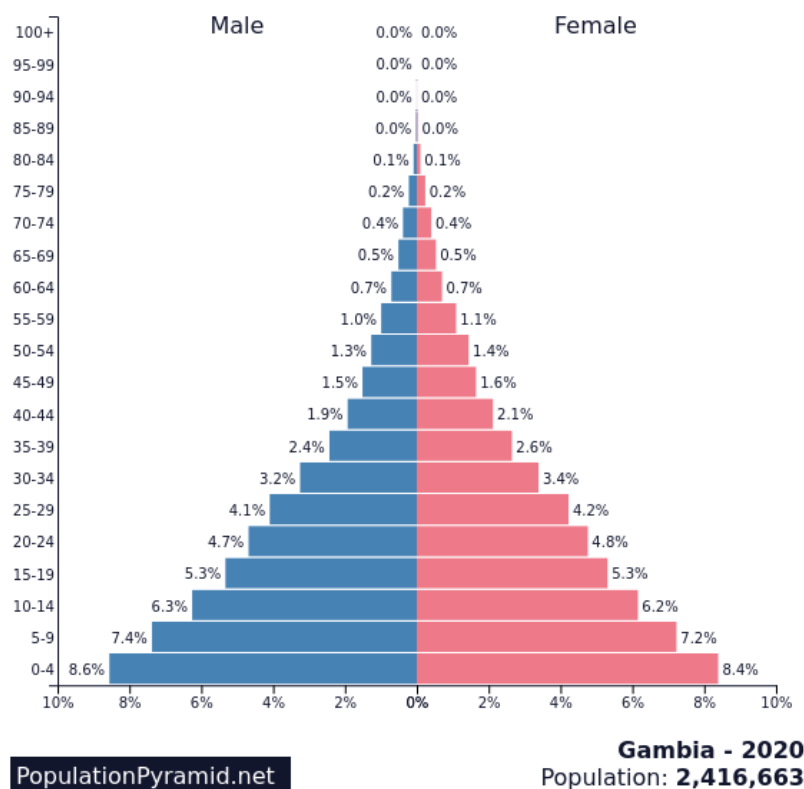
The Gambia is the smallest and most densely populated country in West Africa, with about 2.4 million inhabitants (279). It is a long, narrow country running either side of the river Gambia and bordered on three sides by Senegal, with the fourth, west coast facing the Atlantic Ocean (Figure 4). The majority of The Gambia's inhabitants live in urban and peri-urban neighbourhoods (279). Islam is the predominant religion, polygamy is widely practiced, and families often live in multigenerational compounds within villages (280). About 80% of households in The Gambia are patriarchal (280). Gambians make up 90% of the population, with the remaining 10% being dominated by Senegalese, Guineans, Sierra Leoneans and Nigerians (281). Half of the population is under 18 years of age, with the life expectancy as of 2019 being 62 years of age (Figure 5) (279, 282). Additionally, about 53% of the population is female and 48% male (282). English is the official language, but all communities speak a local dialect, relating to their ethnicity. Mandinka is the most common ethnicity (34%), followed by Fula (22%), and Wolof (12%), with the remaining being a mix of many other ethnicities (282). About 55% of Gambians can read and write, 7.5% can only read, and 37.5% cannot do either (282).

Figure 4. *The Gambia (red) within West Africa.*



From: https://simple.wikipedia.org/wiki/The_Gambia

Figure 5. Population pyramid, The Gambia



From: <https://www.populationpyramid.net/gambia/2020/>

5.1.2 History

The Gambia, a former British colony, gained its independence on the 18th of February, 1965, and became a republic on April 24th, 1970 (283). It was briefly known as Senegambia (1882-89) during a union of Senegal and The Gambia (283). The Gambia is a multiparty republic, with a president democratically elected to five year terms, and no term limit as of 2021 (283). The president appoints a vice president and cabinet members (283). The Gambia's current president is Adama Barrow (2017-present) and he is the third president of The Gambia.

5.1.3 Economy

The Gambia is a poor West African country with an open economy, but limited natural resources (281). The per capita income was estimated at 318 US dollars in 2013 (281). Agriculture contributes 23% and the service sectors (tourism) 62% to Gross Domestic Product (281). The majority of those working are self-employed, 60%, and about 10% of the population is unemployed (279, 282). Most economically active women are involved in services, shops and market sales, and agriculture, with a higher percent of active female workers than males in rural areas (281).

Economic growth in 2019 was robust, at 6%, however with the onset of the Covid-19 pandemic there has been a sharp economic downturn (279). This has been largely due to the disruption of the tourism

industry. This has resulted in the poverty rate increasing from 8.4% in 2019 to 9.2% in 2020 (279). A large source of income in The Gambia comes from remittance payments from relatives living abroad, which has helped prevent the rise in poverty (279). Additionally, The Gambia faced increasing inflation rates in 2021, only further driving household food insecurity (279).

About 80% of Gambians rely on agriculture for their food and cash income (279). The main crops produced are groundnuts, millet and rice (279). Additional contributors to the agricultural income are forestry, fishing, and rearing of livestock (279).

5.1.4 Education

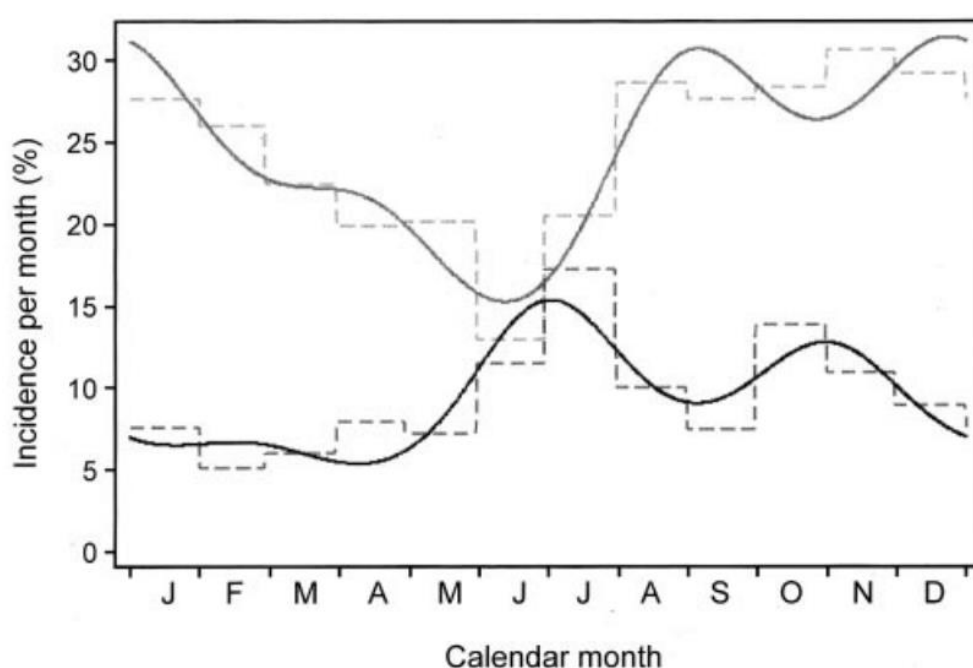
All the following data comes from the most recent Gambia Population and Housing Census, Education Characteristics (2013) (284). Education in The Gambia is divided into three main sectors: early childhood development, basic education (lower and upper), and upper secondary education. Enrolment is from three to 18 years of age, with up to four years in the early childhood development phase (age three to six years), six years of lower basic education (age seven to 12 years), three years of upper basic education (age 13 to 15 years) and three years in upper secondary education (age 16 to 18 years). 45% of Gambians above three years of age have never attended school, with that rate even higher, in rural populations, 53%. Some rural populations have upwards of 70% of people having never attended school. Likewise, women are more likely than men to not attend school; 49% versus 41%, respectively. While rates of enrolment remain relatively equal across most levels of education for the sexes by upper secondary education more men than women are in attendance of school. Women begin childbearing at a young age in The Gambia, especially in more rural regions. Likewise, while rates of attendance are high for both sexes in lower basic education, 52%, by upper basic education and upper secondary, rates drop to 18% and 15%, respectively. An even sharper drop is seen in vocational and tertiary studies (diploma, bachelors, masters and/or doctoral), down to 1% and 1.5%, respectively. The attendance of the early childhood development phase is less common, at only 13% attendance.

5.1.5 Climate

The Gambia has a hot, tropical climate subject to bimodal weather conditions, having a 'wet' (June to October) and 'dry' (November to May) season (285). The average temperature in the coastal region ranges from 17-34° Celsius, whereas inland the average temperature ranges from 15-40° Celsius (285). The seasonal rains determine the farming practices at that time of year, lending to variations in seasonal diets and fluctuating levels of undernutrition (286). These shifts also impact The Gambia's main economic drivers, agriculture and tourism, as staple crops are harvested between September to December (287). Due to the seasonal rains and the short harvest window, the 'wet season' is often

known as the 'hungry season', because food stocks from the previous year's harvest are low (288). The end of the 'dry season' is also the start of the seasonal farm work when the land is cultivated. Women, even those who are pregnant or lactating, provide most of the agricultural work and there is a marked decline in weight at this time of year, between three to six kgs (288). Given this burden of the 'hungry season' followed by laborious work at the end of the 'dry season', weight gain in pregnancy is about one kg/month less during the seasonal farm work (289, 290). Additionally, the number of infants born small for gestational age increases during the 'hungry season' while PTB sees a rise at the start of the seasonal farm work (Figure 6) (291). Analysis of long-term demographic records from The Gambia have shown that infants born during this 'hungry season' have a higher mortality rate in young adulthood (292).

Figure 6. *Monthly trends of small for gestational age and preterm birth, The Gambia.*



Dashed grey lines: small for gestational age. Dashed black lines: preterm infants. Solid lines: Fourier fit analysis for each.

From: Rayco-Solon P, Fulford AJ, Prentice AM. Differential effects of seasonality on preterm birth and intrauterine growth restriction in rural Africans. *Am J Clin Nutr.* 2005 (291).

5.1.6 Maternal health

Chronic energy deficiency is related to heightened morbidity and mortality in women of reproductive age (age 15-49 years). The Gambian 2019-2020 Demographic and Health Survey (DHS) found that 14% of women of reproductive age were underweight (body mass index [BMI] below 18.5), while 36% were overweight or obese (BMI above 25.0) (293). Women living in rural areas are more likely to be thin than those in urban areas, 17% and 14%, respectively, while the proportion of overweight and obese women is higher in urban areas versus rural areas, 40% and 25%, respectively (293).

Anaemia is a concern for women of reproductive age because it leads to increased maternal mortality and poor birth outcomes, as well as decreased work productivity (293). The Gambian 2019-2020 DHS found that 44% of women of reproductive age were anaemic, however, this decreased from 60% in 2013 (293). Additionally, the risk of anaemia increases among Gambian women with increasing number of children ever born, up to 51% with those having had six or more children (293). A rise in anaemia prevalence is seen during pregnancy, up to 55%, as well as in rural versus urban areas, 56% versus 40%, respectively. (293). National anaemia prevention strategies for women during pregnancy include taking IFA tablets, eating iron rich foods, and deworming (293). In the five years preceding The Gambian 2019-2020 DHS, 90% of women reported taking iron during pregnancy, with about 60% taking it for more than 90 days (293). Forty percent of women reported taking deworming medication during their pregnancy, and this rate was higher in rural areas, 48%.

5.1.7 Child health

5.1.7.1 Mortality and morbidity

The trend in under-five mortality in The Gambia showed a national decline of over 90% in the last 55 years, from 341 per 1,000 live births in 1961 to 26 per 1,000 live births in 2017 (282, 294). While under-five mortality in The Gambia drastically decreased, it still remains high in the global context, ranking as the 32nd highest country in the world in 2019 (279, 295). Additionally, 24 deaths per 1,000 births are due to still births and 12% of babies are born preterm (294). Causes of neonatal deaths are due to intrapartum related events (29%), PTB complications (26%), sepsis/tetanus (21%), congenital abnormalities (10%), other conditions (8%), and pneumonia (7%) (294).

5.1.7.2 Vaccination

Childhood vaccinations across The Gambia are provided through the Expanded Programme on Immunisation (EPI) starting from birth, spanning until 18 months of age (293). Routine childhood vaccinations for Gambians are found in Table 8 (293). In 2020, by 12-23 months of age, 85% of Gambians had received all EPI vaccinations and less than 1% had received no vaccinations at all (293).

Table 8. *Expanded Programme on Immunisation vaccine schedule, The Gambia.*

Vaccine	Age administered (months of age)
Tuberculosis (BCG)	0
Hepatitis B (HepB)	0
Oral polio vaccine (OPV)	0, 2, 3, 4, 9
Rotavirus (RV)	2, 3
DPT-HepBHib/pentavalent (diphtheria, tetanus, pertussis, hepatitis B, and <i>Haemophilus influenzae</i> type b)	2, 3, 4
Pneumococcal conjugate vaccine (PCV)	2, 3, 4
Inactivated polio vaccine (IPV)	4
Yellow fever	9

Measles/rubella (MR) (previously given as measles vaccine)	9, 18
Meningitis A	12
DPT booster	12
Polio booster	18

Data from: Gambia Bureau of Statistics G. The Gambia Demographic and Health Survey 2019-20. Banjul, The Gambia: 2021 (293).

5.1.7.3 Growth

The data from this section all derived from The Gambian DHS 2019-20 (293). Eighteen percent of Gambian children under five years of age were stunted (insufficient height for age z-score, HAZ), 5% were wasted (insufficient weight for height z-score, WHZ), 12% were underweight (insufficient weight for age z-score, WAZ), and 2% were overweight, compared to 25%, 12%, 16%, and 3%, respectively, in 2013. Stunting resulted from chronic undernutrition, mostly caused by continued inadequate nutrition and recurrent infections or chronic disease, which impacts intake, absorption, and utilisation of nutrients. Wasting is more acute, often caused by inadequate food intake or a recent illness or infection, causing weight loss. Underweight takes into consideration both acute and chronic undernutrition (wasting and stunting). Stunting was less common in infants from birth to five months of age (9%) and peaked in children from 18-23 months of age (28%). It was more common in rural (20%) than urban areas (16%) and decreased with increasing household wealth. Wasting was more common in children nine to 11 months of age. Overall, boys were more likely to suffer from stunting, wasting, and underweight than girls.

5.1.7.4 Feeding

Early initiation of breastfeeding was seen in over half of the births in The Gambia, with even higher rates in rural areas (294). Almost all children (98%) were breastfed at some point in the first two years of their life, with 36% being breastfed in the first hour, and 93% being breastfed within one day of life. Infants born in rural areas were more likely to begin breastfeeding within one hour of life (45%). Over half of Gambian infants under six months of age (54%) were exclusively breastfed and those living in rural areas were breastfed for a longer duration (293). By six to 23 months of age, only 14% of infants and children were fed a minimum acceptable diet (293). This is a proxy of adequate micronutrient density of foods and meal frequency to ensure appropriate growth and development. This was higher among breastfed infants age six to 23 months (15%) than non-breastfed children (7%) and lower among infants in rural areas (11%) than urban areas (15%) (293). Likewise, decades of Gambian food system data show adequate gross energy supply but micronutrients are suboptimal (296).

5.1.7.5 Anaemia

Anaemia rates in children age six to 59 months of age were high in The Gambia, with 45% of children in this age group being anaemic (293). This is greatly reduced in children of this age group from 73%

in 2013, however in rural areas the anaemia prevalence was still much higher, 60%, and iron supplementation was less common than in urban areas, 9% versus 11%, respectively (293). However, children aged six to 59 months in rural areas were more likely to be given MNPs than those in urban areas, but still at very low levels, 2% versus 1%, respectively (293). A similar trend was seen for rural versus urban areas in children of this age group for vitamin A supplementation, 63% versus 55%, respectively, and deworming medication, 47% versus 36%, respectively (293).

5.1.7.6 Malaria

Despite a decline in malaria rates, it remains a public health issue in The Gambia as it is a leading cause of morbidity and mortality in children under five year of age (293). Malaria has a marked seasonal variation with about 90% of cases occurring in the wet season (293). There is currently a National Malaria Control Programme in The Gambia. In 2020, 79% of households had at least one mosquito net and 77% had at least one insecticide treated net, with an average of 2.8 per household (293). While 36% of households had full coverage of insecticide treated nets (one for every two persons), 23% had none at all (293). The percentage of households with at least one treated net was higher in rural areas (95%) with almost all nets being obtained by the mass distribution campaigns (293). Unfortunately, despite the access to nets increasing since 2013, there was almost no change in the percent of the population sleeping under the nets in 2020, 37% to 38%, respectively (293). Yet, the prevalence of children under five years of age testing positive for malaria decreased from 2% in 2013 to 0.4% in 2020 (293).

5.1.7.7 Respiratory illness

Lower respiratory tract infections and pneumonia are two leading causes of death in children in LMICs, the highest single cause of death outside of the neonatal period (297). In 2015, pneumonia caused 15% of deaths worldwide in children under five, with 90% of deaths occurring in LMICs (297). In The Gambia, acute respiratory infection is a leading cause of childhood morbidity and mortality and treatment is not always sought (293). The 2019-2020 DHS reported a prevalence of 5% of children under five years of age having symptoms of acute respiratory infection, with treatment or advice sought 70% of the time (293)².

5.1.7.8 Diarrhoeal disease

In The Gambia, diarrhoea is frequently caused by consumption of contaminated water and unhygienic food and faecal practices (293). The 2019-2020 DHS reported a prevalence of 20% of children under five years of age having diarrhoea, the highest of all childhood illnesses reported in The Gambia (293).

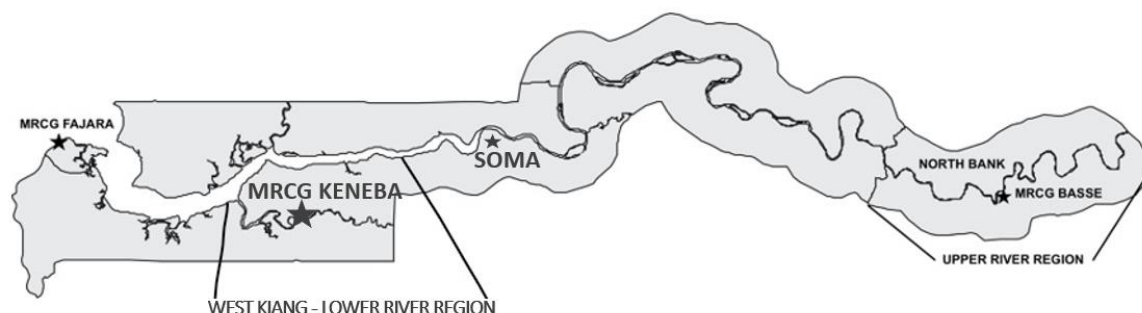
² DHS collected morbidity data as a recall of the previous two weeks.

The prevalence of diarrhoea in Gambian children under five years of age is highest from 12-23 months of age, 31% (293). In sub-Saharan Africa diarrhoeal disease was identified as the second most common cause of under-five mortality, causing approximately 20% of deaths (298, 299). The Global Enteric Multicenter Study (GEMS), done in seven locations in sub-Saharan Africa in the first decade of the millennium, found that moderate to severe diarrhoea (MSD) was fatal if the child could not access care (300). GEMS found that children with MSD had greater growth faltering and an 8.5x increase in the odds of death compared to controls (300).

5.2 STUDY SETTING

The studies presented in Chapters 7 and 8 of this Thesis were based at the MRCG@LSHTM. MRCG@LSHTM has strong community ties thanks to their more than 70 years presence in The Gambia. MRCG@LSHTM has three main study sites: Fajara, Keneba, and Basse, with nutrition studies being based predominantly at the Keneba field station. Keneba is in the district of West Kiang, in the Lower-River Region (LRR) of The Gambia. This study was based at MRCG@LSHTM Keneba as well as the Jarra Soma Regional Hospital in Soma (Figure 7).

Figure 7. Three main MRCG@LSHTM study sites and Soma, The Gambia



5.2.1 MRCG@LSHTM Keneba

The MRCG@LSHTM Keneba field station is a gated research facility containing office space, a research lab, an outpatient clinic and pharmacy, and staff housing. The onsite clinic provides free primary health care to the surrounding village. Being inland, Keneba is subject to extreme weather conditions and large fluctuations during the wet and dry seasons. Keneba is a small, remote village with limited resources and much of the income coming from remittances. The closest town is Soma. The main ethnicity in Keneba is Mandinka with more than $\frac{3}{4}$ the population being Mandinka.

5.2.2 Soma

Study participants were recruited from Jarra West, the communities within and surrounding the town of Soma (Figure 8). Soma is a busy market town where the main east-west road in the country crosses the Trans-Gambia Highway. This places Soma as a major transportation hub for people travelling

through Senegal. Soma has a population of about 10,000 inhabitants, with many small villages surrounding the main town (280). The main ethnicity in Soma is Mandinka. Similarly to the country average, Soma has slightly more females than males in its population, 51.5% and 48.5%, respectively, with the average age of residents being 18 years old (280, 282). The education levels in Soma are slightly lower than the country average, with only 50% of Soma residents able to read and write, and 40% able to do neither (282). The remaining 10% of the population can only read (282). While polygamy is slightly more common in Soma versus the country average, Soma also has the highest proportion of female headed households in The Gambia, 30.5% (280). About 48% of the Soma population has never attended school, slightly higher than The Gambia's average, but lower than other rural areas (284). Likewise, primary school attendance rates are high, roughly 75%, but those making it to lower secondary is lower than The Gambia's average at 28%, and even lower for upper secondary, 18% (284).

Detailed methods of both the quantitative (Chapter 7) and qualitative (Chapter 8) studies are set out in Chapter 6 of this Thesis.

Figure 8. Jarra Soma Regional Hospital and Soma.



6 METHODS

6.1 INTRODUCTION

This Chapter incorporates a published original research protocol paper (Stelle, et al., 2022) from the Iron Babies study. The protocol paper describes both the efficacy supplementation trial to infants (Chapter 7), as well as a qualitative acceptability study with local stakeholders and mothers whose infants are enrolled in Iron Babies (Chapter 8). These studies were based at the MRCG@LSHTM in rural Gambia. For more contextual information about The Gambia, refer to Chapter 5 of this Thesis (Settings).

While most infants are born with an iron endowment intended to maintain iron needs for the first four to six months of life, recent data from The Gambia has shown that despite infants being born with a reasonable endowment, there is a sharp decline in serum iron levels shortly after birth, and this continues until 12 months of age (6). In low resource settings, such as The Gambia, EBF rates are high, and despite breastmilk iron being highly bioavailable, it is in low quantities, so infants born in contexts with high micronutrient deficiencies may be at risk of ID earlier in life than expected. As this is a crucial phase for growth and development, these infants may be compromised before the minimum age (six months) at which the WHO recommends infants in contexts of high ID begin receiving iron supplementation.

This double-blind placebo controlled randomized pilot trial explores if daily iron drops for 98 days starting at six to ten weeks of age impacts serum iron concentrations at the end of the intervention in breastfed Gambian infants. It also explores if iron supplementation at this age will impact the duration of EBF, growth, the gut microbiome, and the frequency of adverse events. The primary outcome (iron status) of the Iron Babies trial are published elsewhere and can be found in Appendix 7 (301). Additionally, a qualitative study assesses the acceptability of the efficacy trial through FGDs and IDIs with local stakeholders and mothers whose infants are enrolled in the trial.

The aim of this Chapter is to describe the methods used for both the efficacy trial and the qualitative component assessing the acceptability. These studies generated the data used in Chapters 7 and 8 of this Thesis. The data analysis in Chapter 7 focuses on the analysis of the secondary outcomes: duration of EBF and growth. One change to the data analysis in the published protocol is that qualitative and quantitative data were analysed separately, rather than together using a mixed-methods approach. Appendix 3 presents additional files associated with this publication.

6.2 AUTHOR ROLES

Conceptualization: Isabella Stelle, Sergio A Silverio, Andrew M Prentice, Sophie E Moore, Carla Cerami

Funding Acquisition: Andrew M Prentice, Sophie E Moore, Carla Cerami

Methodology: All authors

Software: Ebrima Comma

Analysis plan: Isabella Stelle, Mamadou Bah, Sergio A Silverio, Hans Verhoef

Supervision: Sergio A Silverio, Hans Verhoef, Sophie E Moore, Carla Cerami

Original Draft Preparation: Isabella Stelle

Reviewing and Editing: All authors

Approval of final manuscript: All authors

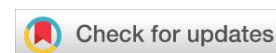
6.3 PUBLISHED PAPER 3: IRON SUPPLEMENTATION OF BREASTFED GAMBIAN INFANTS FROM 6 WEEKS TO 6 MONTHS OF AGE: A RANDOMISED CONTROLLED TRIAL

Stelle I, Bah M, Silverio SA, Verhoef H, Comma E, Prentice AM, Moore SE and Cerami C. Iron supplementation of breastfed Gambian infants from 6 weeks to 6 months of age: protocol for a randomised controlled trial. *Wellcome Open Res.* 2022, 7:16.

<https://doi.org/10.12688/wellcomeopenres.17507.1>

The full paper can be found below and additional files in **Appendix 3** or can be accessed here:

<https://doi.org/10.12688/wellcomeopenres.17507.1>



STUDY PROTOCOL

Iron supplementation of breastfed Gambian infants from 6 weeks to 6 months of age: protocol for a randomised controlled trial [version 1; peer review: 2 approved]

Isabella Stelle¹ , Mamadou Bah^{2,3}, Sergio A. Silverio¹ , Hans Verhoef³, Ebrima Comma², Andrew M. Prentice², Sophie E. Moore^{1,2}, Carla Cerami¹

¹Department of Women and Children's Health, King's College London, 10th Floor North Wing, St. Thomas' Hospital, Westminster Bridge Road, London, SE1 7EH, UK

²Nutrition and Planetary Health Theme, MRC Unit The Gambia @ the London School of Hygiene and Tropical Medicine, PO Box 273, Banjul, The Gambia

³Division of Human Nutrition and Health, Wageningen University, PO Box 17, 6700 AA Wageningen, The Netherlands

v1 First published: 18 Jan 2022, 7:16 <https://doi.org/10.12688/wellcomeopenres.17507.1>

Latest published: 18 Jan 2022, 7:16

<https://doi.org/10.12688/wellcomeopenres.17507.1>

Abstract

Background: A recent analysis showed that plasma iron concentrations decline rapidly from birth in Gambian infants, irrespective of sex or birthweight, to concentrations well below normal expected values for iron-replete children older than two months of age (typically $>10 \mu\text{mol/L}$). The development and function of neural and immune cells may thus be compromised before the minimum age at which children should receive iron supplementation as per World Health Organisation recommendations.

Methods: This study is a two-arm, double-blind, placebo-controlled, randomised superiority trial. Infants will be randomised to receive iron drops (7.5mg/day of iron as ferrous sulphate) or placebo daily for 98 days, to test the impact on serum iron concentrations in healthy, breastfed infants ($n = 100$) aged 6–10 weeks at enrolment. Participants will be visited daily and supplemented by the field team. Daily health and weekly breastfeeding questionnaires will be administered. Anthropometry, and venous blood and faecal samples will be collected at enrolment and after 98 days of supplementation with serum iron as the primary endpoint. Low birthweight (less than 2.5kg at birth) and infants born prematurely (< 37 weeks) will not be excluded. Formula-fed and infants with any illness will be excluded. An additional study exploring maternal stakeholder perspectives of the intervention will be conducted by means of maternal interviews and four focus group discussions with local stakeholders. **Discussion:** Most breast-fed Gambian infants have very low circulating iron levels by five months of age. This study will introduce iron supplements much earlier in infancy than has previously been attempted in a low-income setting with the primary aim of increasing serum iron concentration.

Open Peer Review

Approval Status

	1	2
version 1		
18 Jan 2022	view	view
1. Tor A. Strand , University of Bergen, Bergen, Norway		
2. Vicky Fallon , University of Liverpool, Liverpool, UK		

Any reports and responses or comments on the article can be found at the end of the article.

Trial registration: Clinicaltrials.gov ([NCT04751994](https://clinicaltrials.gov/ct2/show/study/NCT04751994)); 12th February 2021

Keywords iron deficiency, anaemia, supplements, iron, infants, global health, nutrition intervention, breastfeeding

Corresponding author: Isabella Stelle (isabella.stelle@kcl.ac.uk)

Author roles: **Stelle I:** Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Project Administration, Writing – Original Draft Preparation, Writing – Review & Editing; **Bah M:** Data Curation, Formal Analysis, Investigation, Methodology, Project Administration, Writing – Review & Editing; **Silverio SA:** Conceptualization, Data Curation, Methodology, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing; **Verhoef H:** Conceptualization, Data Curation, Formal Analysis, Methodology, Software, Supervision, Writing – Review & Editing; **Comma E:** Data Curation, Methodology, Project Administration, Software, Validation, Writing – Review & Editing; **Prentice AM:** Conceptualization, Funding Acquisition, Investigation, Methodology, Supervision, Writing – Review & Editing; **Moore SE:** Conceptualization, Funding Acquisition, Investigation, Methodology, Supervision, Writing – Review & Editing; **Cerami C:** Conceptualization, Funding Acquisition, Investigation, Methodology, Project Administration, Writing – Review & Editing **Competing interests:** No competing interests were disclosed.

Grant information: This work was supported by funding to from DFID/NIHR/MRC/Wellcome Joint Global Health Trials – Call 9 [MRT003960/1]. This trial is Sponsor is MRCG@LSHTM. SEM is funded by a Wellcome Trust Senior Research Fellowship [220225]. *The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*

Copyright: © 2022 Stelle I *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Stelle I, Bah M, Silverio SA *et al.* Iron supplementation of breastfed Gambian infants from 6 weeks to 6 months of age: protocol for a randomised controlled trial [version 1; peer review: 2 approved] Wellcome Open Research 2022, 7:16 <https://doi.org/10.12688/wellcomeopenres.17507.1>

First published: 18 Jan 2022, 7:16 <https://doi.org/10.12688/wellcomeopenres.17507.1>

Introduction

In a recent study, rural Gambian infants ($n = 317$) had serum iron levels far below the reference range in their first year of life¹. The infants were born with a reasonable endowment of iron despite being born to iron deficient mothers, but following birth there was a rapid deterioration of haemoglobin and ferritin, especially in the fastest growing infants¹. By five months of age about 95% of the infants had low serum iron concentrations (below 5 $\mu\text{mol/L}$ at five months of age) and this continued beyond the first year of life. Figure 1 combines this data with other published and unpublished data from the same setting confirming that serum iron levels fall far below standard ranges for infants. Serum iron is the source of iron for growing tissues such as neuronal and immune cells².

During the first three years of life there is rapid myelination especially of the frontal cortex and basal ganglia (motor control)³. Infants with iron deficiency can have symptoms that are consistent with impaired hippocampal function, reduced myelination, altered temperament and dopamine metabolism. Iron-deficient infants can present with decreased attention and memory⁴ with deficits in visual and auditory systems as well as altered temperament, and social and emotional behaviours⁵. A large body of evidence both from humans and animal models indicates that iron deficiency in early life can alter the brain and nervous system⁶. Since circulating iron is the only iron source for circulating immune cells it may be anticipated that immune function, including appropriate responses to childhood vaccination, might also be compromised⁷.

Therefore, the goal of this pilot study is to introduce iron supplements to breastfed babies much earlier in infancy in a low-resource setting with the aim of improving serum iron concentration. If this trial is successful, we plan to conduct a larger trial that includes behavioural, cognitive and immune assessments.

Study objectives

The primary objective is to measure the impact of daily iron supplements for 98 days starting at 6–10 weeks of age on serum iron concentration at the end of the intervention in breastfed Gambian infants. The secondary objectives are to determine if iron supplementation at this age will affect: (1) the duration of exclusive breastfeeding, (2) growth, (3) pathological processes in the gut, and (4) the frequency of adverse events.

Additionally, a qualitative study will assess the acceptability of the iron supplementation efficacy trial. The supplementary objectives are to:

- Explore mothers' (the main care providers) perspectives on the acceptability of iron supplementation in their young, breast-fed infants through in-depth interviews.
- Explore the acceptability of iron supplementation in young, breast-fed infants through focus group discussions with local stakeholders.
- To integrate the quantitative and qualitative clinical intervention outcomes to explore the acceptability of iron supplementation in young, breast-fed infants.

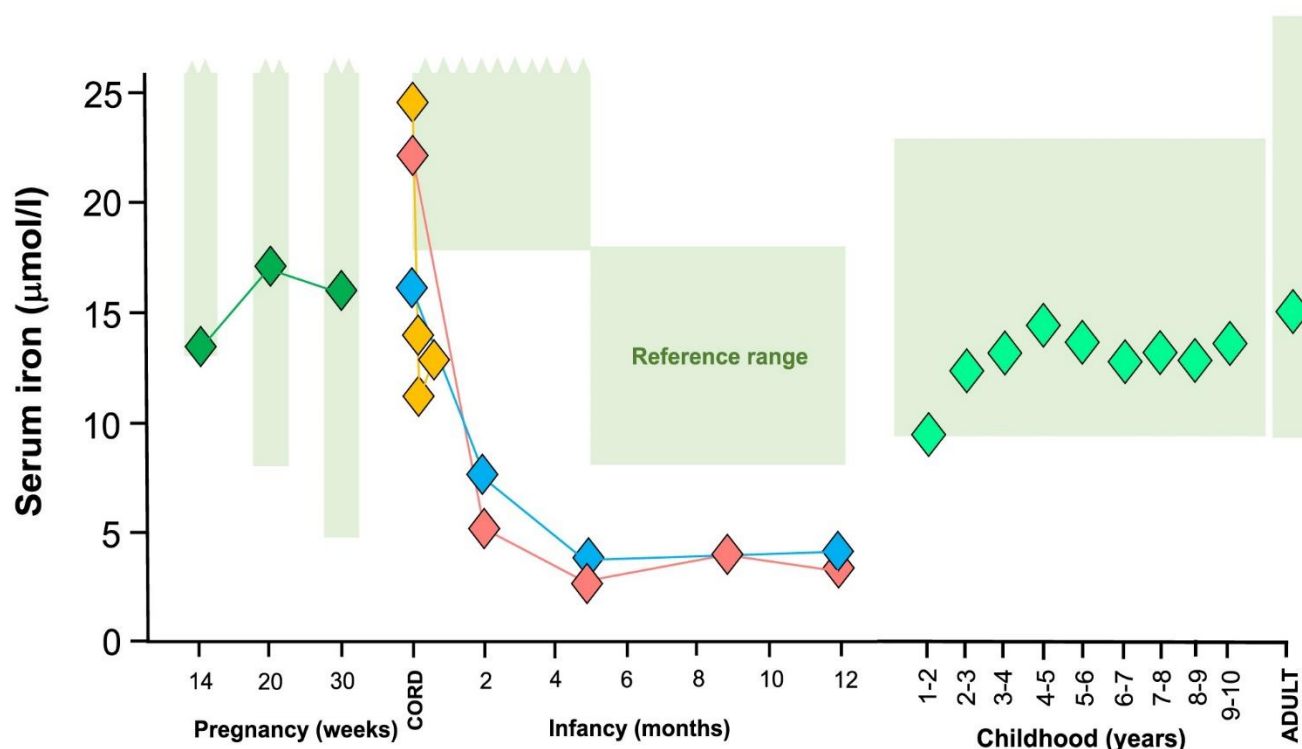


Figure 1. Low serum iron levels in breastfed Gambian infants and pregnant women. Data sources: Reference ranges (shaded green)⁸. Pregnancy (dark green diamonds)⁹. Neonates (yellow diamonds)¹⁰. Infancy (blue diamonds)¹. Infancy (red diamonds; analysis of stored samples¹¹). Childhood and adulthood (light green diamonds); unpublished analysis from the Kenema Biobank¹².

Protocol

Study design and site

This is a two-arm, double-blind, placebo-controlled, individually randomised trial comparing children (n=100) who receive daily supplements with or without iron (7.7mg/day of iron as ferrous sulphate) for a duration of 98 days.

The study is based at the Medical Research Council (MRC) Unit The Gambia (MRCG) at the London School of Hygiene and Tropical Medicine (LSHTM) (MRCG@LSHTM). The study operates out of the MRCG@LSHTM Keneba field station and the Jarra Soma Regional Hospital in the Lower River Region. Study participants will be recruited from the Soma region of The Gambia through community-based clinics and birth attendants.

Participants

Recruitment, informed consent, and screening. Healthy infants from the local communities will be identified at six to ten weeks of age. The eligibility criteria will be explained to their parents/guardians, and they will be invited to join the study by providing consent for their infant. Participants must meet all the inclusion criteria and none of the exclusion criteria to be eligible to participate in the trial.

Informed consent

Individual written consent for the study will be sought. Field workers will be trained to explain the project in full detail to the eligible participants' parents/guardians, covering all aspects of such study as laid out in the 'Participant Information Sheet' (see *Extended data*). Literate parents will be given the Participant Information Sheet whilst illiterate parents will have the full Participant Information Sheet read to them in a language they understand. Illiterate consenting subjects will require an independent literate witness. Any questions that arise will be answered by the field workers and parents/guardians will be given the possibility to obtain further clarifications and explanations by speaking to one of the study investigators. All potentially eligible infants whose parents/guardians provide consent will be invited to a baseline visit.

Eligibility criteria

Inclusion criteria. Inclusion criteria are: infants (male or female) aged between 6–10 weeks. Infants must be exclusively breastfed with plans to continue breastfeeding through six months of age. Parents/guardians (with the participant) must reside in the study site area and be able and willing to adhere to all protocol visits and procedures. Infants must be healthy with no current illness and no chronic health problems. Informed consent must be obtained from the participants parent/guardian. Low birth weight infants (less than 2.5kg at birth) or infants born prematurely (less than 37 weeks) will be included.

Exclusion criteria. Exclusion criteria include formula fed infants or those whose parents/guardians are planning to use commercially available infant formula before six months of age. Infants may not have acute illness, however, once acute illness is resolved, participants may be re-evaluated for eligibility. Infants with fever (a body temperature greater than 37.5°C or mother report of fever)

within three days prior to randomisation will be excluded (once fever/acute illness is resolved, participant may be re-evaluated for eligibility). Infants of parents/guardians unwilling for their infant to avoid the ingestion of supplements or herbal/other traditional medications during the study period will be excluded. Infants with any history of, or evidence for, a chronic clinically significant disorder or disease (including, but not limited to, immunodeficiency, autoimmunity, congenital abnormality, bleeding disorder, and pulmonary, cardiovascular, metabolic, neurologic, renal, or hepatic disease), self-reported history suggestive of meningitis, seizures, Guillain-Barre syndrome, or other neurological disorders, or any condition that in the opinion of the investigator may compromise the safety or well-being of the participant or compromise adherence to protocol procedures, will be excluded.

Baseline

Once parents/guardians of the infant have signed the informed consent document, the infants will be physically examined by a study nurse and, if the infant is considered as generally healthy, their height and weight will be measured. At baseline, a 3 ml blood sample will be collected (0.5 ml in EDTA tube and 2.5 ml in serum tube) to assess the full blood count (including reticulocyte count) and to determine iron status. Parents/guardians of participants will be asked to place nappies inside-out on participants the morning before the baseline or endline visits. Faecal samples will be collected from the nappies by field workers using a spatula. This will be put in universal containers and kept in a cold box. Both blood and stool samples will be transported to the Keneba laboratory for processing and storage into -80°C within 4hrs after collection. Participants who do not pass stool will be provided with labelled universal containers and a cold box to collect faecal samples. The sample will be transported and processed by the field supervisor. This will be stored immediately into a -20°C freezer in Jarra Soma. Frozen stool sample will be transported and stored in Keneba at -80°C. Infants with haemoglobin concentration < 70 g/L will be referred to the regional health centre for treatment according to national guidelines. For safety purposes, all infant's health will be monitored by the field workers for two weeks post supplementation. See [Figure 2](#) for the full study scheme.

Randomisation and blinding

Participants will be individually and randomly allocated on a 1:1 ratio to daily oral supplementation for 98 days with either iron or placebo. A randomization code will be produced in R software (see *Extended data*) and incorporated in REDCap software by the database manager (who is not part of the field team), with support from the trial statistician. The data manager, all investigators, and the entire study team are blinded to the intervention codes. The randomisation module will be protected by the RedCap "user access privilege" function to ensure that the randomization codes are not changed. Each participant will be assigned to a unique intervention code that is only known by the dedicated person independent from the study. This will prevent the unblinding of the entire study should there be a request for unblinding from the Data Safety Monitoring Board (DSMB) in the case of a serious adverse event. A list of identification numbers will be generated in advance by the MRCG@LSHTM data team with the oversight of the database manager. The study identification number (Study ID) will comprise

a three-letter study code (IRN), a three-digit unique number (e.g. 001–140) and a check letter (e.g. A-Z). Every consented participant will be assigned a Study ID (e.g. IRN001X). At the end of the baseline visit, after eligibility has been fully established, the database manager/sub-investigator will allocate each Study ID to one of the six intervention codes (prelabelled bottles of either intervention or placebo) using the randomisation module on Redcap.

We will use permuted block randomisation with a fixed block size of six (three replications of two treatment groups within each block) to produce a list with the Study IDs. To assist in the blinding to treatment, the six numbers (1–6) will be linked to two intervention groups, so that each intervention (iron or placebo) will be replicated 3-fold within a permuted block, and arbitrarily assign the alphabetical letters to intervention groups (e.g., 1, 4, 5 indicate iron; 2, 3 and 6 indicate placebo).

A pharmacist will pre-label and re-bottle the intervention and placebo using the randomisation codes 1–6. Only the pharmacist (an independent person not involved in the trial) will know the interpretation of the randomisation codes (1–6). The codes will be put in a sealed opaque envelope and stored in a locked box. Once a participant's eligibility to participate in the trial has been fully established and confirmed in REDCap, the participant will automatically be allocated to the next available treatment code. This treatment code with the corresponding study ID will be sent to the study nurses via email for labelling and dispensing to the field workers.

Interventions

Supplements will be administered as drops from a liquid formulation containing either iron (7.5mg contained in 0.5mL) or its placebo (sorbitol solution USP 70%, an inactive ingredient in the iron supplements). The intervention and placebo will be issued in identical dark brown plastic bottles. The ferrous sulphate (intervention) and sorbitol (placebo) will be manufactured such that they are identical in terms of taste and appearance thus, allowing robust allocation concealment. Infants will be visited in their homes daily for 98 days by the field workers who will administer the drops. Products will be stored at ambient temperature (15–30°C), away from direct sunlight, at both the MRCG@LSHTM Keneba Field Station main store and Jarra Soma health clinic in a locked room. The procured products have a 12-month shelf-life and expiry dates will be checked by the sub-investigators during study implementation.

Follow up

The final study visit will be on Day 99 after 98 days of supplementation. Procedures for blood and faecal collections and storage and anthropometry will be as per baseline.

An early termination visit may occur because of a participant's voluntary withdrawal, trial team decision, or at the discretion of the DSMB. Apart from the safety evaluations, no other evaluations required for the final study visit will be done.

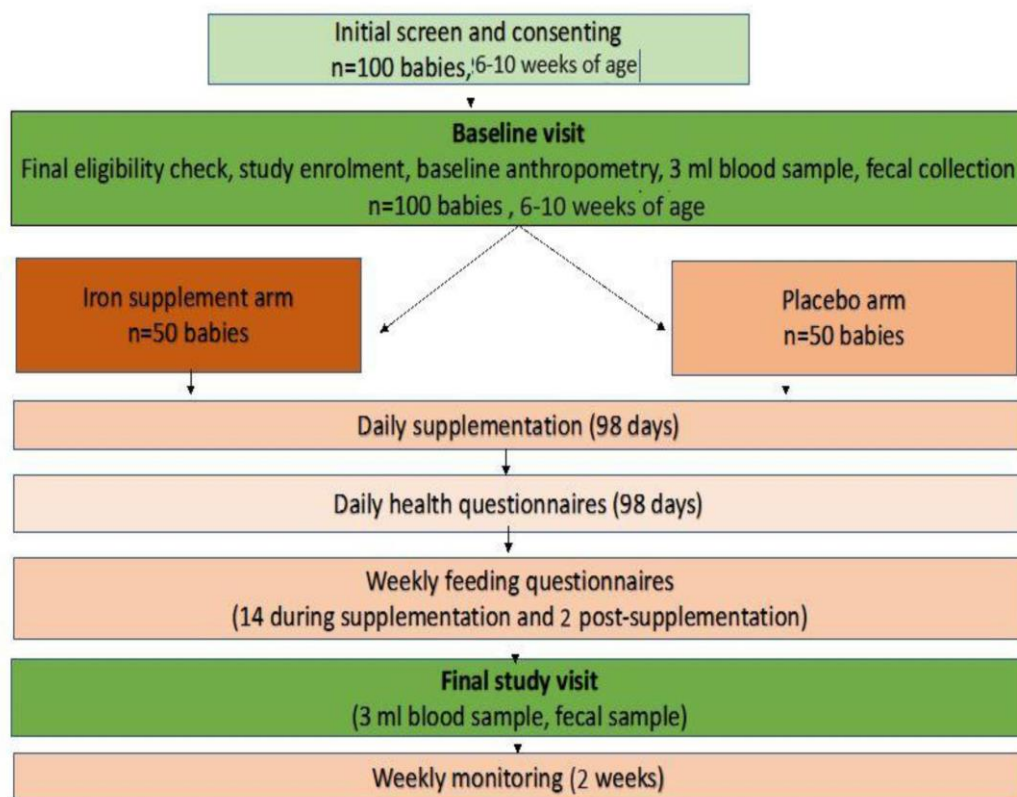


Figure 2. Pilot study schematic

Laboratory evaluations

Blood samples: Blood samples will be taken by venipuncture into EDTA and serum tubes and kept at 2–8°C in cool boxes. Within four hours of collection the samples will be processed by the MRCG@LSHTM laboratory. EDTA samples will be run on an automated haematology analyser (Sysmex XN 1500) to measure haemoglobin concentration, to obtain a full blood count and to assess reticulocytes. Serum samples will be centrifuged and aliquots will be stored at -80°C. Samples will be analysed using a biochemistry analyser (Cobas Integra 400 plus) for iron markers (ferritin, transferrin, transferrin saturation, unbound iron-binding capacity, soluble transferrin receptor, and inflammatory markers [c-reactive protein/alpha-1 acid glycoprotein]). One of the serum aliquots will also be used to measure hepcidin, erythroferrone and erythropoietin concentrations using commercially available ELISAs.

Faecal samples: At baseline and endline a faecal sample will be collected, and iron content measured. Gut pathogens including bacteria, protozoa, intestinal helminths and viruses will be detected using real-time polymerase chain reaction multiplex assays.

Outcomes

Primary: Serum iron concentration on Day 99, after 98 days of iron supplementation and a one day wash-out period. Serum iron will be measured in venous blood collected at trial enrolment and 98 days after initiation of iron supplementation.

The primary endpoint is serum iron concentration at Day 99.

Secondary endpoints relating to iron status (all at Day 99) are:

- a) Haemoglobin concentration.
- b) Percentage of infants with anaemia (defined as: haemoglobin < 110 g/L).
- c) Percentage of infants with iron deficiency anaemia (defined as: haemoglobin < 110 g/L, soluble transferrin receptor /logferritin ratio < 2.0, and ferritin < 12 ug/L or < 30 ug/L in the presence of inflammation).
- d) Hepcidin concentration.
- e) Reticulocyte number.
- f) Erythropoietin concentration.
- g) Erythroferrone concentration.

The secondary objectives are to test whether iron supplementation at this age is safe.

Endpoints related to this objective are:

- a) Maternal reports of illness.
- b) Adverse events.
- c) Serious adverse events.
- d) Changes in gut pathogens and microbiota during the study period.

- e) Markers of environmental enteric dysfunction (EED).

The study will also assess potential impacts on growth, breastfeeding and faecal iron losses:

Endpoints related to these objectives are:

- a) Anthropometric Z-scores relative to the World Health Organization (WHO) Growth Reference.
- b) Duration of exclusive breastfeeding.
- c) Faecal haemoglobin and iron.

Mixed methods study of parent and stakeholder attitudes

The mixed methods approach will explore the effects of the clinical intervention on relevant outcomes while observing and gathering information on implementation. We will use qualitative and quantitative methods to explore the acceptability of the intervention proposed in the main study. Given that the main study is an efficacy trial, it is not possible to assess the full implementation and scale up of such a trial to the real-world setting. Instead, the proposed research will explore the acceptability of iron supplementation in young infants.

Consent for mixed methods study. Prior to conducting focus group discussions and in-depth interviews, topic guides and interview schedules will be piloted among the study team. One investigator and a second female interviewer will pilot the focus group topic guides on members of the study team and adjustments will be made from the feedback. For practice, mock in-depth interviews will then be conducted by the female interviewer on the study team members. Not only will this help adjust the focus group topic guides and in-depth interviews schedules, but likewise, this will act as an opportunity for the interviewers to familiarise themselves with the questions and structure of the focus group discussions and in-depth interviews.

All potential participants for the qualitative data collection will be given written information in English in the form of a participant information leaflet and given adequate time to read it before agreeing to take part. Illiterate participants will have the information leaflet read to them by a trained member of the research team in their preferred language who will also sign to confirm their participation in consent. A copy of the signed informed consent form will be given to the participants.

Data collection, management, and processing

Type of data: Four types of data, quantitative (demographic questionnaire and anthropometric) and qualitative (focus group discussions and in-depth interviews), will be collected.

Quantitative. Data collected from questionnaires on demography, daily review of 24-hour recall of health/morbidity from parents/guardians and weekly feeding questionnaires.

Anthropometric measurements, vital signs, adverse events, and stool questionnaire.

The clinical intervention from which we will gain the quantitative materials will be from the pilot study. We will use data collected from health questionnaires and from study endpoints. This data will be collected on site and stored via a secure data capturing platform (REDCap). This data will be collected by the field workers and will be fully anonymised. The data used for quantitative analysis will include:

- Baseline demographic data
- Laboratory data
- Adherence rates
- Daily health data (adverse events and serious adverse events)
- Weekly infant feeding data

For the quantitative data, no patient-identifiable data will be included. The consent and data collection for this component will fall under the remit of the pilot study.

Qualitative

Focus group discussions with local stakeholders Initial focus group discussions will take place with local stakeholders to further inform the in-depth interviews with infants' mothers. Four focus group discussions will take place with:

- Members of the National Nutrition Agency
- MRCG@LSHTM field workers working on the study
- MRCG@LSHTM field workers not working on the study

- Mothers from surrounding villages whose infants are not enrolled in the study

These focus group discussions will last on average one hour and will be conducted in English or Mandinka (the predominant local language) by an investigator and a female local team member trained in qualitative research. The location will be chosen to add comfort and will most likely include the offices of National Nutrition Agency, the facilities of MRCG@LSHTM, local health clinics, and within local villages. Both MRCG@LSHTM staff from the main study and those not working for the pilot study will be interviewed to ensure a wide range of opinions are sought given that staff hiring at MRCG@LSHTM is done by application and staff applying to work on the main study may have different pre-conceived perceptions of the acceptability and necessity of iron supplementation.

In-depth interviews with infants' mothers

The in-depth interviews will discuss the acceptability of iron supplementation in infancy from the perspective of mothers, but data obtained from the focus group discussions will be used to finalize the interview schedules. Using these interview schedules, 30–60-minute individual in-depth interviews will be conducted with mothers whose infants are enrolled in the trial. Interviews will be conducted, in Mandinka, by a qualitatively trained, female member of the study team, and will be audio recorded. In-depth interviews will employ concurrent and retrospective data collection by interviewing mothers during the last four weeks of the trial and in the two-week follow-up window. The location will be chosen by the interviewee to add comfort and will most likely include the homes of participants and within local villages. Data will be

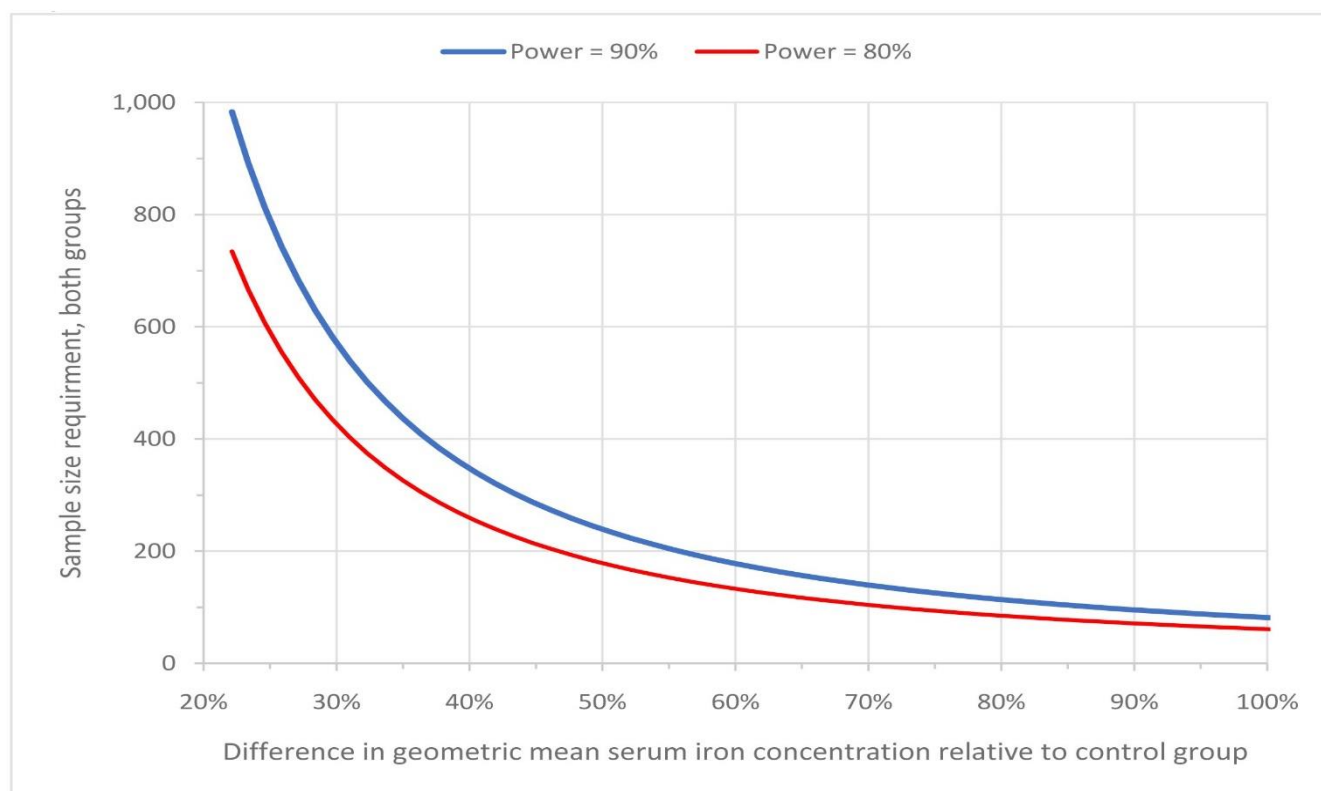


Figure 3. Sample size requirements for various intervention effect sizes.

collected towards the end of the infant's enrolment so that mothers have had mostly the full experience until that point.

All answers obtained and information given through both focus group discussions and in-depth interviews will be anonymised, collated, and analysed. NVivo (2020 release) software will be used to analyse qualitative data.

Data quality and standards

As per MRCG@LSHTM guidelines, principles of good clinical practice will be adhered to, and all training will be documented. The studies are monitored by the MRCG@LSHTM Clinical Trial Unit. To ensure standardisation of processes, MRCG@LSHTM standard operating procedures with respect to trial management, quality assurance, data management, IT security and statistics will be followed.

Sample size

We intend to enrol 100 infants in the trial, which will be sufficient to have 80% probability that, if the true increase in geometric mean serum iron concentration in the iron group increases by 70% or more relative to the control group, the 95% confidence interval for the ratio would exclude zero (with the additional assumptions that the variance of $\log_e$ serum iron concentration [in $\mu\text{mol/L}$] is 0.76 for each group¹³; 10% of infants in the iron group stop taking iron supplements in the course of the intervention period; and the percentage of infants in the control group who stop taking placebo supplements is negligible) (Figure 3). In absolute terms, an increase by 70% corresponds to a change of geometric mean serum iron concentration of $3.0\mu\text{mol/L}$, assuming that this concentration is $4.3\mu\text{mol/L}$ in the placebo group (as observed at Day 0 in Armitage *et al.* 2019¹), so that it will have increased $7.3\mu\text{mol/L}$ in the iron group. We consider intervention effects below $3.0\mu\text{mol/L}$ (absolute group difference in geometric means) to be unimportant from medical and public health points of view.

With regards to the mixed methods study, we aim to recruit roughly five participants to each focus group discussion, in accordance with general recommendations for focus group size in qualitative research (20 participants total)¹⁴. Maximum variation purposive sampling of the mothers whose infants are enrolled in the main study will be used to ensure a wide variety of the dimensions of interest, aiming to identify central themes across this diverse sampler^{15–17}. This will take into consideration various regions, religions, families, ages, and schooling levels. However, all mothers must be Mandinka (the most common local tribe in anticipation that the majority of participants recruited into the main trial will be Mandinka) to provide homogeneity in the translation of the in-depth interviews. An equal number of mothers from the intervention and control arms of the trial will be included, to ensure both perspectives are considered. We anticipate an approximate sample size of 10–20 infants' mothers.

Statistical analysis

Main study. Statistical analyses will be performed using R studio and STATA software. Intervention groups will be described using conventional summary statistics (e.g., means or counts with standard deviations, medians with 25- and 75-centiles). The

primary analysis concerns the effect of intervention on serum iron concentration at Day 99 (i.e., 98 days of intervention with a 1-day wash-out period after administration of the last supplement, assuming that a transient increase in serum iron concentration following iron supplementation will disappear within this period), adjusted for serum iron concentration measured at baseline. Continuous outcomes will be analysed by linear regression, with transformation of the outcome as needed to normalise the distribution of residuals. Analysis will be by modified intention-to-treat, i.e., all infants who were randomised and who received at least one supplemental dose will be included. We also plan to perform per protocol analysis (i.e., to assess the efficacy that can be attained under controlled conditions) to determine the sensitivity of our modified intention-to-treat analysis. To account for missing data, we will use multiple imputations. In secondary analyses, we will adjust for any group imbalances that may occur in prognostic variables measured at baseline. Serum iron concentrations are known to undergo diurnal fluctuation and they are known to be reduced in inflammation¹⁸. Thus, to account for these effects, we will also conduct secondary analyses with adjustment for the time of blood collection and for plasma concentrations of C-reactive protein and alpha-1 acid glycoprotein at the time of blood collection.

Group differences in adverse events will be analysed using negative binomial regression. In this analysis, we will count adverse events as the number of days with adverse events divided by the total number of days that the infant was observed during the intervention period. In the analysis of diarrhoea, we will count an event if the parent/guardian reported three or more fluid stools (as indicated by a WHO stool chart) in the previous 24-hour recall period¹⁹. To produce conservative effect estimates, analyses of adverse events will be per protocol.

Lastly, we will explore to what extent the magnitude of the intervention effect on serum iron concentration depends on iron status at baseline, as indicated by plasma concentrations of ferritin and soluble transferrin receptor (with adjustment for plasma concentrations of c-reactive protein/alpha-1 acid glycoprotein at baseline as inflammation markers).

Mixed methods study. The focus group discussions and in-depth interviews will be recorded and later transcribed and translated (where needed) into English. Translation will occur at MRCG@LSHTM by a trained employee literate in both Mandinka and English.

Data will be analysed with respect to the implementation framework known as *Acceptability of healthcare interventions: an overview of reviews and development of a theoretical framework* (Figure 4)²⁰.

The qualitative data will be uploaded into, managed, and analysed in NVivo (2020 release) using a Template Analysis Method^{21,22}. The template will be based on the six themes in the acceptability framework, with some minor variation to make analytical sense with regards to our data. By this we mean the framework itself is a macro-level conceptual framework and will require semantic changes to some terminology for it to be useful to analyse at the meso-level (i.e., data from focus group discussions with

stakeholders) and at the micro-level (i.e., data from in-depth interviews with women enrolled in the trial). Template analysis follows a methodical six-stage process: 1) Data re-familiarization; 2) Preliminary coding; 3) Thematic organization within the template; 4) Defining the template; 5) Application of template to the full dataset; and 6) Finalization of template definitions. By utilising the acceptability framework as the basis for the analytical template in both the focus group discussions and in-depth interviews datasets-, the qualitative data will be able to be linked coherently across all levels. An analysis in this detail should provide valuable insight about the acceptability of the intervention. We acknowledge that the domains may not all reach saturation, but as these are based on an extensive search of the literature by the framework authors, and have been tested in different fields, we propose to use these as a basis for our analysis. We will later analyse the extent to which the framework was valuable in this evaluation study.

This coding will be conducted by one researcher and 10–20% will be independently coded by another researcher to cross-check appropriateness of coding. Throughout the coding process, these two researchers will meet regularly to discuss the codes and applicability of the framework and subsequently derived templates for analyses. Any disagreements will be resolved by discussion with a third researcher.

The quantitative data will be summarised and analysed using descriptive statistics to further understand the acceptability of the intervention proposed in the main study. This data will likewise be modelled to the implementation acceptability framework. This quantitative data will help further interpret results from the qualitative data, highlighted triangulated key factors which may have varied the level of acceptability.

Data and safety monitoring

Field workers will record any adverse events and ensure the safety of participants. Field workers will visit all infants daily during the supplementation period to administer the drops as described above and to check on the infant's health status. If an infant is found unwell, the study nurse will check on the infant and decide on treatment/referral to the MRCG@LSHTM Keneba health centre in consultation with the Research Clinician. Parents/guardians will complete a weekly questionnaire asking whether they have offered foods or fluids other than breast milk.

The trial will be overseen by a DSMB. Safety data will be reviewed by the DSMB as overall pooled data (open session) and tabulated by coded treatment arm (closed session) at given time intervals. Unblinding of the treatment will only be requested by the DSMB in individual cases if there is a pattern of serious adverse events which may be related to study treatment where the DSMB feels there is 'potential for harm'. Monitoring the rate of recruitment and level of retention of infants and to examine any trends apparent related to

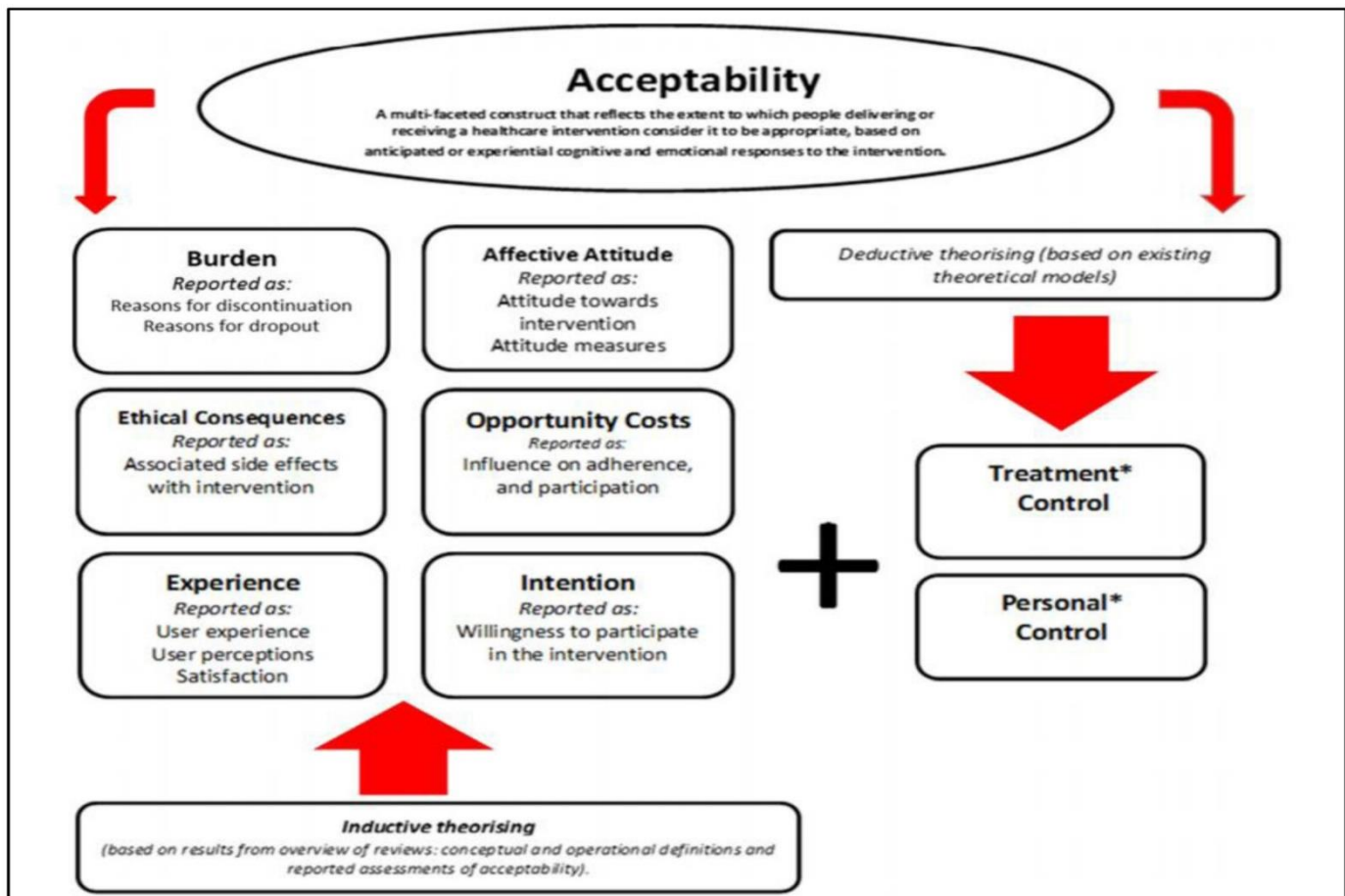


Figure 4. Acceptability of healthcare interventions: an overview of reviews and development of a theoretical framework (v1).

non-retention (e.g., consent withdrawal, loss to follow-up etc.) and to provide advice accordingly.

Recruitment to the trial may be paused upon discretion of the DSMB if any infant has a serious adverse reaction to any of the interventions administered during the trial.

In addition to the DSMB, the Local Safety Monitor will regularly review all adverse events and serious adverse events. This review will focus particularly on adverse events causality and reasons for losses to follow up, raising any concerns or issues that present immediate safety concern with the principal investigators for reporting to the DSMB, while protecting the confidentiality of the trial data and the results of monitoring.

Experimental supplements shall be withheld for seven days following detection of any of the following conditions: confirmed fever (axillary temperature $>37.5^{\circ}\text{C}$) not associated with teething or vaccination³; visually confirmed bloody diarrhea; hospitalization for somatic infection; treatment with antibiotics for any confirmed or suspected somatic infection⁴. Withdrawal of participants

In case the participant decides to withdraw participation or consent during the study, we will not work on the participant's samples without permission, but any information already generated from the samples until the time of withdrawal will be used and samples already collected, for which they have given consent, will also be analysed and data used. The study clinician may also ask for tests for the participant's safety. The principal investigator/sub-investigator will ask about the reason for any withdrawal and follow-up with the participant regarding any unresolved adverse events.

Participant confidentiality

Any identifiable data collected will be stored securely and their confidentiality protected in accordance with the UK General Data Protection Regulation (UK GDPR) and the Data Protection Act (2018). Participant confidentiality, privacy and anonymity will always be ensured. All data will be anonymised, and individuals will not be identifiable.

Future use of stored specimens and data

Aliquots of blood and faecal samples will be kept frozen at -80°C for future analysis. This may include export of samples. We will obtain informed consent from the parents/guardians for this within informed consent. The blood and faecal samples collected during the trial may be used to support other research in the future, and may be shared anonymously with other researchers, for their ethically approved projects. Any future use of data or samples will require approvals from the principal investigator, MRCG@LSHTM Scientific Coordinating Committee, and Ethics Committee.

³ This includes malaria (confirmed fever plus dipstick-test confirmed parasitemia). In case of 48-hour history of fever as reported by the mother (or guardian) that is not confirmed axillary temperature $\leq 37.5^{\circ}\text{C}$, ask the parent/guardian to bring the infant after six hours, and repeat measurement.

Ethics

These studies will be conducted in accordance with the principles set forth in the ICH Harmonised Tripartite Guideline for Good Clinical Practice and the Declaration of Helsinki in its current version whichever affords the greater protection to the participants. The studies have been reviewed by the MRCG@LSHTM Scientific Coordinating Committee, The Gambian Government/MRC Unit The Gambia Joint Ethics Committee and the Ethics Committee at LSHTM (Approval IDs: 19092; 21257). All amendments will go through the same process and the changes will be communicated with investigators, trial participants, trial registries, journals, and regulators.

Funding

Funding for the trial is from DFID/NIHR/MRC/Wellcome Joint Global Health Trials - Call 9. All funds will be used in The Gambia. The study will benefit from the infrastructure already in place at MRCG@LSHTM which are funded by the UK Medical Research Council (MRC) and the Department for International Development (DFID) under the MRC/DFID Concordat agreement.

Dissemination and data access

All key findings from this study will be submitted for publication in peer-reviewed journals. Our planned dissemination avenues include at least three publications in high impact peer reviewed open-access scientific journals with a wide readership, presentation at international conferences (e.g., the Micronutrient Forum) and dissemination of the trial findings to international and Gambian organizations.

Any request for use of study data will go through approval from the Sponsor and the Ethics Committee. All data will be in an anonymous format for external users. Data sharing will agree with the Sponsor policy on research data sharing.

Study status

The pilot study has recruited all 100 infants (November 2021) with the final infant due for follow up in March 2022. The qualitative study focus group discussions have been completed, with the interviews commencing in late December 2021.

Discussion and conclusion

In infants under six months of age, with normal birth weight, exclusive breastfeeding and delayed cord clamping are the only practices recommended to prevent anaemia by the WHO guidelines²³. For low-birth-weight infants, WHO guidelines recommend an external source of iron before six months of age. Maternal iron status is important during pregnancy as iron stores for the first few months of life are passed from mother to infant in utero²⁴. The iron received in utero is used for growth and development in the first six months of life, acting as a buffer for the nutritional needs of new tissue. Randomised trials have shown that

⁴ Suspected ear infections or urinary tract infections in children aged less than six months shall be treated with antibiotics (check with physician) - thus, iron shall be withheld in these cases. If antibiotics are given for localized infections and there is no confirmed fever or a reported 48-hour history of fever, then supplemental iron can be given.

antenatal iron supplementation and delayed cord clamping each increase iron stores in neonates²⁵.

This study aims to determine if early supplementation of infants with iron will improve serum iron concentrations without additional adverse effects and without undermining exclusive breastfeeding. This is the first time such an intervention will be done in this age group for this population. Further, the planned mixed methods research will explore the effects of this clinical intervention on relevant outcomes while observing and gathering information on implementation. The use of qualitative and quantitative methods will shed light on acceptability of iron supplementation in young infants through the perspective of local stakeholders and mothers as the primary caregivers.

Evaluation of risks and benefits

There has been a long-standing controversy regarding iron and infections after the Pemba Trial indicated an increase in malaria-related hospital admissions²⁶. Malaria rates in West and East Kiang are now low. Although there is limited data on young infants in the Gambia, malaria prevalence is low²⁷ and recent data from trials by our group in The Gambia has demonstrated that children in Gambia are not put at risk by receiving iron (personal communication with D.I.A. Pereira; IHAT-GUT)⁹. Nonetheless, all infants in this trial will be visited daily, both to ensure compliance with taking the iron or placebo and to ensure their safety.

A 2020 systematic review of multiple micronutrients and/or iron supplementation in infants under six months of age concluded that infants less than six months of age benefit biochemically from early supplementation with iron, but the impacts on growth, morbidity.

and/or mortality, and neuro-behavioural outcomes remain unclear²⁸. Well-powered randomised controlled trials are required to determine whether routine supplementation with iron or multiple micronutrients containing iron should commence before six months of life in exclusively breastfed infants in low-resource settings²⁸. Furthermore, a large portion of published and registered on-going trials have initiated supplements after six months of age; by which time children living in poor communities in rural Africa and elsewhere are already likely to be iron deficient.

In conclusion, before testing the impact of iron supplementation in early infancy in a larger scale trial it is important to run this proof-

of-concept trial aiming to show a reverse in decline in serum iron without undermining breast feeding in this setting. Additionally, the mixed methods study will be crucial to help understand the acceptability of this approach. If supplementation works well in this group, it will provide the background information for a larger multi-center, multi-country randomised control trial with behavioural and developmental outcomes.

Data availability

Underlying data

No data is associated with this article.

Extended data

Figshare: Extended Data: Iron supplementation of breastfed Gambian infants from 6 weeks to 6 months of age: Protocol for a randomised controlled trial. <https://doi.org/10.6084/m9.figshare.17206319.v1>²⁹.

This project contains the following extended data:

- Participant information sheet and consent form
- R code for randomization Reporting guidelines

Figshare: SPIRIT Checklist for ‘Iron supplementation of breastfed Gambian infants from 6 weeks to 6 months of age: Protocol for a randomised controlled trial’. <https://doi.org/10.6084/m9.figshare.17143019.v2>³⁰.

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

Acknowledgments

We would like to thank the MRCG@LSHTM SCC and The Gambian EC Board for their support and input into this trial. We would like to thank Mortala Ndow for his help as project manager, along with Babucarr Touray and the Clinical Trials Support Office. We would like to thank the DSMB and Trial Steering committee members for their dedication to and expert insight into this project. We acknowledge the National Nutrition Agency and the Jarra Soma Regional Hospital for their support. For the qualitative study we would like to thank our collaborators Prof. Jane Sandall CBE, Dr Phil James and, Dr Cristina Fernandez Turienzo.

References

1. Armitage AE, Agbla SC, Betts M, *et al.*: **Rapid growth is a dominant predictor of hepcidin suppression and declining ferritin in Gambian infants.** *Haematologica*. 2019; **104**(8): 1542–53. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
2. Cusick SE, Georgieff MK, Rao R: **Approaches for Reducing the Risk of EarlyLife Iron Deficiency-Induced Brain Dysfunction in Children.** *Nutrients*. 2018; **10**(2): 227. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
3. Wachs TD, Georgieff M, Cusick S, *et al.*: **Issues in the timing of integrated early interventions: contributions from nutrition, neuroscience, and psychological research.** *Ann N Y Acad Sci*. 2014; **1308**: 89–106. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
4. Burden MJ, Westerlund AJ, Armony-Sivan R, *et al.*: **An event-related potential study of attention and recognition memory in infants with iron-deficiency anemia.** *Pediatrics*. 2007; **120**(2): e336–45. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
5. Georgieff MK: **Iron assessment to protect the developing brain.** *Am J Clin Nutr*. 2017; **106**(Suppl 6): 1588s–93s. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
6. Algarin C, Nelson CA, Peirano P, *et al.*: **Iron-deficiency anemia in infancy and poorer cognitive inhibitory control at age 10 years.** *Dev Med Child Neurol*. 2013; **55**(5): 453–8. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
7. Drakesmith H, Pasricha SR, Cabantchik I, *et al.*: **Vaccine efficacy and iron deficiency: an intertwined pair?** *Lancet Haematol*. 2021; **8**(9): e666–e69. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
8. Johns Hopkins Hospital, Kleinman K, McDaniel L, *et al.*: **The Harriet Lane Handbook.** 22nd ed: Elsevier, 2020. [Reference Source](#)
9. Bah A, Muhammad AK, Wegmuller R, *et al.*: **Hepcidin-guided screen-and-treat interventions against iron-deficiency anaemia in pregnancy: a randomised controlled trial**

- in The Gambia. *Lancet Glob Health*. 2019; 7(11): e1564–e74. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
10. Prentice S, Jallow AT, Sinjanka E, *et al.*: Hepcidin mediates hypoferrremia and reduces the growth potential of bacteria in the immediate post-natal period in human neonates. *Sci Rep*. 2019; 9(1): 16596. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 11. Darboe MK, Thurnham DI, Morgan G, *et al.*: Effectiveness of an early supplementation scheme of high-dose vitamin A versus standard WHO protocol in Gambian mothers and infants: a randomised controlled trial. *Lancet*. 2007; 369(9579): 2088–96. [PubMed Abstract](#) | [Publisher Full Text](#)
 12. Jallow MW, Campino S, Saidykhan A, *et al.*: Common Variants in the *TMPRSS6* Gene Alter Hepcidin but not Plasma Iron in Response to Oral Iron in Healthy Gambian Adults: A Recall-by-Genotype Study. *Curr Dev Nutr*. 2021; 5(3): nzab014. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 13. Cross JH, Jarjou O, Mohammed NI, *et al.*: Early postnatal hypoferrremia in low birthweight and preterm babies: A prospective cohort study in hospital-delivered Gambian neonates. *EBioMedicine*. 2020; 52: 102613. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 14. Carlsen B, Glenton C: What about N? A methodological study of sample size reporting in focus group studies. *BMC Med Res Methodol*. 2011; 11: 26. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 15. Coyne IT: Sampling in qualitative research. Purposeful and theoretical sampling; merging or clear boundaries? *J Adv Nurs*. 1997; 26(3): 623–30. [PubMed Abstract](#) | [Publisher Full Text](#)
 16. Higginbottom GM: Sampling issues in qualitative research. *Nurse Res*. 2004; 12(1): 7–19. [PubMed Abstract](#)
 17. Farrugia B: WASP (Write a Scientific Paper): Sampling in qualitative research. *Early Hum Dev*. 2019; 133: 69–71. [PubMed Abstract](#) | [Publisher Full Text](#)
 18. Nemeth E, Ganz T: Anemia of inflammation. *Hematol Oncol Clin North Am*. 2014; 28(4): 671–81, vi. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 19. Gustin J, Gibb R, Kenneally D, *et al.*: Characterizing Exclusively Breastfed Infant Stool via a Novel Infant Stool Scale. *JPEN J Parenter Enteral Nutr*. 2018; 42 Suppl 1: S5–S11. [PubMed Abstract](#) | [Publisher Full Text](#)
 20. Sekhon M, Cartwright M, Francis JJ: Acceptability of healthcare interventions: an overview of reviews and development of a theoretical framework. *BMC Health Serv Res*. 2017; 17(1): 88. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 21. Brooks J, McCluskey S, Turley E, *et al.*: The Utility of Template Analysis in Qualitative Psychology Research. *Qual Res Psychol*. 2015; 12(2): 202–22. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 22. King N, Brooks JM: *Template analysis for business and management students*. Los Angeles; Thousand Oaks, CA: Sage, 2017. [Reference Source](#)
 23. World Health Organisation: *Nutritional anaemias: tools for effective prevention and control*. In: Development NfHa, ed. Geneva, Switzerland, 2017. [Reference Source](#)
 24. Armitage AE, Moretti D: The Importance of Iron Status for Young Children in Low- and Middle-Income Countries: A Narrative Review. *Pharmaceuticals (Basel)*. 2019; 12(2): 59. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 25. Mwangi MN, Mzembe G, Moya E, *et al.*: Iron deficiency anaemia in sub-Saharan Africa: a review of current evidence and primary care recommendations for high-risk groups. *Lancet Haematol*. 2021; 8(10): e732–e43. [PubMed Abstract](#) | [Publisher Full Text](#)
 26. Sazawal S, Black RE, Ramsan M, *et al.*: Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial. *Lancet*. 2006; 367(9505): 133–43. [PubMed Abstract](#) | [Publisher Full Text](#)
 27. Ceasay SJ, Koivogui L, Nahum A, *et al.*: Malaria Prevalence among Young Infants in Different Transmission Settings, Africa. *Emerg Infect Dis*. 2015; 21(7): 1114–21. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 28. Stelle I, Venkatesan S, Edmond K, *et al.*: Acknowledging the gap: a systematic review of micronutrient supplementation in infants under six months of age [version 1; peer review: 2 approved]. *Wellcome Open Res*. 2020; 5: 238. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Stelle I, Bah M, Silverio SA, *et al.*: Extended Data: Iron supplementation of breastfed Gambian infants from 6 weeks to 6 months of age: Protocol for a randomised controlled trial. *figshare*. Dataset. 2021. <http://www.doi.org/10.6084/m9.figshare.17206319.v1>
- Stelle I, Bah M, Silverio SA, *et al.*: SPIRIT Checklist: Iron supplementation of breastfed Gambian infants from 6 weeks to 6 months of age: Protocol for a randomised controlled trial. *figshare*. Dataset. 2021. <http://www.doi.org/10.6084/m9.figshare.17143019.v2>

Open Peer Review

Current Peer Review Status:



Version 1

Reviewer Report 19 January 2023

<https://doi.org/10.21956/wellcomeopenres.19357.r53882>

© 2023 Fallon V. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Vicky Fallon ✓

Institute of Population Health, University of Liverpool, Liverpool, UK

This is a very well written protocol with excellent clarity on design and methods. The background provides a very clear rationale is well substantiated with recent and appropriate evidence. My only (minor) comment is that it would be useful to use the term 'exclusively breastfed infants' throughout as it was not until the methods section that I understood this to be the case.

Is the rationale for, and objectives of, the study clearly described? Yes

Is the study design appropriate for the research question? Yes

Are sufficient details of the methods provided to allow replication by others?

Yes

Are the datasets clearly presented in a useable and accessible format? Not applicable

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Reproductive psychology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 18 May 2022 <https://doi.org/10.21956/wellcomeopenres.19357.r50161>

© 2022 Strand T. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Tor A. Strand ✓

Innlandet Hospital Trust, Department of Research, Norway and the Centre for International Health, University of Bergen, Bergen, Norway

This is a well-written protocol on daily iron supplementation in young Gambian infants. The primary outcome is serum iron concentration. Other more commonly used markers of iron status such as ferritin, transferrin receptor, or iron-binding capacity will also be measured.

The introduction builds a good case for the research questions, which are reflected in the main objective; to measure the effect of iron supplementation until six months of age on serum iron concentration.

The secondary objectives (effects of iron supplementation on) exclusive breastfeeding, growth, and gut functions lack appropriate justification in the introduction. Moreover, these outcomes are not mentioned when the secondary outcomes are listed later in the manuscript. I suggest that the authors align their description of the secondary outcomes and secondary objectives.

The qualitative objectives make sense as the project's purpose is to prepare for a more extensive iron supplementation trial with clinical outcomes.

Specifics:

Consenting:

◦A significant proportion of the mothers will be illiterate, and a witness will sign. Will there be a registry of the witnesses?

Baseline:

◦Please explain what is meant by a "cold box" or, later in the manuscript, "cool box" (maybe the authors should use the same term).

Randomization and blinding:

◦The description of the randomization process, allocation concealment, and blinding is unclear and unnecessarily lengthy. To me, it seems as if two different procedures are described. Please sharpen this part.

Laboratory parameters:

◦The hematology and biochemical assays are well described. However, the method to measure serum iron (the primary outcome) is not mentioned. Please do.

◦All the biochemical parameters make sense, but I suggest that the authors should offer a couple of sentences to justify these parameters. I assume that hematology (i.e., analyses of whole blood) is quite resource intensive (full blood that needs to be analysed within a relatively short time).

◦Justify and explain the method of analyzing faecal iron.

Outcomes:

◦It makes sense that Hb, anemia, and iron status parameters are included as 2nd outcomes. However, they are not mentioned as 2nd objective.

◦The iron status parameters will be used as categorical variables; I think that it makes more sense to include them as continuous variables, particularly in this study. Please justify the analytical approach (of using categorical outcome variables).

Data collection:

◦Four types of data are mentioned, but laboratory data is not included in this description.

◦Missing data, which variables do you consider to impute? And why?

Is the rationale for, and objectives of, the study clearly described? Yes

Is the study design appropriate for the research question? Yes

Are sufficient details of the methods provided to allow replication by others? Yes

Are the datasets clearly presented in a useable and accessible format? Not applicable

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Epidemiology, clinical trials, micronutrition, child health, development.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

7 RESULTS: DAILY IRON SUPPLEMENTATION DOES NOT IMPACT ON RATES OF EXCLUSIVE BREASTFEEDING OR GROWTH IN YOUNG BREASTFED GAMBIAN INFANTS

7.1 INTRODUCTION

This Chapter is presented as a manuscript for publication, as it has been submitted (but is not yet published). This Chapter is a secondary analysis of the main Iron Babies trial, with the aim to measure the effect of iron supplementation on a) time to cessation of exclusive breastfeeding; b) age at cessation of exclusive breastfeeding; c) linear and ponderal growth; as well as examining the impact of age at baseline and sex on anthropometry. The previously highlighted Iron Babies trial was designed to assess the effect of 14 weeks of iron supplementation on iron status in rural Gambian infants who, at enrolment, were aged six to ten weeks and exclusively breastfed. The main results are published elsewhere (301). The Iron Babies study was powered to measure serum iron as the main trial outcome.

For normal birthweight infants (>2.5 kilograms) under six months of age, exclusive breastfeeding and delayed cord clamping are the only practices recommended by the WHO to prevent ID and anaemia. However, evidence suggests that some young, exclusively breastfed infants may be at risk of ID (6). In terms of feeding practices, the WHO recommends EBF until six months of age, and continued breastfeeding until two years of age or beyond (8). Iron supplementation is commonly noted to cause adverse gastrointestinal side effects in children such as diarrhoea, nausea, constipation, and vomiting, therefore limiting adherence in supplementation trials (129). Parallel to possible effects on feeding, more rapid growth has been correlated with a faster decline in infant iron status (6, 102-104), although evidence around the impacts of supplementation on growth is unclear. There is concern that mothers may be confused by seemingly contradictory messages that a) they should administer oral iron supplements; b) they should not administer any foods or liquids (to adhere to EBF). We expected that daily iron supplementation for 98 days would not adversely impact on infant feeding practices or growth in breastfed Gambian infants.

This study was based at the MRCG@LSHTM in rural Gambia. For more contextual information about The Gambia, refer to Chapter 5 of this Thesis (Settings) and for more information on the Iron Babies study refer to Chapter 6 of this Thesis (Methods, published Iron Babies protocol paper).

Statistical analyses were performed using STATA (version 17). Study groups were described using conventional summary statistics, z-score were calculated from growth data collected both at baseline

and endline. Kaplan-Meier analysis (with log-rank tests) and Cox regression analysis were used to measure the effect of intervention (i.e., iron supplementation) on the time from randomisation until cessation of EBF and age of cessation of EBF. Linear regression analysis was used to compare groups regarding the change in z-scores, and analysis of variance (ANOVA) to measure group differences in the proportion of infants being underweight (WAZ <-2 SD), stunted (LAZ <-2 SD), and wasted (WLZ <-2 SD) at endline (302). As well as explore the impacts of sex and age at baseline, and mode of feeding and length of time of EBF, (adjusted for baseline variables selected a priori: infant sex and age) on infant growth outcomes.

7.2 AUTHOR ROLES

Conceptualization & funding acquisition: Carla Cerami

Methodology: Carla Cerami, Sophie E More

Analysis plan: Isabella Stelle, Hans Verhoef, Sophie E Moore

Trial conduct and management: Isabella Stelle, Mamadou Bah

Paper specific data management and main analysis: Isabella Stelle

Supervision: Sophie E Moore

Original Draft Preparation: Isabella Stelle

Reviewing and Editing: All authors

Approval of final manuscript: All authors

7.3 ABSTRACT:

Background: In a randomised placebo-controlled trial among exclusively breastfed rural Gambian infants aged 6-10 weeks at randomisation (n=101), daily iron supplementation for 14 weeks led to improved iron status. As adverse consequences have been observed in some trials of early iron interventions, this secondary analysis explores the impact of iron supplementation on duration of exclusive breastfeeding and growth.

Methods: Breastfed infants from six to ten weeks of age were supplemented for 14 weeks with either daily iron or placebo (n=101). Infant feeding practices were assessed weekly through questionnaires. Survival analysis was used to measure the effect of iron supplementation on age at and time to cessation of exclusive breastfeeding. Infant size was measured at baseline (Day 1) and endline (Day 99), and z-score computed using World Health Organization growth reference standards. Groups were compared regarding the change in these z-scores between baseline and endline.

Results: At endline, 31% (n=31/101) of infants were exclusively breastfed. There was no evidence that iron supplementation reduced the time to cessation of exclusive breastfeeding (median: 70 days [range: 7-105 days], iron: 67 days; placebo 71 days; Kaplan-Meier, log-rank test: $p=0.15$; Cox regression, crude hazard ratio: 1.42, 95% CI: 0.86-2.34, $p=0.17$; hazard ratio adjusting for infant age and sex: 1.40, 95% CI: 0.85-2.31, $p=0.19$) or age at cessation of exclusive breastfeeding (median time: 18 weeks [range:1-24 weeks], iron: 16 weeks; placebo 18 weeks; Kaplan-Meier, log-rank test: $p=0.13$; crude hazard ratio=1.47, 95% CI: 0.89, 2.43; $p=0.13$; hazard ratio adjusting for infant age and sex=1.44, 95% CI: 0.87, 2.39 $p=0.16$) There was no evidence that iron supplementation affected infant weight ($p=0.79$) or length ($p=0.64$) at endline or change in z-scores during the intervention period for weight-for age ($p=0.99$), length-for-age ($p=0.70$) and weight-for length ($p=0.89$). There was no evidence that duration of exclusive breastfeeding impacted endline anthropometric outcomes.

Conclusion: Although requiring replication in larger trials, these findings suggest that giving iron to exclusively breastfed infants, in addition to having positive effects on iron status, is safe and does not adversely impact on infant feeding patterns or growth.

Keywords: iron, supplementation, infants, growth, breastfeeding, global health, nutrition, Gambia

7.4 BACKGROUND

Iron stores received in utero to support exclusively breastfed young infants are vital for growth and development and act as a buffer for the nutritional needs of new tissue (4). Infants born to women with iron deficiency anaemia may start life with sub-optimal iron stores and if exclusively breastfed, this may put infants at earlier risk of iron deficiency and anaemia. Therefore, approaches to improve iron status to prevent anaemia during this critical phase of development are needed. According to World Health Organisation (WHO) guidelines, in normal birthweight infants (>2.5 kg) under six months of age, exclusive breastfeeding and delayed cord clamping are the only practices recommended to prevent anaemia (8). However, in a recent study in rural Gambia, where the prevalence of exclusive breastfeeding is high and delayed cord clamping is recommended, infants frequently had serum iron levels far below the 95% reference range in their first year of life (6). The infants were born with a reasonable endowment of iron despite some being born to iron deficient mothers, but following birth there was a rapid decline in concentrations of haemoglobin and ferritin (6). By five months of age about 95% of the infants had low serum iron concentrations (<5µmol/L) and this continued beyond the first year of life (6). These data highlight a potential risk of iron deficiency during this critical phase of development and support the need for novel interventions.

In response, a recent trial ('Iron Babies') in rural Gambia comparing daily supplements of 7.5mg/day iron as ferrous sulphate or placebo as sorbitol solution USP 70% for a duration of 98 days starting at six to ten weeks of age was conducted (301). The primary objective was to measure the impact of daily iron supplements on serum iron concentration at the end of the intervention. This trial showed that supplementary iron led to an increased serum iron concentration (crude difference in means: 2.5 µmol/L; 95%CI: 0.6 to 4.3µmol/L, p=0.009) and meaningfully improved additional markers of iron and haematological status, with no evidence of differences in adverse events or deaths reported between trial arms (301). This was the first time such an intervention was undertaken in this age group for this population and adds to the very limited number of previously published trials of iron supplementation in exclusively breastfed infants particularly in low-income setting (105).

The WHO recommends exclusive breastfeeding until six months of age, and continued breastfeeding until two years of age or beyond (8). In The Gambia, the prevalence of exclusive breastfeeding at six months of age have previously been reported at 32% (7). In a nationally representative survey, over half of Gambian infants under six months of age (54%) were exclusively breastfed and those living in rural areas were breastfed for a longer duration (293). Iron supplementation is commonly noted to cause adverse gastrointestinal side effects in children such as diarrhoea, nausea, constipation, and vomiting, therefore limiting adherence in supplementation trials (129). A systematic review found side

effects to be one of the leading barriers to acceptability in nutrition intervention trials in infants under 24 months of age (132). There is concern that mothers may be confused by seemingly contradictory messages that a) they should administer oral iron supplements; b) they should not administer any foods or liquids (to adhere to exclusive breastfeeding).

Parallel to possible effects of iron on exclusive breastfeeding, more rapid growth has been correlated with a faster decline in infant iron status (6, 102-104), although evidence around the impacts of supplementation on growth is unclear. Studies in infant populations over six months of age with a high prevalence of iron deficiency anaemia have shown a beneficial impact of iron on growth (106-108). Other studies found no effect, with some even reporting adverse effects (107, 109-111). However, these impacts were only seen in iron replete children (110, 111). A 2020 systematic review of multiple micronutrients and/or iron supplementation in infants under six months of age concluded that infants less than six months of age benefit biochemically from early supplementation with iron, but the impacts on growth, morbidity and/or mortality, and neuro-behavioural outcomes remain unclear (105). However, this review was limited by the small number of studies looking at supplementation of infants under six months of age.

The previously highlighted Iron Babies trial was designed to assess the effect of 14 weeks of iron supplementation on iron status in rural Gambian infants who, at enrolment, were aged six to ten weeks and exclusively breastfed. The main results are published elsewhere (301). The present study presents a secondary analysis of this trial, with the aim to measure the effect of iron supplementation on a) time to cessation of exclusive breastfeeding; b) age at cessation of exclusive breastfeeding; c) linear and ponderal growth; as well as examining the impact of age at baseline and sex on anthropometry. We expected that daily iron supplementation for 98 days would not adversely impact on infant feeding practices or growth in breastfed Gambian infants.

7.5 METHODS

Full details of the *Iron Babies* trial can be found in the published trial protocol (14) with additional details on the trial results in the manuscript on the primary trial outcome (301). A summary of the key methods for this analysis are provided below.

7.5.1 Study setting

Data collection for this study took place in Jarra West in the Lower River Region of The Gambia, West Africa, and was conducted by the Medical Research Council Unit The Gambia at the London School of Hygiene and Tropical Medicine (MRCG@LSHTM).

7.5.2 Study design and field implementation

Participants were identified at vaccination clinics as well as in the communities in Jarra West by the field staff. After written informed consent was obtained, infants were screened for eligibility at the Jarra Soma Health Centre. Inclusion criteria were healthy infants aged between six to ten weeks, being exclusively breastfed with mothers reporting that they planned to continue breastfeeding through to six months of age. Birthweight, collected to the closest 100 g, and gestational age at birth were recorded from birth records. Low birth weight infants (less than 2.5 kg) or infants born prematurely (less than 37 weeks) were included. Further inclusion and exclusion criteria can be found in the published trial protocol (14).

Infants were individually and randomly allocated on a 1:1 ratio to daily oral supplementation for 98 days with either iron or placebo. Supplements were administered as drops from a liquid formulation containing either iron (7.5mg as ferrous sulphate contained in 0.5mL of a sorbitol solution USP 70%,) or its placebo (0.5ml of sorbitol solution USP 70%). Infants were visited daily for supplement administration in their homes for the duration of the trial by field assistants.

Daily questionnaires assessing infant health were administered throughout the study duration by field staff to mothers. Weekly questionnaires were administered to collect data on breastfeeding practices. Exclusive breastfeeding was defined as per WHO recommendations as no substance outside of breastmilk, besides drops or syrups containing vitamins, minerals, supplements or medicine, given during the infants first six months of life (24). Data on adverse events were collected up to two weeks post supplementation. Infant length and weight were measured at baseline (Day 1) and at endline (Day 99). Recumbent length was measured using a Seca 417 length board to the nearest 0.1 centimetre (cm). Weight was measured using a Seca 336 digital weighing scale to the nearest 10 g, with the infant in minimal clothing.

7.5.3 Consent and ethics

Full written informed consent was provided by all study participants. The study was reviewed and approved by the MRCG@LSHTM Scientific Coordinating Committee, The Gambian Government/MRC Unit The Gambia Joint Ethics Committee, the Ethics Committee at LSHTM, and King's College London (Approval IDs: 19092; 21257; LRS/DP-21/22-17625). This trial was registered with clinicaltrials.gov (NCT04751994).

7.5.4 Data processing and analysis

Data were downloaded from the REDCap database as Microsoft Excel, where they were collated, cleaned, and prepared for analysis. The process of cleaning involved removal of any unrealistic outliers (such as unfeasible anthropometric measurements). At each weekly survey visit, infants were coded as exclusively breastfed or not. Statistical analyses were performed using STATA (version 17).

Study groups were described using conventional summary statistics (e.g., means or counts with standard deviations [SD]). z-scores for weight-for-age (WAZ), length-for-age (LAZ), weight-for-length (WLZ) were generated to indicate how far a child's measurement deviates from the median value of the WHO reference population of breastfed children with the same age and sex (303). This was done for growth data collected both at baseline and endline, using the STATA 'zscore06' command and cross-checked using WHO Anthro software (304).

Kaplan-Meier analysis (with log-rank tests) and Cox regression analysis were used to measure the effect of intervention (i.e., iron supplementation) on the time from randomisation until cessation of exclusive breastfeeding, defined as the first recorded instance of introduction of a non-breastmilk feed. For infants who did not experience this outcome (i.e., they were exclusively breastfed by the end of the intervention period) or who were lost to follow-up, observation time was censored at the time at which they were last observed. A secondary survival analysis was used to measure the effect of intervention on age of cessation of exclusive breastfeeding, defined as age in weeks of the first recorded instance of introduction of a non-breastmilk feed.

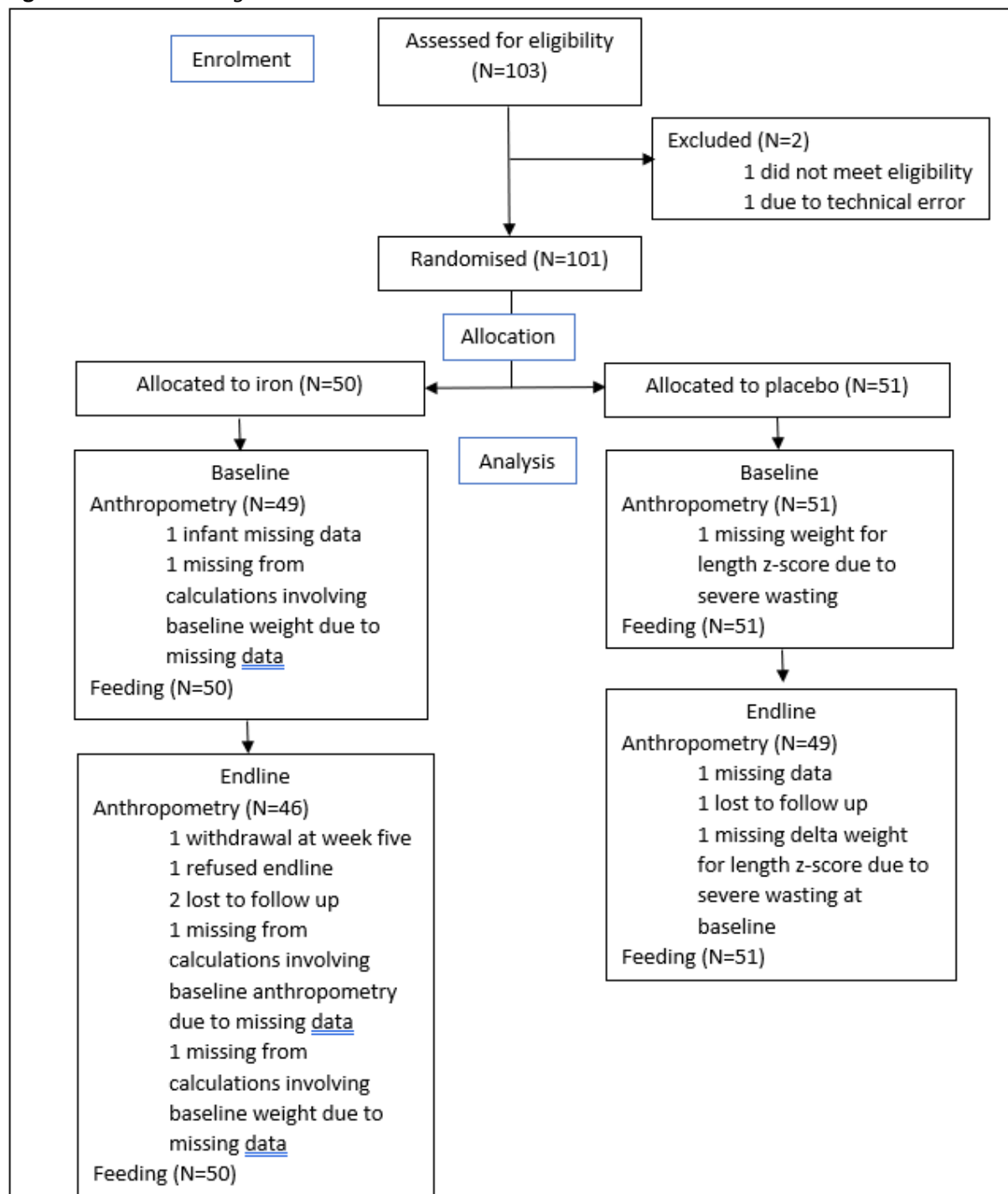
Linear regression analysis was used to compare groups regarding the change in z-scores, and analysis of variance (ANOVA) to measure group differences in the proportion of infants being underweight (WAZ <-2 SD), stunted (LAZ <-2 SD), and wasted (WLZ <-2 SD) at endline (302). As well as explore the impacts of sex and age at baseline, and mode of feeding and length of time of exclusive breastfeeding, (adjusted for baseline variables selected a priori: infant sex and age) on infant growth outcomes.

The STATA code for these analyses can be found in Appendix A.

7.6 RESULTS

Of 103 infants who were screened for eligibility, 101 were randomised to receive either iron or placebo. Participant flow through the trial is described in Figure 1. Because few data were missing, we used complete-case analysis to handle incomplete data. This procedure produces unbiased estimates if data are missing completely at random. For seven infants, data on breastfeeding status were missing at baseline; of those, five infants also had missing anthropometry indices missing at baseline.

Figure 1. CONSORT diagram.



Baseline infant and maternal characteristics are summarised in Table 1. On average, infants were aged 7.5 weeks (range: 6.1-10.0 weeks); the prevalence of being underweight, stunted and wasted was 12.1%, 9.0% and 5.1%, respectively. Mean maternal age was 26.4 years and median parity was 4 (IQR: 2,5) children. A little over half (52%) of the mothers had not completed primary school. The mean (SD) length of enrolment was 99.0 (2.45) days (range: 78.0-105 days, due to late collection of endline data). One infant moved away from the study area after 78 days of enrolment, so the infant's endline data was collected after 78 days. Study groups were similar regarding birthweight (mean [SD]: 2.99 [0.51] vs 2.97 [0.64] kgs), baseline age (7.5 [1.0] vs 7.6 [1.1] weeks), weight (4.73 [0.72] vs 4.75 [0.81] kgs), and length (55.8 [2.7] vs 55.7 [3.6] cms) between the iron and placebo groups.

Table 1. Baseline characteristics for infants and their mothers, by study group.

	Entire cohort	Iron group	Placebo group
n	101	50	51
Sex			
Male	45.5% [46]	48.0% [24]	43.1% [22]
Female	54.5% [55]	52.0% [26]	56.9% [29]
Tribe			
Mandinka	73% [74]	74% [37]	73% [37]
Fula	23% [23]	20% [10]	25% [13]
Wolof	3.0% [3]	4.0% [2]	2.0% [1]
Serer	1.0% [1]	2.0% [1]	0.0% [0]
Birthweight (in kg) (SD)	2.97 (0.58) [79]	2.99 (0.51) [36]	2.97 (0.64) [43]
Infant age (in weeks) (SD)	7.5 (1.1) [101]	7.5 (1.0) [50]	7.6 (1.1) [51]
Infant weight (in kg) (SD)	4.74 (0.76) [99]	4.73 (0.72) [48]	4.75 (0.81) [51]
Infant length (in cm) (SD)	55.7 (3.2) [100]	55.8 (2.7) [49]	55.7 (3.6) [51]
Weight for age z-score (WAZ) (SD)	-0.60 (1.18) [99]	-0.59 (1.11) [48]	-0.61 (1.25) [51]
Length for age z-score (LAZ) (SD)	-0.53 (1.38) [100]	-0.52 (1.13) [49]	-0.54 (1.60) [51]
Weight for length z-score (WLZ) (SD)	-0.09 (1.12) [98]	-0.07 (1.14) [48]	-0.11 (1.11) [50]
Underweight[‡]	12.1%, [12/99]	8.3% [4/48]	15.7% [8/51]
Stunted[‡]	9.0% [9/100]	6.1% [3/49]	11.8% [6/51]
Wasted[‡]	5.1% [5/98]	2.1% [1/48]	8.0% [4/50]
Maternal age (years)	26.4 (6.1)	24.8 (5.5)	28.0 (6.2)
Maternal parity*	4 (2, 5)	4 (2, 5)	4 (2, 5)
Maternal educational level completed			
Less than primary	52% [53]	48% [24]	57% [29]
Primary	16% [16]	16% [8]	16% [8]
Secondary	27% [27]	30% [15]	23% [12]
Higher	5.0% [5]	6.0% [3]	4.0% [2]

Values indicate percentage [n] or mean (standard deviation) [n]. * Maternal parity is expressed as median (inner quartile range) Z-scores were calculated using WHO Anthro (304). [‡] WHO definition of underweight (WAZ <-2 SD), stunting (LAZ <-2 SD), and wasting (WLZ <-2 SD) (302)

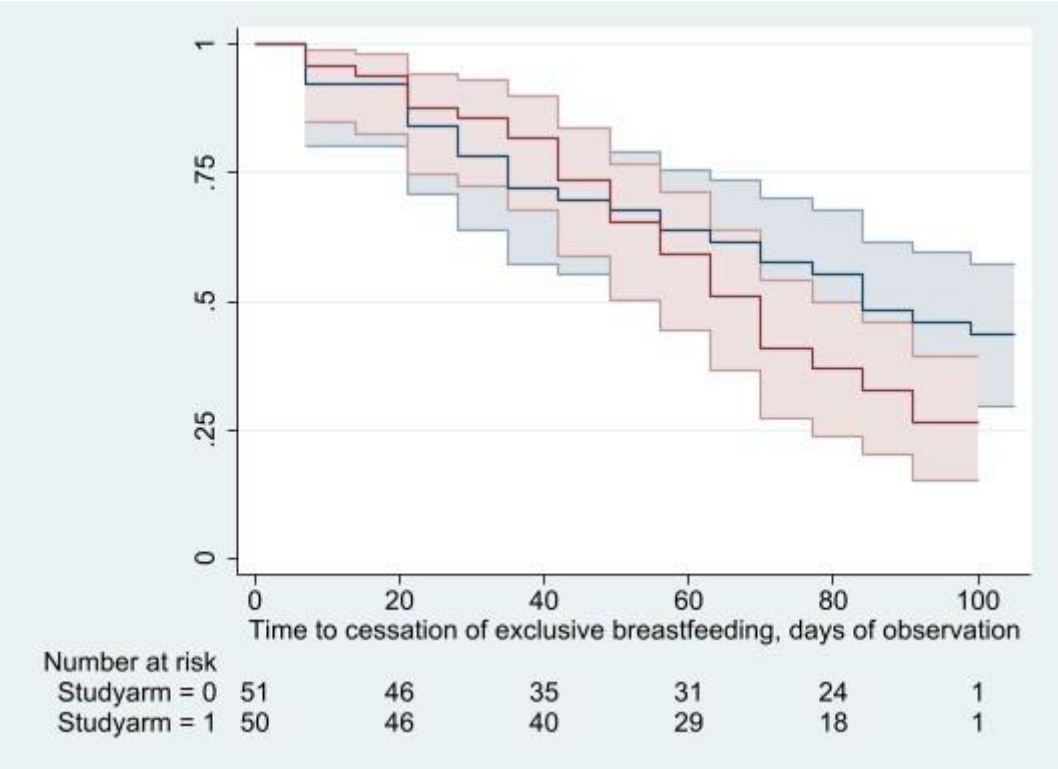
7.6.1 Infant feeding practices

Of the 101 infants who were randomised, 31% (n=31) were still being exclusively breastfed at endline (n=13 iron, n=18 placebo), while 62% (N=63) were partially breastfed (n=36 iron, n=27 placebo), and 7% (n=7) infants were missing endline data (n=1 iron, n=6 placebo). There was no difference in rates of exclusive breastfeeding at endline by treatment group (iron: 26.5%, placebo: 35.4%; p=0.17).

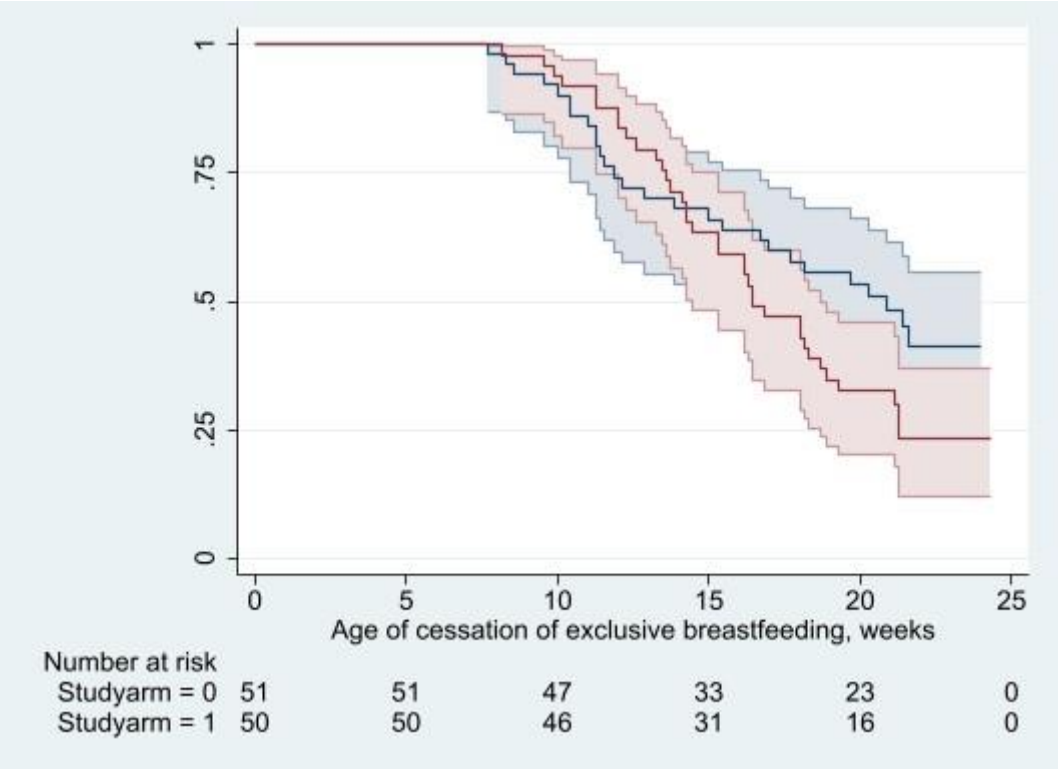
Of 63 infants for whom non-breastmilk feeds were introduced throughout the study, 21 were only given water on top of breastmilk. From the weekly feeding questionnaires across the 14 weeks study period, 535 instances were reported when non-breastmilk feeds were given. When non-breastmilk feeds were given, water was the most common (76% of instances), followed by local foods (7%), powdered milk (6%), local drinks and commercial formula (4% each), powdered potato (2%), and other substances (1%).

There was no evidence that iron supplementation reduced the time to cessation of exclusive breastfeeding (median: 70 days [range: 7-105 days], iron: 67 days; placebo 71 days; Kaplan-Meier, log-rank test: p=0.15; Cox regression, crude hazard ratio: 1.42, 95% CI: 0.86-2.34, p=0.17; hazard ratio adjusting for infant age and sex: 1.40, 95% CI: 0.85-2.31, p=0.19) (Figure 3) or age at cessation of exclusive breastfeeding (median time: 18 weeks [range:1-24 weeks], iron: 16 weeks; placebo 18 weeks; Kaplan-Meier, log-rank test: p=0.13; crude hazard ratio=1.47, 95% CI: 0.89, 2.43; p=0.13; hazard ratio adjusting for infant age and sex=1.44, 95% CI: 0.87, 2.39 p=0.16) (Figure 4). On average, 9.5 infants stopped exclusive breastfeeding per 1,000 infant-days of observation time (iron: 8.0; placebo: 11.2), and 3.7 infants (iron: 4.3; placebo: 3.1) stopped exclusive breastfeeding per 1,000 infant-days.

237 **Figure 3.** Kaplan Meier curves with 95% CIs and corresponding risk table, by intervention arm (red:
 238 iron; blue placebo)



239
 240
 241 **Figure 4.** Kaplan Meier curve with 95% CIs and corresponding risk table, by intervention arm (red: iron;
 242 blue placebo)



7.6.2 Anthropometry

When looking at the whole cohort, mean weight and length (SD) at endline were 6.81 (0.95) kgs and 63.8 (2.9) cms, respectively. There was a decrease in the number of infants who were underweight and stunted between baseline and endline (underweight: 12.1%, N=12/99 vs 9.47%, N=9/95; stunted: 9.00%, N=9/100 vs 8.42%, N=8/95; for baseline vs endline, respectively) and a marginal increase in the number of infants who were wasted (5.10%, N=5/98 vs 5.26%, N=5/95; for baseline vs endline, respectively).

There was no evidence of meaningful differences between iron and placebo groups in any of the anthropometric outcomes (Table 2).

When looking at the impact of infant sex and age at baseline on growth, there was statistical evidence for an association between infant sex and change in WAZ, with males showing a greater decrease in WAZ over the duration of the trial than females ($p=0.03$, coefficient: -0.34, 95% CI: -0.65, -0.03). The mean (SD) change in WAZ for males was -0.08 (0.70), versus 0.26 (0.78) for females. Males' mean (SD) WAZ changed from -0.46 (0.87) at baseline to -0.55 (1.03) at endline, whereas females changed from -0.70 (1.38) to -0.49 (1.31). A statistical association was observed between infant age at baseline and change in LAZ with younger infants at baseline having greater change in LAZ ($p=0.01$, coefficient: -0.26, 95% CI: -0.45, -0.06) over the duration of the trial than older infants. No other associations with z-scores and infant age or sex at baseline were observed. There was no statistical evidence for associations between intervention arm and endline anthropometry outcomes for both unadjusted and adjusted (sex and age at baseline) models.

264

Table 2. *Crude effect of iron supplementation on anthropometric outcomes.*

	Entire cohort	Iron group	Placebo group	p-value: iron vs placebo	Group difference (95% CI)
Infant age (in weeks) (SD)*	21.7 (1.1) [100]	21.7 (1.0) [49]	21.7 (1.2) [51]	-	0.18 (-3.03, 3.38)
Weight (in kg) (SD)*	6.81 (0.95) [95]	6.83 (0.97) [46]	6.78 (0.95) [49]	0.79	-0.05 (-0.44, 0.34)
Length (in cm) (SD)*	63.8 (2.9) [95]	63.6 (2.8) [46]	63.9 (3.0) [49]	0.64	0.28 (-0.89, 1.45)
Weight for age z-score (WAZ) (SD)*	-0.52 (1.19) [95]	-0.49 (1.26) [46]	-0.54 (1.12) [49]	0.83	-0.05 (-0.54, 0.43)
Length for age z-score (LAZ) (SD)*	-0.50 (1.18) [95]	-0.57 (1.21) [46]	-0.43 (1.16) [49]	0.57	0.14 (-0.35, 0.62)
Weight for length z-score (WLZ) (SD)*	-0.16 (1.08) [95]	-0.08 (1.09) [46]	-0.25 (1.07) [49]	0.44	-0.17 (-0.61, 0.27)
Change in WAZ (SD)*	0.11 (0.76) [93]	0.11 (0.79) [44]	0.11 (0.74) [49]	0.99	0.00 (-0.31, 0.32)
Change in LAZ (SD)*	0.09 (1.05) [94]	0.05 (1.01) [45]	0.13 (1.08) [49]	0.70	0.08 (-0.35, 0.51)
Change in WLZ (SD)*	-0.10 (1.31) [92]	-0.08 (1.39) [44]	-0.12 (1.24) [48]	0.89	-0.04 (-0.58, 0.51)
Underweight [‡]	9.47% [9/95]	8.70% [4/46]	10.2% [5/49]	0.80	0.02 (-0.11, 0.14)
Stunted [‡]	8.42% [8/95]	8.70% [4/46]	8.16% [4/49]	0.93	-0.01 (-0.12, 0.11)
Wasted [‡]	5.26% [5/95]	6.52% [3/46]	4.08% [2/49]	0.60	-0.02 (-0.12, 0.07)

265

*Expressed as mean (standard deviation) [n]. SD = standard deviation; kg = kilograms; cm = centimetres. Z-scores were calculated using WHO Anthro (304)

266

[‡] WHO definition of underweight (WAZ <-2), stunting (LAZ <-2), and wasting (WLZ <-2) (302)

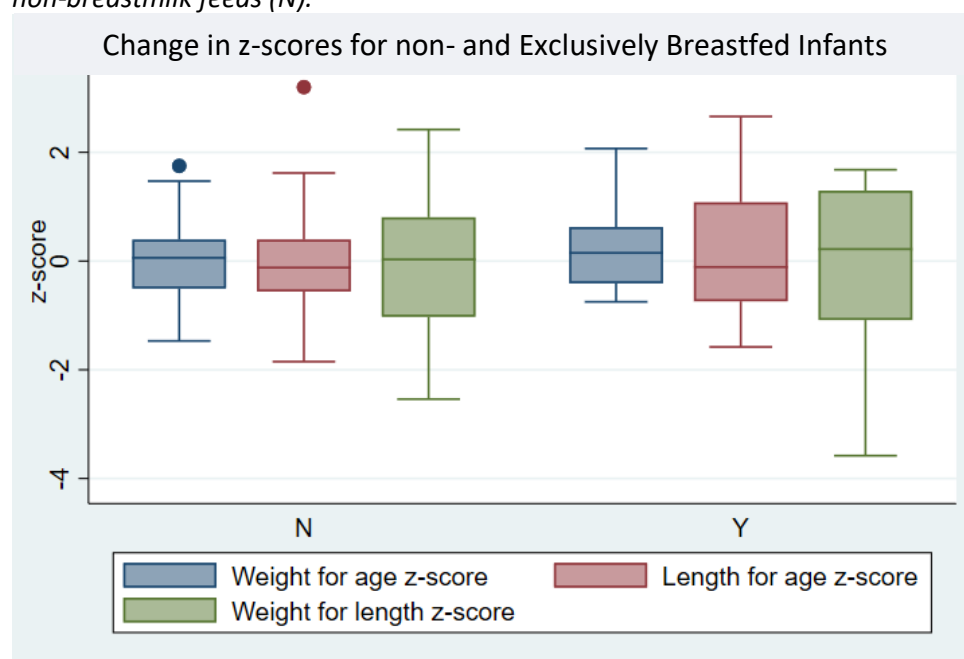
7.6.3 Impact of mode of feeding on infant growth

When looking at differences in growth by mode of feeding (being exclusively breastfed at endline or those who had non-breastmilk feeds introduced [N=94]), the prevalence of infants underweight (p=0.16), stunted (p=0.32), or wasted (p=0.94) at endline versus baseline (change in WAZ, LAZ, and WLZ) for in those infants who were exclusively breastfed and those infants who were not at endline were similar (Figure 5).

In the unadjusted model, whether an infant was exclusively breastfed or not throughout the trial resulted in similar endline anthropometric outcomes. However, following adjustment for infant sex and age at baseline, there was statistical support that being exclusively breastfed (p=0.02, coefficient: 0.37, 95% CI: 0.05, 0.68), resulted in a greater positive change in WAZ over the duration of the trial. The mean (SD) change in WAZ for exclusively breastfed infants was 0.24 (0.74), versus 0.02 (0.67) for non-exclusively breastfed infants at endline. Exclusively breastfed infants' mean (SD) WAZ changed from -0.82 (1.16) at baseline to -0.61 (1.16) at endline, whereas non-exclusively breastfed infants changed from -0.46 (1.10) to -0.47 (1.23).

There was no statistical support in the unadjusted or adjusted model (infant sex and age at baseline), that duration of exclusive breastfeeding had any impact on endline anthropometric outcomes.

Figure 5. Change in weight for age (WAZ), length for age (LAZ), and weight for length (WLZ) z-scores between baseline and endline for exclusively breastfed (Y) infants at endline and those introduced to non-breastmilk feeds (N).



7.7 DISCUSSION

This secondary analysis of data collected from a pilot trial in rural Gambia has shown that daily iron supplementation to young, breastfed infants did not adversely impact on patterns of infant feeding or growth. This finding, among a population of breastfed young infants with marginal iron status supports that supplementary iron does not impact on mode of feeding, does not compromise infant growth, and adds to our previously published observations that infant iron status is significantly improved and that supplementary iron in this age group is safe (301).

The prevalence of exclusive breastfeeding in this setting at six months of age has previously been reported at a similar rate as found in this trial (7), however when looking at country wide statistics, the rates of exclusive breastfeeding in The Gambia are reported as higher than in this trial (293). All infants were exclusively breastfeeding at entry into the trial and only 31 remained exclusively breastfeed until the end of the trial, 63 remained partially breastfed, and seven had unknown endline feeding status. In the *Iron Babies* cohort, instances when non-breastmilk feeds were given, the most common substance was water, 76% of the time. Formula had a low percent of usage (4% of instances) whereas local foods (7%) or powdered milk (6%) were more often given. In a neighbouring rural region, watery millet cereals have been noted to be introduced as early as three months of age (305). In *Iron Babies*, the remaining non-breastmilk feeds consisted of local juices (4%), powdered potato (2%) and other substances (1%). Supplementary iron did not impact on any of the infant feeding practices monitored. While there are concerns around gastrointestinal side effects of iron supplementation, which may in turn impact infant feeding practice, the findings from this study are important as data around this topic is lacking. A systematic review of iron and/or multiple micronutrient interventions in infants under six months of age, found no side effects around infant feeding practices (105). However, a systemic review of caregiver perspectives of early nutrition interventions in infants under 24 months of age noted increased infant appetite as the main reason for high acceptability of interventions (132).

Consistent with published data from rural Gambia, infant z-scores decreased with age in this cohort, reflecting growth faltering across infancy (7, 306). Infant mean birthweight in *Iron Babies* was 2.97kg, which is similar to previously reported in a cohort of rural Gambian infants (7).

Previous trials and commentaries have raised concerns that supplementary iron given to young, breastfeeding infants may impact on development or feeding (105, 307). There was no statistical support for any growth impacts due to iron intervention. Some studies in infant populations over six months of age have reported adverse effects from iron on growth, but these impacts were only seen

in iron replete children (110, 111). When looking at feeding and adjusting for infant sex and age at baseline, being exclusively breastfed at endline ($p=0.02$) meant greater positive change in WAZ over the duration of the trial. However, duration of exclusive breastfeeding did not impact endline anthropometric outcomes.

This study has several limitations. The primary limitation is the sample size of this pilot study which was not powered to test the secondary outcomes considered in this paper. Nevertheless, given the concerns around the safety of early iron interventions and the lack of data from well conducted trials in this age group these findings make an important contribution to the field. Daily administration of the intervention by the field workers, whilst ensuring efficacy, may have impacted on caregivers' usual behaviours, especially in relation to feeding practices. A qualitative adjunct study found that feasibility to scale up with self-administration was a concern for some involved in the study, but overall there was enthusiasm for the intervention (307). In the survival analysis, the 95% CI was about 2.5 on the upper end, so a larger study would be required to verify that intervention did not have an impact on feeding practices. Additionally, in the Kaplan Meier curves, by the end of the analysis time, the iron group sees an earlier time since enrolment of introduction of non-breastmilk feeds as well as earlier age of cessation of exclusive breastfeeding, and while the p-values indicate this is due to chance in these graphs, this should be explored in larger studies. Lastly, infant anthropometric indices were only assessed at baseline and endline so more sophisticated growth modelling could not be performed.

7.8 CONCLUSION

In summary, this study has shown that giving daily iron from six to ten weeks of age to breastfed rural Gambian infants was safe and improved several markers of iron status without any adverse impacts on the prevalence of exclusive breastfeeding or added risk to growth (301). No adverse effects were seen on growth outcomes from the early iron intervention. These findings are important as, together with the primary trial outcomes they add to the evidence of importance of early iron interventions in contexts of marginal status and negate any concerns around the impact of iron supplementation on safety outcomes, including feeding practices and growth (301). Although requiring replication in larger trials, these findings suggest that giving iron to young breastfeeding infants, in addition to having positive effects on iron status, is safe and does not adversely impact on infant feeding patterns or growth.

Appendix A. Stata Code

```
stset Timetofollowup, failure(SurvivalAnalysis==1) OR stset AgeEBFc cessationweeks,  
failure(SurvivalAnalysis==1)
```

```
sort ID
```

```
stdescribe
```

```
stsum
```

```
stvary
```

```
sts test Studyarm, logrank
```

```
stcox Studyarm
```

```
stcox Studyarm cagebaseline csex
```

```
estat phtest
```

```
stphplot, by (Studyarm)
```

```
stphplot, by (Studyarm) adjustfor (cagebaseline csex)
```

```
sts graph
```

```
sts graph, ci
```

```
sts graph, by (Studyarm)
```

```
sts graph, by (Studyarm) ci
```

```
sts graph, by (Studyarm) risktable
```

```
sts graph, by (Studyarm) ci risktable
```

```
sts graph, by (Studyarm) adjust for (cagebaseline csex)
```

```
regress chtendline cagebaseline
```

```
regress chtendline csex
```

```
regress chtendline Studyarm
```

```
regress chtendline EBFYN
```

```
regress chtendline AgeEBFc cessationweeks
```

```
regress chtendline EBFYN csex cagebaseline
```

```
regress chtendline AgeEBFc cessationweeks csex
```

8 RESULTS: MATERNAL ACCEPTABILITY OF IRON SUPPLEMENTATION IN YOUNG BREASTFED RURAL GAMBIAN INFANTS

8.1 INTRODUCTION

This Chapter incorporates a published original research article from the Iron Babies study (Stelle, et al., 2023) published in *Reproductive, Female, and Child Health*. This publication reports on an acceptability study with local stakeholders and mothers whose infants are enrolled in Iron Babies. This study was based at the MRCG@LSHTM in rural Gambia. For more contextual information about The Gambia, refer to Chapter 5 of this Thesis (Settings) and for more information on the Iron Babies study refer to Chapter 6 of this Thesis (Methods, published Iron Babies protocol paper).

Acceptability is a key consideration in evaluating and implementing healthcare interventions, but definitions are often vague (308-313). Successful implementation of an intervention requires acceptability from both the perspective of the care providers and recipients (314, 315). If care providers find an intervention acceptable it is often delivered as intended, in turn increasing the effectiveness of the intervention (316-319).

This embedded qualitative study assessed the acceptability of an iron supplementation efficacy trial (Iron Babies) in infants under six months of age in rural Gambia. Mothers' (as the main care providers) perspectives on the acceptability of iron supplementation in their young infants, as well as their willingness to involve their young infants in clinical trials on iron supplementation, were explored through IDIs. Additionally, the acceptability of iron supplementation in young infants was explored through FGDs with local stakeholders.

Data was analysed with respect to an implementation framework known as: Acceptability of healthcare interventions: an overview of reviews and development of a theoretical framework (308). Template analysis was employed for analysing the qualitative data (320).

Appendix 4 presents additional files associated with this publication.

8.2 AUTHOR ROLES

Conceptualization: Isabella Stelle, Sophie E Moore, Sergio A Silverio

Funding Acquisition: Sophie E Moore, Carla Cerami

Methodology: Isabella Stelle, Sergio A Silverio

Analysis plan: Isabella Stelle, Sergio A Silverio

Trial conduct and management: Isabella Stelle, Mamadou Bah, Mariama Saidykhan

Paper specific data management and main analysis: Isabella Stelle, Sergio A Silverio

Supervision: Sophie E Moore

Original Draft Preparation: Isabella Stelle

Reviewing and Editing: All authors

Approval of final manuscript: All authors

8.3 PUBLISHED PAPER (4): MATERNAL ACCEPTABILITY OF IRON SUPPLEMENTATION IN YOUNG BREASTFED RURAL GAMBIAN INFANTS

Stelle I, Bah M, Saidykhan M, Verhoef H, Prentice AM, Cerami C, Silverio SA and Moore SE. Maternal acceptability of iron supplementation in young breastfed rural Gambian infants. *Reproductive, Female and Child Health*. 2023, <https://doi.org/10.1002/rfc2.29>

The full paper can be found below and additional files in **Appendix 4** or accessed here:

<https://onlinelibrary.wiley.com/doi/full/10.1002/rfc2.29>

ORIGINAL ARTICLE



Maternal acceptability of iron supplementation in young breastfed rural Gambian infants

Isabella Stelle¹ | Mamadou Bah^{2,3} | Mariama Saidykhan² | Hans Verhoef³ | Andrew M. Prentice² | Carla Cerami² | Sergio A. Silverio¹ | Sophie E. Moore^{1,2}

¹Department of Women and Children's Health, King's College London, London, UK

²Medical Research Council Unit The Gambia at The London School of Hygiene and Tropical Medicine, Fajara, The Gambia

³Division of Human Nutrition and Health, Wageningen University, Wageningen, The Netherlands

Correspondence: Isabella Stelle, Department of Women and Children's Health, King's College London, London, UK. Email: Isabella.stelle@kcl.ac.uk

Funding information: Wellcome Trust Senior Research Fellowship, Grant/Award Number: 220225/Z/20/Z; DFID/NIHR/MRC/Wellcome Joint Global Health Trials, Grant/Award Number: MRT003960/1; UK MRC and the UK Department for International Development (DFID) under the MRC/DFID Concordat agreement, Grant/Award Number: MC-A760-5QX00; National Institute for Health Research Applied Research Collaboration South London [NIHR ARC South London] at King's College Hospital NHS Foundation Trust

Abstract

Introduction: A recent analysis of Gambian infants showed that plasma iron concentrations decline rapidly from birth to levels below the 95% reference range within 5 months of age. To address this issue, a clinical trial was initiated to assess the efficacy of early iron supplementation in breastfed infants under 6 months of age ('Iron Babies'; NCT04751994). To understand if such an intervention is feasible for scaleup, the acceptability, through the lens of local stakeholders and mothers (as the primary caregiver), must be considered.

Methods: An embedded qualitative study, therefore, explored acceptability through focus group discussions with local stakeholders and interviews with mothers. Four focus group discussions with local stakeholders (n = 19) and individual interviews (n = 14) with mothers whose infants were enrolled in the clinical trial were conducted. Qualitative data were analysed with respect to an implementation acceptability framework using Template Analysis.

Results: From the focus group discussions, there was a general sense of enthusiasm for the intervention, but also a concern due to confusion around nutritional messaging. The intervention was acceptable to mothers with limited mention of side effects and enthusiasm for participating in clinical trials on iron supplementation. However, when looking at scaleup, there were mixed opinions on the practicality of daily supplementation, as well as concerns around costs. In addition, it was suggested that proper dissemination of information and inclusion of all family members in decision-making, especially fathers, is key to acceptability. **Conclusion:** There was no indication that stakeholders or mothers were resistant to iron supplementation of breastfed infants.

KEY WORDS

nutrition, qualitative research

Abbreviations: EBF, exclusive breastfeeding; FGDs, focus group discussions; IDIs, individual interviews; NaNA, National Nutrition Agency.

Sergio A. Silverio and Sophie E. Moore contributed equally to this study.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2023 The Authors. Reproductive, Female and Child Health published by John Wiley & Sons Ltd.

INTRODUCTION

Iron deficiency anaemia, the largest nutritional deficiency worldwide, remains the leading cause of years lived with disabilities in low- and middle-income countries and is associated with more than 120 000 maternal deaths per year.¹ The prevalence of anaemia is five times higher in low and middle-income countries than in high-income countries, with about 43% of children between the ages of 6–59 months being anaemic.^{1,2}

A recent pooled analysis of 317 rural Gambian infants found that they were born with a reasonable endowment of iron despite being born to deficient mothers.³ However, following birth there was a rapid deterioration of markers of iron status and a simultaneous increase in markers of iron tissue demands.³

While efficacy studies in infants have shown that early nutrition interventions improve infant nutritional status, to examine whether such interventions can be effectively taken to scale, a better understanding of maternal acceptability is required.^{4–6}

Acceptability has become a key consideration in evaluating and implementing healthcare interventions.⁷ Definitions of acceptability are vague in the literature, but vary from social to treatment acceptability.^{8–12} Successful implementation requires the acceptability of an intervention from both the perspective of the providers and recipients.^{13,14} When an intervention is deemed acceptable, adherence is often higher, leading to improved clinical outcomes.^{15,16} Additionally, providers must find an intervention acceptable to ensure it is delivered as intended, in turn increasing the effectiveness of the intervention.^{17,18}

On this backdrop, the aim of the current study was to assess the acceptability of an iron supplementation efficacy trial in infants under 6 months of age in rural Gambia (efficacy trial: Iron Babies). The following objectives were assessed:

- To explore mothers' (as the main care providers) perspectives on the acceptability of iron supplementation in their young infants through individual interviews (IDIs).
- To explore mothers' willingness to involve their young infants in clinical trials on iron supplementation.
- To explore the acceptability of iron supplementation in young infants, under evaluation in the primary clinical trial on which this work is nested, through focus group discussions (FGDs) with local stakeholders.

To address the objectives of the study, data were analysed with respect to an implementation framework. Frameworks act as a guide to conceptualise an intervention to a specific aspect of implementation.¹⁹ The clinical trial in which this study is embedded (Iron Babies) is an efficacy study; therefore, not all components of this framework are relevant to the data collected. However, it was used as a starting point.

Template analysis, as defined by Brooks et al.²⁰ (p. 203), 'is a form of thematic analysis which emphasise the use of hierarchical coding but balances a relatively high degree of structure in the process of analysing textual data with the flexibility to adapt it to the needs of a particular study'. Key to this technique is the use of a coding template, based on a subset of the data, which is then applied to the remaining data and revised and refined.²⁰ This form of analysis does not first set a sequence of coding levels, but instead lets the researcher develop themes where the richest data are found.²⁰ Template analysis often utilises interview transcripts, FGDs, and questionnaires.²⁰ It follows a methodical six-stage process: (1) data refamiliarization; (2) preliminary coding; (3) thematic organisation within the template; (4) defining the template; (5) application of template to the full data set; and (6) finalisation of template definitions.

METHODS

To assess the efficacy of early iron supplementation in infants under 6 months of age in this setting, a double-blind, placebo controlled, randomised pilot study was conducted. To measure the impact of daily iron supplementation on serum iron concentrations, healthy, breastfed infants (n = 101), aged 6–10 weeks at enrolment, were randomised to receive daily iron (7.5 mg/day of iron as ferrous sulphate) or placebo drops for 98 days. The primary and secondary endpoints were serum iron, serum ferritin, haemoglobin and other haematological markers of anaemia, iron deficiency and iron deficiency anaemia after 98 days of supplementation. Full details of the Iron Babies trial and this qualitative adjunct study have been published in detail in the trial protocol.²¹ The methods applicable to this qualitative study are provided below.

Study setting

Data collection for the current study took place in The Gambia, West Africa. The Iron Babies trial was based at the Jarra Soma Regional Hospital with study participants recruited from the Soma region of The Gambia through community-based clinics and birth attendants. The current study was conducted in the same community, recruiting local mothers and stakeholders.

Study design and methodology

This approach explores the effects of a clinical intervention on relevant outcomes while observing and gathering information on implementation. Qualitative methods were used to explore the acceptability of such an intervention proposed in the Iron Babies trial.

The two following components compromise this qualitative study:

1. Qualitative: FGDs with local stakeholders.

Four FGDs took place with local stakeholders to inform the IDIs with infants' mothers (Supporting Information: Appendix 1):

- Members of the National Nutrition Agency (NaNA).
- Iron Babies trial health workers.
- Non-Iron Babies trial health workers.
- Mothers from surrounding villages whose infants are not enrolled in the Iron Babies trial.

The FGDs were audio recorded, lasting on average 1 h, and were conducted in English or Mandinka (the predominant local language). The location was chosen by the interviewee to add comfort. Both staff from the main Iron Babies trial and those not working for the main trial were interviewed to ensure a wide range of opinions were sought. We aimed to recruit around five participants to each FGD, in accordance with general recommendations for focus group size in qualitative research (20 participants total).²²

2. Qualitative: IDIs with infants' mothers.

The IDIs discussed the acceptability of iron supplementation in infancy from the perspective of mothers whose infants were enrolled in the Iron Babies trial. The data from the FGDs were used to finalize the interview schedules (Supporting Information: Appendix 2). Using these schedules, IDIs, not lasting longer than 1 h, were conducted in Mandinka by a qualitatively trained female member of the study team and were audio recorded. All mothers were Mandinka (the most common local tribe making up 75% of the Iron Babies trial participants) to provide homogeneity in the translation of the IDIs. The location was chosen by the interviewee to add comfort and included the homes of participants and within local villages. IDIs employed concurrent and retrospective data collection. Both the mothers and researchers were blinded. Maximum variation purposive sampling of the mothers whose infants were enrolled in the main study was used to ensure many opinions are represented.^{23,24} We anticipated an approximate sample size of 10–20 mothers to reach data saturation.

Data collection

Before conducting FGDs and IDIs, the guides and schedules were piloted among the Iron Babies trial study team. Two female interviewers piloted the FGD guides on members of the Iron Babies trial study team and adjustments were made from the feedback. Mock IDIs were then conducted on other

study team members of the Iron Babies trial study team. This helped to adjust the FGD guides and IDI schedules, but likewise, acted as an opportunity for the interviewers to familiarise themselves with the structure of the FGDs and IDIs.

Consent and ethics

Full written informed consent was provided by all study participants. The studies have been reviewed and approved by the applicable Scientific Coordinating Committee and Ethics Committees.

Data analysis

The FGDs and IDIs were audio recorded and later transcribed and concurrently translated (where needed) into English. Data were analysed with respect to the implementation framework known as Acceptability of healthcare interventions: an overview of reviews and development of a theoretical framework using Template analysis.^{7,20,25}

The six themes used to analyse the data were burden, ethical consequences, opportunity costs, experience, affective attitude and intention. The acceptability framework was adapted, and the themes were placed on a spectrum of being the most inductive or evidence-based, to the most deductive or preconceived/cultural, as well as ethical consequences being adapted to ethical considerations (Figure 1).

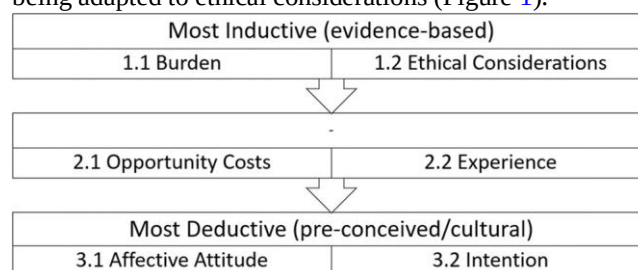


FIGURE 1 Adapted acceptability framework.

The qualitative data were uploaded into, managed and analysed in NVivo using Template analysis.^{20,25} Data were coded to the six themes in the framework, with minor variations to make analytical sense of the data. The acceptability framework used to develop the template for analysis is a macro-level conceptual framework, which can be applied to data that consider both meso-levels (data from the FGDs) and microlevels (data from IDIs). While acknowledging the domains may not all reach saturation, these are based on an extensive search of the literature by the framework authors and have been tested in different fields, so were used as a basis for our analysis. The extent to which the framework was valuable in this study is later discussed. This coding was conducted by one researcher and 25% of the data was independently coded by another researcher to ensure

rigour of coding. Throughout the coding process, the two researchers met to discuss the codes and applicability of the acceptability framework. Any disagreements were resolved by discussion with a third researcher.

RESULTS

Qualitative data analyses

Four FGDs were conducted (n = 19 individuals). All participants were Gambian. Characteristics of the FGDs participants are detailed in Table 1. IDIs were conducted with mothers whose infants were enrolled in the Iron Babies clinical trial (n = 14). All mothers were Mandinka (the most common local tribe) and married. Demographic data from the IDI participants can be found in Table 1. The Medical Research Council Unit The Gambia at the London School of Hygiene and Tropical Medicine is referred to as 'the unit'.

TABLE 1. Demographics of the four focus group discussions (n = 19), and individual interviews (n = 14).

Focus group discussion demographics (n = 19)					
	Participant ID	Gender	Age (years)	Years working for their organisation	Number of children
Members of the National Nutrition Agency	Participant 1	Male	56	21	NA
	Participant 2	Male	53	21	
	Participant 3	Female	48	21	
	Participant 4	Female	31	6	
Iron Babies trial health workers	Participant 1	Male	50	19	NA
	Participant 2	Male	51	3	
	Participant 3	Male	26	7	
	Participant 4	Male	38	8	
	Participant 5	Male	51	23	
Non-Iron Babies trial health workers	Participant 1	Female	29	6	NA
	Participant 2	Female	19	1	
	Participant 3	Female	39	14	
	Participant 4	Female	19	1	
Mothers from surrounding villages whose infants were not enrolled in the Iron Babies trial	Participant 1	Female	24	NA	4
	Participant 2	Female	28		3
	Participant 3	Female	24		1
	Participant 4	Female	35		5
	Participant 5	Female	36		7
	Participant 6	Female	37		3
Individual interview demographics (n = 14)					
	Age (years)	Number of children	Location of births	Educational attainment	Employment
Participant 1	40	6	Hospital	None	Cook
Participant 2	27	2	Health centre	Primary	Housewife
Participant 3	40	5	Health centre	None	Housewife
Participant 4	24	3	Hospital	Primary	Housewife
Participant 5	28	3	Home (1) and hospital (2)	Primary	Housewife
Participant 6	28	7	Hospital	Secondary	Housewife
Participant 7	28	4	Hospital	Primary	Housewife
Participant 8	23	2	Hospital	Secondary	Housewife
Participant 9	20	3	Hospital	Primary	Housewife
Participant 10	19	1	Hospital	Secondary	Housewife

Participant 11	26	1	Hospital	Secondary	Housewife
Participant 12	25	4	Hospital	None	Housewife
Participant 13	30	6	Home (2) and hospital (4)	None	Cleaner
Participant 14	30	5	Hospital	Primary	Housewife

Abbreviation: NA, not applicable.

Template analysis

The below findings are based on the constructs of the acceptability framework,⁷ which was used as a template for analysis. Illustrative quotes and analysis were separated by FGD (Tables 2–4) and IDI for each of the six themes.

Illustrative quotes are presented using the following nomenclature:

- NaNA: NaNA FGD-PX (where PX indicates participant number, i.e., P1).
- Iron Babies trial health workers: IB FGD-PX.
- Non-Iron Babies trial health workers: N-IB FGD-PX.
- Mothers from surrounding villages whose infants were not enrolled in the Iron Babies trial: M FGD-PX.
- IDIs: IDI-PX.

TABLE 2 Illustrative quotes from the focus group discussions with local stakeholders for Theme 1.

1.1 Burden	‘They [mothers] will not be able to give their infants [supplements] as regularly as the field assistant, because this is their jobs...because they monitor everything and make sure the supplements are given to the child’. (N-IB FGD-P1) ‘The fathers should be included in this discussion, because maybe at times the mothers might forget [to give the supplement]...but if the two of them are informed at the same time, if one forgets the other will not’. (N-IB FGD-P2) ‘We are capable of giving it [supplements] to them’. (M FGD-P2) ‘Make a community sensitisation first...but other family members who aren't present...some of them would say they don't want to participate...and start complaining. Just have the patience, explain it to them and what the study is and the benefits that this iron supplement is going to give to the infants’. (N-IB FGD-P1) ‘Most of the mothers...understand by explaining to them what they have not learnt at school...it may change some of those mothers’ [minds about enrolling]’. (IB FGD-P5) ‘Because they will ask you “if anything happens, are you going to take us to the unit?”...The family may say, “we are in the project”. What would they expect from you? Like if somebody falls sick what will happen?’ (IB FGD-P5) ‘NaNA with the support of the communities, created those [antenatal education] groups...We are in...over 1,000 communities in The Gambia...The Gambia has about 2,000 settlements. So almost we are in half of the country...We frequently talk to mothers, educate them. Anaemia is one thing we educate these mothers on’. (NaNA FGD-P3)
1.2 Ethical considerations	‘Why is the unit collecting blood from babies...I heard that they are collecting blood and selling it’. (N-IB FGD-P3) ‘It's also important to tell them [families] how many times you will be taking the blood of their babies because they are very sensitive about that...That may discourage many participants. To want to remove from the study’. (N-IB FGD-P4) ‘Some of the participants will ask about the safety of the iron...Is it going to cause harm to their infants?...Talk about the iron supplements given to the child, that will just break the barriers...Like telling the mothers that this iron is a medicine’. (N-IB FGD-P1) ‘Once we start supplementing the child...they will have iron in their systems which we know iron is a good medium for the growth of bacteria...The most common infections, childhood infections here, malaria is one of course, but you also have diarrhoea diseases, pneumonia, these could be bacterial infections as well’. (NaNA FGD-P1) ‘In The Gambia a lot of women still die as a result of anaemia. So they are not taking the iron tablets just to build up their stores for their babies, but it's to protect themselves...So the messaging should be in such a way that there is a link between the iron in the mother and the iron in the child’. (NaNA FGD-P1) ‘Giving the infants the supplements now, can it discourage the mothers from taking [iron themselves]? That can be also another un-intended consequence...there are issues mothers complain of...constipation, taking different excuses as to why they were not taking it’. (NaNA FGD-P4) ‘If you explain to the mother, they will understand [why this supplement is important]...We are talking about medicine’. (IB FGD-P1)

'They [mothers] would be worried because this is the norm, exclusive breastfeeding is everywhere. One the news, on the television...We are told to not even give a drop of water to the kids. Now comes a study that is telling you...we will be giving an iron supplement to your baby daily...We won't be comfortable taking it'. (IB FGD-P3)

Abbreviations: FGD, focus group discussions; NaNA, National Nutrition Agency.

TABLE 3 Illustrative quotes from the focus group discussions with local stakeholders for Theme 2.

2.1 Opportunity Costs	No data were present in FGDs for this subtheme.
2.2 Experience	'These messages have been given to them [communities] at the right time by the right people...We have that trust...between them and their community structures. And they believe in what they told them previously, they have seen benefits in that'. (NaNA FGD-P2) 'Mothers will have questions...and their questions are relevant...Let's make sure that whenever they need clarification, they get that clarification...from the right person'. (NaNA FGD-P4) 'If the feedback [trial result] is not given, people are discouraged. You just come here, do your business, get your profit. But you just leave. Whether they are healthy or not healthy, they have no information'. (IB FGD-P5) 'I will thank and praise them [the unit] for the wonderful work they are doing... We don't have to buy medicine, we are given it free of charge'. (M FGD-P6)

Abbreviations: FGD, focus group discussions; NaNA, National Nutrition Agency.

TABLE 4 Illustrative quotes from the focus group discussions with local stakeholders for Theme 3.

3.1 Affective Attitude	'The mothers...will ask:...“why is my infant taking supplementation at this early stage of six weeks?” And other family members too might intervene:...“why is the unit doing such and such...why is your child taking these supplements? Is your child sick? What is the problem?”' (N-IB FGD-P1) 'The immediate benefit of the study. Most [families] are almost always concerned with that...They will be asking, “I have been giving you my child's blood, still now, I don't see anything”.' (N-IB FGD-P2) 'All the mothers are aware of not giving even a drop [of water] to their babies because of the health implications of giving that...It is very important if we are going to give iron...it doesn't mean that you can give the child water. They need to understand this is a health routine'. (IB FGD-P2) '“Now they are telling us, our babies from six weeks can take these drops”. So it [the trial] has to go with education that will clarify the issues to these mothers'. (NaNA FGD-P3)
3.2 Intention	'Their in laws [may say]: “I have not given that [iron] to all my children and they have all grown well”...During the consenting, if the in laws were present, call them and explain everything to them...Acceptability is very crucial in this'. (N-IB FGD-P1)

'They have village health workers. People who are there to lead, the Alkali or the head...So you have a meeting with those close people, so they can disseminate [information]...that will also help...And the field workers...they should also have a clear understanding, or knowledge about what is the importance of this iron...If you cannot answer it [the questions] they will say he or she is here giving my child medicine and...does not even know the benefit...or the importance of the medicines'. (N-IB FGD-P4)

'The messaging should be consistent...uniform...But once the messaging starts to be disjointed, the acceptability starts to be a problem...To be successful, obviously the fathers are the key decision makers, but the in-laws, the grandparents of the lady, are very key in making decisions...Childcare is shared within the household, lots of people are involved with care of the child in the household'. (NaNA FGD-P1)

Abbreviations: FGD, focus group discussions; NaNA, National Nutrition Agency.

Burden

Burden is the burden (when not financial) of being involved in the clinical trial or supplementing infants. Burden was categorised as the most inductive, along with ethical considerations.

From the FGDs, burden was frequently perceived as the mothers struggling to give the supplement to their infant in a real-world setting. However, not all participants anticipated this as a burden. Additionally, study enrolment and community sensitisation were perceived as a sensitive issue and must be approached correctly and with patience.

Likewise, education levels were noted as important to consider when sensitising the community so participants can have a clear understanding of the purpose of the study. When enrolled in a clinical trial, a participants' full health needs are covered by the study, regardless of whether it is linked to the intervention or not. This level of care was noted as then being expected by other family members and potentially posing a burden on the trial. Illustrative quotes can be found in Table 2.

When not involved in trials, access to proper medical care in The Gambia was noted as reoccurring burden in the maternal IDIs.

Sometimes they give me medicines and other times, I will be asked to go and buy it at the pharmacy because not all the medicines are available [at the hospital]...When there are many patients it takes a long time to get treatment. (IDI-P13)

Additionally, when not involved in trials, access to transportation can pose a burden.

It's difficult to get transportation. Even when I was about to deliver my infant, I walked to the hospital. (IDI-P2)

As with the FGDs, mixed opinions about the act of supplementing infants in the real-world setting were raised in the IDIs. From issues with the infant accepting the drops to mothers remembering to administer the supplement.

I will forget sometimes...I will be needing their [health workers] assistance. (IDI-P8)

We sometimes struggle just to have the infant take it...but once it's in his mouth, he takes it easily. (IDI-P11)

Ethical considerations

Ethical considerations are the associated side effects of the intervention. Ethical considerations were categorised as the most inductive, along with the burden.

Ethical considerations were more frequently noted in the FGDs than the IDIs, despite none of the FGD participants being enrolled in the study. A common reoccurring theme was the dislike of providing blood samples for research and concerns about selling participant's blood. Additionally, subsequent side effects around the supplementation were expressed as a concern for mothers. Therefore, it was noted that ensuring adequate community sensitisation and addressing all concerns are important for easing any hesitations around the intervention. Antenatal care guidelines in The Gambia include the provision of iron and folic acid. However, members of NaNA commented on the associated side effects of iron, causing compliance issues, so if policy changes to recommend young infants to also take iron, it was believed that this may further complicate noncompliance in mothers, therefore exacerbating the problem of antenatal iron deficiency. Lastly, proper dissemination of information was noted as crucial to decrease this potential issue. NaNA endorse the World Health Organisation policy of exclusive breastfeeding (EBF) to 6 months of age, and this is promoted through their Baby Friendly Hospital Initiative. EBF in infants under 6 months of age is common, so NaNA mentioned it is important to ensure mothers understand why the study is giving iron, a medicine, during this period of EBF.²⁶ Illustrative quotes can be found in Table 2.

The issue of side effects during the intervention only came up a few times in the IDIs, however, like in the FGDs, the issue of blood samples when participating in clinical trials was noted.

What scares people away from the unit...the little blood that they take from people. That is what they say: "the unit takes people's blood". (IDI-P1)

Opportunity costs

Opportunity costs are those that influence adherence and trial participation. Given this was an efficacy study, opportunity costs were often hypothetical. It was considered both inductive and deductive. Opportunity costs were not noted in the FGDs; however, it was a common theme in maternal IDIs. When mothers were asked in IDIs about the affordability of supplements and if they could afford iron supplements in a nonclinical trial setting, the issue of cost was continuously noted.

It would be difficult for me...Our income is dependent on fishing and at times my husband does not have any fish, hence no money in the household. (IDI-P4)

It will be hard for me as medicine is very expensive nowadays. (IDI-P8)

Experience

Experience is the perception, and satisfaction with the intervention. It was considered in the middle of both inductive and deductive.

Within FGDs, community and individual sensitization before study initiation was considered a critical element of ensuring study success. Further, it was stated that failure to disseminate study results on completion can have a negative effect on participant's experience. However, when enquiring with the mothers about their past experiences with studies, the responses were all positive. Illustrative quotes can be found in Table 3.

Mothers from the IDIs had positive experiences with their participation in the clinical trial and often praised the unit for their work and the healthcare they provided. Most noted was the quality of care that was given if an infant ever had adverse events.

Since they started giving the medicine [daily drops], I haven't seen any negative effects... The medicine has helped my infant to gain weight...Even when your infant got sick in the night they came and checked to know what happened...They will give you medicine, but when it's something serious they will take both of you [to the health facility and]...they will drive you back home. (IDI-P2)

Since I joined the study, my infant has not been having fever and all those health issues...I must thank the unit for their support because after my infant fell ill without my knowledge, we were taken to Keneba to get admitted and later referred to Fajara, where we spent more than two weeks treating my infant and during these periods everything was free for us including food...If not for the unit it would have been difficult for me...I cannot ever pay for what the unit gave me, because without them my infant would have died. (IDI-P4)

I am very happy...I have noticed many positive changes in my infant's health...It has turned my infant into a much stronger and healthier infant because I have seen the difference between him and other infants that didn't take it...both mentally and physically. (IDI-P12)

Mothers not only reflected on their experience of the intervention but also breastfeeding. Specifically, the importance of breastfeeding was noted, especially EBF until 6 months of age as well as the daily drops causing the infant to feed better.

It is very important because since my infant was born, I didn't give her water yet, all I give her is breastmilk...Some people were saying she is premature due to her size, but I kept on breastfeeding her and I didn't leave too much of a gap between the next feeding...She is much stronger compared to before...The more she drinks the medicine [daily drops] the more she breastfeeds too. (IDI-P2)

The medicine [daily drops] can even help the infant to eat more. You know if you are not healthy you won't be able to breastfeed as well, so the medicine will help to keep the infant healthier. (IDI-P5)

Affective attitude

Affective attitude is reported as mothers' attitudes once the trial had been initiated or hypothetically for the FGDs. It was categorised as the most deductive, along with intention. In the FGDs, issues were raised around the varying attitudes in the communities towards the intervention, and how this may affect participation in the trial. A noted theme was the desire to see immediate impacts from an intervention. Additionally, it was suggested that the iron supplementation could cause confusion around the need for continuous EBF and that to correct this issue, education around the intervention is key. Illustrative quotes can be found in Table 4.

However, in the IDIs, mothers seemed enthusiastic about the offer to have an intervention that may benefit their infant's health and were very happy with the treatment they received during the study.

The unit, the way they work is what satisfies me. And how they handle people if they are sick. It is civilised that's why. I know they will not do any harm to the infant. Everything they do will be towards the health of the infant, that's what I admire. (IDI-P2)

I am very impressed with the work of this study, because they have the interest of their patients at heart and are ready to help anytime they are needed or called...I encouraged anyone that wants to join to join because their medicine [daily drops] is very good for infant and...it is for the betterment of your infant. (IDI-P11)

Intention

Intention was mothers' intention to join the study or hypothetically for the FGDs. It was categorised as the most deductive, along with affective attitude.

It was noted in FGDs that mothers may find the guidance of giving iron supplements during the period of EBF confusing, but to help mothers be more inclined to be involved in a study, sensitisation and information sharing were important. Additionally, it was stressed that the sharing of information must be done through culturally accepted channels such as village heads and local stakeholders. Illustrative quotes can be found in Table 4.

In the IDIs, mothers expressed their desire to be involved in the study, because they knew it could enhance their infant's access to healthcare. Sometimes this even meant disregarding concerns other mothers had expressed about clinical trials. Often noted was the need for their husbands to give permission for the infant to join the study.

I said "ah my infant won't join the unit, because I hear that they say the unit used to take people's blood". When my husband came...he said, "take the infant to the unit, don't you know that the unit takes more care than these other hospitals...when your infant is sick, they will come up to your house to attend to your infant". (IDI-P5)

I have seen that their work was very good, they give help to people and there are good results in the health of the infants...I joined it willingly after consulting my husband, who also agreed to it...to maintain my infant's good health. (IDI-P7)

DISCUSSION

Overall, there was no indication that stakeholders or mothers were resistant to iron supplementation of breastfed infants. The FGDs indicated a general sense of enthusiasm for the intervention, but also a concern due to confusion around nutritional messaging. However, the intervention was acceptable to mothers with limited mention of side effects and enthusiasm for participating in clinical trials on iron supplementation. Key to acceptability is the proper dissemination of information and inclusion of all family members in decision-making, especially fathers. When considering scaleup there were mixed opinions on the practicality of daily supplementation, as well as concerns around costs.

IDIs

The IDIs explored maternal acceptability of iron supplementation in infants on the microlevel. Significant burdens among the Gambian population were indicated to be access to healthcare and transportation to hospitals and health centres. It was often noted that outside of the trial,

prescription medicine needed to be paid for, which was a financial burden. While the answers were varied, some mothers did flag that supplementing the infants themselves in a real-world setting may be a burden due to other commitments or forgetting. A qualitative study conducted in The Gambia looking at issues around care found that a main barrier to getting the necessary treatment was associated transport costs.²⁷ A meta-analysis of iron and folic acid supplements in Ethiopian women found forgetfulness to be a major barrier to adherence.²⁸

For ethical considerations, side effects were rarely noted, and when they were it was in the context of how helpful the trial was in responding and providing healthcare for these incidences. No mother saw the side effects as negative or due to the intervention itself. The issue around fear of blood sample collection was raised as there are concerns about what happens with the blood once it is drawn and some confusion that the research unit may sell blood. A study looking at the misconceptions around blood samples and its impacts on trial participation in a village in rural Gambia found that originally finger-pricking posed no problem, but as soon as 'rumours' about the use of the blood and concerns around the health implications of blood loss started to spread, recruitment rates suffered.²⁹

Some mothers were also worried about the cost of purchasing the iron if it was for sale in the future. This has been seen in the past in The Gambia with barriers to healthcare due to related costs.³⁰

Maternal experiences were very positive when describing the daily drops as bringing them 'happiness' and being 'very good'. Mothers also appreciated the ability to be able to call the team at any time if there was a health issue with their infant, noting prompt free care and transportation, if needed. A previous qualitative study in The Gambia also found this increase in healthcare to be the main facilitator in an iron supplementation trial in children.³¹ Some mothers even noted physical health increases in their infants such as weight gain, getting 'stronger' and less sicknesses. It was believed that the supplements helped the infants breastfed more effectively, which made mothers happy due to breastfeeding being beneficial for the infant.

Again, like with experiences, mothers' affective attitudes towards the intervention were positive. Mothers often noted that if someone wants to help you in the healthcare of your infant, and at no cost, why would one reject such help, especially in a setting such as The Gambia where access to and affordability of healthcare was as a noted burden. The care given to the infants in the clinical trial was described in a positive light, encouraging some mothers to express that they would never want to quit such an intervention. The desire to be involved in additional trials was expressed. These experiences are reflective of other qualitative literature looking at the barriers, facilitators, and benefits of an iron supplementation trial in children in The Gambia.³¹

Lastly, maternal enthusiasm to join the study was high. Like their affective attitude, mothers mentioned that the study would enhance their infant's health and if someone made such an offer, of course, they would take it. The issue around blood sample collection was noted, but that this was not a problem in deterring them from joining the study. The mothers also highlighted how crucial the father of the infant was in making the final decision for the infant's healthcare and needs. A study looking at participant retention in sub-Saharan Africa found that consent withdrawal due to the father not being involved at the point of consenting or parents no longer being comfortable with blood samples were the main reasons for not completing the trial.³²

FGDs

The FGDs explored the acceptability of iron supplementation in young infants with local stakeholders at the mesolevel. Multiple burdens were raised in the FGDs. For example, there were concerns around supplementing infants in a real-world setting, although not all agreed this would be a burden. It was noted that community sensitisation would be difficult as other family members may get involved with negative opinions, so it is crucial to be clear and consider education levels when doing so. Additionally, it must be made apparent that there would be no remuneration for joining the study, as other family members may ask how they would benefit. A previous study in The Gambia concluded that to overcome these burdens one must better inform and educate the participants and their caregivers.²⁹

In terms of ethical considerations, there may be confusion around the acceptability of supplementing infants during EBF. As mentioned in the NaNA FGD, there have been noncompliance issues with mothers taking iron and folic acid in The Gambia, so making it clear that mothers must still take this even if infants are supplemented, as well as the potential side effects infants may experience, must be well explained. These considerations can be managed through proper sensitisation within the communities. To facilitate clinical trials, health education has been found to be crucial to avoid misconceptions in The Gambia.²⁷

Maternal experience was perceived as positive by the FGDs, noting that with the right people being involved in sensitisation and answering all mothers' questions, they would be happy. An issue in the past was that study findings were not always disseminated to the community, so to ensure a positive experience, it was noted that findings should be shared with the local communities. From the maternal FGD, those who had past experience in trials were all positive. In the literature, a fundamental feature of community-based research is engaging with community members and disseminating the research findings.³³

Affective attitudes were influenced by other family members intervening, mothers wanting to see immediate benefits to their infants, and confusion around the ability to give iron during EBF. Again, it was mentioned that it was important to include husbands in these discussions and educate the potential participants on the reason for the intervention.

Intention may be inhibited by the confusion around the acceptability of giving iron during the period of EBF. It was noted as vital that information is shared through socially accepted channels and respects cultural hierarchies. In The Gambia, childcare was highlighted as a household decision, not just that of the mother. So informing the communities of the intervention through key stakeholders such as the community health workers, the village and compound head, in-laws and especially fathers were seen as crucial. Likewise, it was highlighted that to ensure families are willing to join a study, the messaging is consistent around the purpose of the intervention. Community sensitisation, allowing information to be passed through the appropriate hierarchy in villages in The Gambia, has been successful in the past.³⁴

Strengths and limitations

This study involved a wide array of stakeholders from mothers as the primary caregivers, to local field staff, to nutrition-focused government workers. This helps strengthen the growing body of implementation research needed for scale-up of clinical interventions, of which more is needed in low- and middle-income countries. In implementation studies, it is crucial to take a special interest in the user of the research, not just the outcomes, as well as involving a diverse array of key stakeholders.³⁵

Efforts were made to increase the comfort and reduce any power dynamic in the IDIs by having a young local female trained in qualitative interview practices conduct the interviews and in a chosen location by the participant. However, the interviewer was an employee of the research unit; therefore, answers from mothers may be slightly biased as praise for the unit was often expressed. A study in Zambia, South Africa, and Kenya emphasises the importance of matching the interviewer with the participant by age, sex, and native language to provide comfort for the participants.³⁶

Another limitation of this study is that it may be hard to translate to other settings. The research unit has been working in The Gambia for over 70 years, so the communities are more familiar with clinical trials.

Lastly, given that the main study was an efficacy trial, it was not possible to assess the full implementation and scaleup of such a trial in a real-world setting.

Applicability of the acceptability framework and future directions

Overall, the framework was useful for categorising the data into six main themes. However, the division of an inductive and deductive arm was not applicable. Instead, the data were interpreted to be on a spectrum of inductive to deductive, and the framework was therefore adapted accordingly. Additionally, some of the themes were felt to overlap as data could often be coded to more than one of the themes. The issue around blood samples was ethical, but also impacted intention. The issue of advising to give a supplement during the window of EBF and how this may cause confusion was an ethical consideration, but also affected intention, and fell under affective attitude. EBF also came up in experience during mothers' reflections. The need for proper sensitisation was also reoccurring in all themes apart from opportunity costs. The struggles experienced with sensitisation were raised as burdens in the FGDs. Iron supplements can cause side effects, which is an ethical consideration, and the need for proper sensitisation here was noted. In terms of improving mothers' experiences, proper sensitisation was also noted in the FGDs. Likewise, in the FGDs, the affective attitude was noted as being influenced by confusion around the intervention, which can be fixed with proper sensitisation and being sure all key community stakeholders, especially fathers, are involved. Lastly, for intention, proper sensitisation and the need for the father's consent greatly influenced if mothers wanted to be involved. Issues around the act of supplementing came up in opportunity costs, due to financial struggles, but also appeared as a burden as some mothers found they would struggle to remember and self-administer the drops in a real-world setting. A noted burden, when not enrolled in trials with the research unit, was transportation and access to proper healthcare in The Gambia, this came up in intention, affective attitude and experience as many mothers wanted to join the study for better access to healthcare and transport. They also found this enhanced their experience and improved their attitude towards the intervention. Lastly, the mother's gratitude towards the research unit and the intervention was apparent in her affective attitudes and experiences. While it was apparent that the framework covered all aspects of local stakeholder and maternal acceptability, the frequent overlap between themes implies it may help to view the framework as a continuum (Figure 1). This revised framework allows the researcher to look at the data as a continuum, as healthcare research is often complex and multifaceted. Additionally, this framework does not group findings as either inductive or deductive, because in cultural contexts such as The Gambia, views are often to some extent both inductive and deductive. This revised framework should facilitate future healthcare research around acceptability.

CONCLUSIONS

Overall, at the micro-level, the intervention was found to be acceptable, with limited mention of side effects or concerns around the intervention or participation in the clinical trial. However, when looking at the scale-up to the real-world setting, there were mixed opinions on the burden of daily supplementation, as well as concern around the cost of the iron. At the meso-level, there was excitement about this new intervention, but also concern over the acceptability of the intervention due to confusion around nutritional messaging. Noted as central to ensuring the acceptability of an intervention was the need for proper dissemination of information and inclusion of all family members, especially fathers, in decision making. This study provides insight into factors central to the acceptability of nutritional interventions in young infants. Given there was no indication that stakeholders or mothers were resistant to the intervention, this data support further effectiveness trials of targeted iron in this age group.

AUTHOR CONTRIBUTIONS

This study was conceptualised by Sophie E. Moore, Isabella Stelle and Sergio A. Silverio. Isabella Stelle and Sergio A. Silverio formulated the study design. Isabella Stelle was responsible for conducting and managing the study, along with the help of Mamadou Bah and Saidy Khan. Isabella Stelle carried out data analysis with 25% of data being analysed by Sergio A. Silverio to ensure rigour. Any disputes were mediated by Sophie E. Moore. Carla Cerami is the PI on the parent study in which this study was imbedded. Data synthesis was carried out by Isabella Stelle. Isabella Stelle wrote the first draft of this manuscript. Final drafts were edited and approved by all authors. Sophie E. Moore and Sergio A. Silverio are joint senior authors.

ACKNOWLEDGEMENTS

We would like to thank the MRCG@LSHTM Scientific Coordinating Committee, The Gambian Ethics Committee Board and the King's College London Ethics Committee for their support and input into this trial. We would like to thank our collaborators for their invaluable input, specifically, Prof Jane Sandall CBE, Dr Phil James and Dr Cristina Fernandez Turienzo. We would like to thank Pa Ansu Sanneh. We would like to thank the entire study field team, especially Mr Kebba Bajo, as without their help this study would not have been possible. Lastly, we would like to thank all study participants for their time and invaluable insight. This work was supported by funding from DFID/NIHR/MRC/Wellcome Joint Global Health Trials—Call 9 [MRT003960/1] to Carla Cerami. This trial was sponsored by MRCG@LSHTM. Sophie E. Moore is supported by a Wellcome Trust Senior Research Fellowship (220225/Z/20/Z). Sergio A. Silverio is supported by the National Institute for Health Research Applied Research Collaboration South

London (NIHR ARC South London) at King's College Hospital NHS Foundation Trust. The views expressed are those of the authors and not necessarily those of the NIHR or the Department of Health and Social Care. The MRC Unit The Gambia at LSHTM is supported by core funding MC-A760-5QX00 by the UK MRC and the UK Department for International Development (DFID) under the MRC/ DFID Concordat agreement. Mamadou Bah, Andrew M. Prentice, and Carla Cerami funded in part through this arrangement.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy.

ETHICS STATEMENT

The study was conducted in accordance with the principles set forth in the ICH Harmonised Tripartite Guideline for Good Clinical Practice and the Declaration of Helsinki in its current version, whichever affords greater protection to the participants. The studies have been reviewed by the MRCG@LSHTM Scientific Coordinating Committee, The Gambian Government/MRC Unit The Gambia Joint Ethics Committee, the Ethics Committee at LSHTM, and the Ethics Committee at King's College London. Amendments went through the same process, and as necessary, changes were communicated with investigators, trial participants and registries, journals and regulators.

ORCID

Isabella Stelle  <http://orcid.org/0000-0003-2023-7614>
Sergio A. Silverio  <http://orcid.org/0000-0001-7177-3471>
Sophie E. Moore  <http://orcid.org/0000-0003-1650-3238>

REFERENCES

- Wang H, Naghavi M, Allen C, et al. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet*. 2016;388(10053):1459-1544. doi:10.1016/S0140-6736(16)31012-1
- Miller JL. Iron deficiency anemia: a common and curable disease. *Cold Spring Harbor Perspect Med*. 2013;3(7):a011866. doi:10.1101/cshperspect.a011866
- Armitage AE, Agbla SC, Betts M, et al. Rapid growth is a dominant predictor of hepcidin suppression and declining ferritin in Gambian infants. *Haematologica*. 2019;104(8):1542-1553. doi:10.3324/haematol.2018.210146
- Bhutta ZA, Ahmed T, Black RE, et al. What works? Interventions for maternal and child undernutrition and survival. *Lancet*. 2008;371(9610):417-440. doi:10.1016/S0140-6736(07)61693-6
- Christian P, Mullany LC, Hurley KM, Katz J, Black RE. Nutrition and maternal, neonatal, and child health. *Semin Perinatol*. 2015;39(5): 361-372. doi:10.1053/j.semperi.2015.06.009
- Goudet SM, Bogin BA, Madise NJ, Griffiths PL. Nutritional interventions for preventing stunting in children (birth to 59 months) living in urban slums in low- and middle-income countries (LMIC). *Cochrane Database Syst Rev*. 2019;2019:CD011695. doi:10.1002/14651858.CD011695.pub2
- Sekhon M, Cartwright M, Francis JJ. Acceptability of healthcare interventions: an overview of reviews and development of a theoretical framework. *BMC Health Serv Res*. 2017;17(1):88. doi:10.1186/s12913-017-2031-8
- Becker CB, Darius E, Schaumberg K. An analog study of patient preferences for exposure versus alternative treatments for posttraumatic stress disorder. *Behav Res Ther*. 2007;45(12):2861-2873. doi:10.1016/j.brat.2007.05.006
- Sidani S, Epstein DR, Bootzin RR, Moritz P, Miranda J. Assessment of preferences for treatment: validation of a measure. *Res Nurs Health*. 2009;32(4):419-431. doi:10.1002/nur.20329
- Tarrier N, Liversidge T, Gregg L. The acceptability and preference for the psychological treatment of PTSD. *Behav Res Ther*. 2006;44(11): 1643-1656. doi:10.1016/j.brat.2005.11.012
- Dillip A, Alba S, Mshana C, et al. Acceptability—a neglected dimension of access to health care: findings from a study on childhood convulsions in rural Tanzania. *BMC Health Serv Res*. 2012;12(1):113. doi:10.1186/1472-6963-12-113
- Staniszewska S, Crowe S, Badenoch D, Edwards C, Savage J, Norman W. The PRIME project: developing a patient evidencebase. *Health Expect*. 2010;13(3):312-322. doi:10.1111/j.1369-7625.2010.00590.x
- Diepeveen S, Ling T, Suhrcke M, Roland M, Marteau TM. Public acceptability of government intervention to change health-related behaviours: a systematic review and narrative synthesis. *BMC Public Health*. 2013;13(1):756. doi:10.1186/1471-2458-13-756
- Stok FM, de Ridder DTD, de Vet E, et al. Hungry for an intervention? Adolescents' ratings of acceptability of eating-related intervention strategies. *BMC Public Health*. 2016;16(1):5. doi:10.1186/s12889-0152665-6
- Fisher P, McCarney R, Hasford C, Vickers A. Evaluation of specific and non-specific effects in homeopathy: feasibility study for a randomised trial. *Homeopathy*. 2006;95(4):215-222. doi:10.1016/j.homp.2006.07.006
- Hommel KA, Hente E, Herzer M, Ingerski LM, Denson LA. Telehealth behavioral treatment for medication nonadherence: a pilot and feasibility study. *Eur J Gastroenterol Hepatol*. 2013;25(4):469-473. doi:10.1097/MEG.0b013e32835c2a1b
- Borrelli B, Sepinwall D, Ernst D, et al. A new tool to assess treatment fidelity and evaluation of treatment fidelity across 10 years of health behavior research. *J Consult Clin Psychol*. 2005;73(5):852-860. doi:10.1037/0022-006x.73.5.852
- Proctor EK, Landsverk J, Aarons G, Chambers D, Glisson C, Mittman B. Implementation research in mental health services: an emerging science with conceptual, methodological, and training challenges. *Admin Policy Ment Health Ment Health Serv Res*. 2009;36(1):24-34. doi:10.1007/s10488-008-0197-4
- Nilsen P. Making sense of implementation theories, models and frameworks. *Implement Sci*. 2015;10(1):53. doi:10.1186/s13012-015-0242-0
- Brooks J, McCluskey S, Turley E, King N. The utility of template analysis in qualitative psychology research. *Qual Res Psychol*. 2015;12(2):202-222. doi:10.1080/14780887.2014.955224
- Stelle I, Bah M, Silverio SA, et al. Iron supplementation of breastfed Gambian infants from 6 weeks to 6 months of age: protocol for a randomised controlled trial. *Wellcome Open Res*. 2022;7(16):1-16. doi:10.12688/wellcomeopenres.17507.1
- Carlsen B, Glenton C. What about N? A methodological study of sample-size reporting in focus group studies. *BMC Med Res Methodol*. 2011;11:26. doi:10.1186/1471-2288-11-26

23. Coyne I. Sampling in qualitative research. Purposeful and theoretical sampling; merging or clear boundaries? *J Adv Nurs*. 1997;26(3): 623-630. doi:10.1046/j.1365-2648.1997.t01-25-00999.x
24. Wang H, Naghavi M, Allen C, et al. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet*. 2016;388(10053):1459-1544. doi:10.1016/S0140-6736(16)31012-1
25. Miller JL. Iron deficiency anemia: a common and curable disease. *Cold Spring Harbor Perspect Med*. 2013;3(7):a011866. doi:10.1101/cshperspect.a011866
26. Armitage AE, Agbla SC, Betts M, et al. Rapid growth is a dominant predictor of hepcidin suppression and declining ferritin in Gambian infants. *Haematologica*. 2019;104(8):1542-1553. doi:10.3324/haematol.2018.210146
27. Bhutta ZA, Ahmed T, Black RE, et al. What works? Interventions for maternal and child undernutrition and survival. *Lancet*. 2008;371(9610):417-440. doi:10.1016/S0140-6736(07)61693-6
28. Christian P, Mullany LC, Hurley KM, Katz J, Black RE. Nutrition and maternal, neonatal, and child health. *Semin Perinatol*. 2015;39(5): 361-372. doi:10.1053/j.semperi.2015.06.009
29. Goudet SM, Bogin BA, Madise NJ, Griffiths PL. Nutritional interventions for preventing stunting in children (birth to 59 months) living in urban slums in low- and middle-income countries (LMIC). *Cochrane Database Syst Rev*. 2019;2019:CD011695. doi:10.1002/14651858.CD011695.pub2
30. Sekhon M, Cartwright M, Francis JJ. Acceptability of healthcare interventions: an overview of reviews and development of a theoretical framework. *BMC Health Serv Res*. 2017;17(1):88. doi:10.1186/s12913-017-2031-8
31. Becker CB, Darius E, Schaumberg K. An analog study of patient preferences for exposure versus alternative treatments for posttraumatic stress disorder. *Behav Res Ther*. 2007;45(12):2861-2873. doi:10.1016/j.brat.2007.05.006
32. Sidani S, Epstein DR, Bootzin RR, Moritz P, Miranda J. Assessment of preferences for treatment: validation of a measure. *Res Nurs Health*. 2009;32(4):419-431. doi:10.1002/nur.20329
33. Tarrier N, Liversidge T, Gregg L. The acceptability and preference for the psychological treatment of PTSD. *Behav Res Ther*. 2006;44(11): 1643-1656. doi:10.1016/j.brat.2005.11.012
34. Dillip A, Alba S, Mshana C, et al. Acceptability—a neglected dimension of access to health care: findings from a study on childhood convulsions in rural Tanzania. *BMC Health Serv Res*. 2012;12(1):113. doi:10.1186/1472-6963-12-113
35. Stanisewska S, Crowe S, Badenoch D, Edwards C, Savage J, Norman W. The PRIME project: developing a patient evidencebase. *Health Expect*. 2010;13(3):312-322. doi:10.1111/j.1369-7625.2010.00590.x
36. Diepeveen S, Ling T, Suhrcke M, Roland M, Marteau TM. Public acceptability of government intervention to change health-related behaviours: a systematic review and narrative synthesis. *BMC Public Health*. 2013;13(1):756. doi:10.1186/1471-2458-13-756
37. Stok FM, de Ridder DTD, de Vet E, et al. Hungry for an intervention? Adolescents' ratings of acceptability of eating-related intervention strategies. *BMC Public Health*. 2016;16(1):5. doi:10.1186/s12889-0152665-6
38. Fisher P, McCarney R, Hasford C, Vickers A. Evaluation of specific and non-specific effects in homeopathy: feasibility study for a randomised trial. *Homeopathy*. 2006;95(4):215-222. doi:10.1016/j.homp.2006.07.006
39. Hommel KA, Hente E, Herzer M, Ingerski LM, Denson LA. Telehealth behavioral treatment for medication nonadherence: a pilot and feasibility study. *Eur J Gastroenterol Hepatol*. 2013;25(4):469-473. doi:10.1097/MEG.0b013e32835c2a1b
40. Borrelli B, Sepinwall D, Ernst D, et al. A new tool to assess treatment fidelity and evaluation of treatment fidelity across 10 years of health behavior research. *J Consult Clin Psychol*. 2005;73(5):852-860. doi:10.1037/0022-006x.73.5.852
41. Proctor EK, Landsverk J, Aarons G, Chambers D, Glisson C, Mittman B. Implementation research in mental health services: an emerging science with conceptual, methodological, and training challenges. *Admin Policy Ment Health Ment Health Serv Res*. 2009;36(1):24-34. doi:10.1007/s10488-008-0197-4
42. Nilsen P. Making sense of implementation theories, models and frameworks. *Implement Sci*. 2015;10(1):53. doi:10.1186/s13012-015-0242-0
43. Brooks J, McCluskey S, Turley E, King N. The utility of template analysis in qualitative psychology research. *Qual Res Psychol*. 2015;12(2):202-222. doi:10.1080/14780887.2014.955224
44. Stelle I, Bah M, Silverio SA, et al. Iron supplementation of breastfed Gambian infants from 6 weeks to 6 months of age: protocol for a randomised controlled trial. *Wellcome Open Res*. 2022;7(16):1-16. doi:10.12688/wellcomeopenres.17507.1
45. Carlsen B, Glenton C. What about N? A methodological study of sample-size reporting in focus group studies. *BMC Med Res Methodol*. 2011;11:26. doi:10.1186/1471-2288-11-26
46. Coyne I. Sampling in qualitative research. Purposeful and theoretical sampling; merging or clear boundaries? *J Adv Nurs*. 1997;26(3): 623-630. doi:10.1046/j.1365-2648.1997.t01-25-00999.x
47. Higginbottom GMA. Sampling issues in qualitative research. *Nurse Res*. 2004;12:7-19. doi:10.7748/nr2004.07.12.1.7.c5927
48. King N, Brooks JM. *Template Analysis for Business and Management Students*. Sage; 2017.
49. Eriksen KG, Johnson W, Sonko B, Prentice AM, Darboe MK, Moore SE. Following the World Health Organization's Recommendation of exclusive breastfeeding to 6 months of age does not impact the growth of rural Gambian infants. *J Nutr*. 2017;147(2):248-255. doi:10.3945/jn.116.241737
50. Eastwood SV, Hill PC. A gender-focused qualitative study of barriers to accessing tuberculosis treatment in The Gambia, West Africa. *Int J Tuberc Lung Dis*. 2004;8(1):70-75.
51. Desta M, Kassie B, Chanie H, et al. Adherence of iron and folic acid supplementation and determinants among pregnant women in Ethiopia: a systematic review and meta-analysis. *Reprod Health*. 2019;16(1):182. doi:10.1186/s12978-019-0848-9
52. O'Neill S, Dierickx S, Okebe J, et al. The importance of blood is infinite: conceptions of blood as life force, rumours and fear of trial participation in a fulani village in rural Gambia. *PLoS One*. 2016;11(8):e0160464. doi:10.1371/journal.pone.0160464
53. Broekhuizen H, Fehr A, Nieto-Sanchez C, et al. Costs and barriers faced by households seeking malaria treatment in the Upper River Region, The Gambia. *Malar J*. 2021;20(1):368. doi:10.1186/s12936021-03898-6
54. Stelle I, McDonagh LK, Hossain I, Kalea AZ, Pereira DIA. The IHATGUT iron supplementation trial in rural Gambia: barriers, facilitators, and benefits. *Nutrients*. 2021;13(4):1140. doi:10.3390/nu13041140
55. Idoko OT, Owolabi OA, Odutola AA, et al. Lessons in participant retention in the course of a randomized controlled clinical trial. *BMC Res Notes*. 2014;7:706. doi:10.1186/1756-0500-7-706
56. McDavitt B, Bogart LM, Mutchler MG, et al. Dissemination as dialogue: building trust and sharing research findings through community engagement. *Prev Chronic Dis*. 2016;13:150473. doi:10.5888/pcd13.150473
57. Dierickx S, Gryseels C, Mwesigwa J, et al. Factors associated with non-participation and non-adherence in directly observed mass drug administration for malaria in the Gambia. *PLoS One*. 2016;11(2):e0148627. doi:10.1371/journal.pone.0148627

58. Peters DH, Adam T, Alonge O, Agyepong IA, Tran N. Republished research: implementation research: what it is and how to do it: implementation research is a growing but not well understood field of health research that can contribute to more effective public health and clinical policies and programmes. This article provides a broad definition of implementation research and outlines key principles for how to do it. *Br J Sports Med.* 2014;48:731-736. doi:10.1136/bmj.f6753
36. Milford C, Kriel Y, Njau I, et al. Teamwork in qualitative research: descriptions of a multicountry team approach. *Int J Qual Methods.* 2017;16(1):160940691772718. doi:10.1177/1609406917727189

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Stelle I, Bah M, Saidykhan M, et al. Maternal acceptability of iron supplementation in young breastfed rural Gambian infants. *Reprod Female Child Health.* 2023;1-12. doi:10.1002/rfc2.29.

9 DISCUSSION

9.1 KEY FINDINGS

ID remains one of the leading contributors to the global burden of disease. Further, ID during critical phases of development can have lasting impacts on growth and developmental outcomes across the life course. This Thesis highlights the first six months of life as a missed opportunity for iron interventions, when often only EBF and delayed cord clamping are recommended. This final section of the Thesis serves to contextualize this area of research within the broader global health framework, while addressing the remaining questions, and making recommendations for future programmes, policy, and research, as well as identifying the strengths and limitations of the research conducted.

Published research on iron supplementation in infants under six months of age is limited, with even less evidence around the impacts of early interventions on behavioural outcomes, including infant feeding practices at this early age. The systematic review of nutrition interventions in infants under six months of age (N=24 trials) conducted as part of the research included in this Thesis (Chapter 3), showed that infants benefit biochemically from iron supplementation (105). An RCT conducted as part of this Thesis (Chapter 6) in rural breastfed six to ten week old Gambian infants (*Iron Babies*; N=101) for 98 days with daily oral iron (versus placebo), likewise found that supplementation improved iron status, with no adverse consequences (301). However, the systematic review found less clear evidence of benefit from additional micronutrients, or of iron on outcomes for growth, morbidity and/or mortality, and neuro-behavioural development. This was largely a consequence of low study numbers and design heterogeneity. In the Iron Babies trial, there was also no evidence that early iron intervention had any adverse impact on infant mode of feeding or growth, but the trial was limited by a small sample size (Chapter 7). A large portion of the trials (88%) included in the systematic review were conducted in LMICs, reflecting the large burden that ID plays in such resource limited settings. These findings suggest that giving iron to young, breastfed infants benefits iron status, while not adversely impacting on feeding practices or early growth. Given the beneficial effects, and no safety issues raised, from the Iron Babies trial, larger well-powered randomised controlled effectiveness trials are required to determine whether routine supplementation with iron or MMNs containing iron should commence before six months of life in contexts with diets low in iron and high rates of breastfeeding.

Efficacy studies show early nutrition interventions can improve infant nutrition status, however, understanding caregiver (most often mothers') acceptability is required for implementation of such interventions. The systematic review of qualitative research included in this Thesis (Chapter 4)

identified 37 relevant studies and found that positive perceptions and enthusiasm for interventions were frequently reported (132). The qualitative study embedded into Iron Babies exploring acceptability through FGDs with local stakeholders (four FGDs, N=19) and IDIs with mothers whose infants were enrolled in the clinical trial (N=14) likewise found no indication that stakeholders or mothers were resistant to early iron supplementation of breastfed infants (Chapter 8) (307). In the systematic review, some studies cited both high and low acceptability, with 89% of studies (N=33) noting high acceptability and 57% of studies (N=21) citing low acceptability. The main benefit from early nutrition interventions in infants and children under 24 months of age from the review was noted as increased appetite, with low acceptability most often a consequence of apparent side effects such as gastrointestinal issues, appetite loss, and stained teeth. From the Iron Babies IDIs with mothers, there was high acceptability and enthusiasm for the intervention, with limited mention of side effects, as well as no difference in adherence rates by intervention arm, and overall extremely high compliance rates with no indication that participation in the trial disrupted infant feeding practices. From the FGDs there was a general sense of enthusiasm for the intervention, but also concern due to potential confusion around nutritional messaging. When looking at scaleup to the real-world setting, mothers had mixed opinions on the practicality of remembering supplementation daily, as well as concerns around costs. In addition, it was suggested that proper dissemination of information and inclusion of all family members in decision making, especially fathers, is key to acceptability. For the development and scale-up of future interventions, mitigation strategies and education around common side effects are important for caregiver acceptability, as well as understanding both positive and negative caregiver perceptions. Given no indication that stakeholders or mothers from both the literature and Iron Babies were resistant to the intervention, this data supports further effectiveness trials of targeted iron supplementation in this age group.

The following section reviews these findings in relation to current policy and programmatic considerations around early interventions.

9.2 CAUTIONS AROUND EARLY INTERVENTIONS

As highlighted in Section 2.3.3 of this Thesis, concerns exist regarding potential adverse consequences of iron overload in infants. Given routine iron supplementation is only recommended for infants from six to 23 months of age when the anaemia prevalence is over 40%, the safety of interventions must be considered.

Iron overload should be monitored, and so supplementation should be avoided among population groups with iron repletion or among individuals with certain genetic disorders such as hemochromatosis (321). The WHO acknowledges that robust evidence around adverse effects of iron

overload are lacking in the current literature around infants, children, and in post-partum women (24, 322).

From the wider literature, there is inconsistent data of the impact of iron supplementation on growth, which seem dependent on infant iron status at baseline. A systematic review looking at the effects of iron overload in infants and children found that iron replete infants in a high income setting given iron supplementation from four to nine months of age had lower length gain than infants with low iron status supplemented in the same manner (80). Another study found that breastfed infants in a HIC given daily iron drops from four to nine months of age had reduced weight gain and a trend towards reduced length gain compared to the placebo group (323). Lastly, a third study showed no adverse effects on growth in breastfed Canadian infants supplemented daily with iron drops from one to six months of age (324). A systematic review and meta-analysis over 40,000 children from four to 23 months of age showed that daily iron supplementation vs placebo showed slightly lower length and weight gains but no difference in final length, length of age, weight, or weight for age (325). Iron Babies showed no adverse effects from the early iron intervention on mode of feeding or growth parameters (110, 111), and the quantitative and qualitative systematic reviews found no conclusive outcomes of negative impacts or perceptions of early iron supplementation on growth (105, 132).

Given compliance issues around iron supplementation in pregnancy due to gastrointestinal side effects, these side effects should also be carefully considered for infants (326). In the high income setting where infants were given iron supplements from four to nine months of age, iron replete infants had a higher rate of diarrhoea, whereas iron deplete infants had lower rates than those given placebo (80). In the large metanalysis and systematic review of children from four to 23 months of age, iron supplementation increased the risk of vomiting and fever (98).

This Thesis explored both quantitatively and qualitatively the impact of iron interventions on infant feeding practices and infant growth and there is little evidence that iron impacts on mode of feeding or growth (105, 132). While the data presented in this Thesis raised no concerns for such cautions, future programmes and policy should always consider cautions around iron overload and side effects.

9.3 CAREGIVER ACCEPTABILITY OF EARLY INTERVENTIONS

A novel component of the research presented in this Thesis was the inclusion of a focus on maternal, caregiver, and stakeholder perceptions around early iron interventions. Given the concerns over the safety of iron, layered on other potentially conflicting public health messaging, such as EBF campaigns, it is essential to understand facilitators and barriers to future iron intervention programmes. Caregiver acceptability is a key component for implementation.

From the wider scientific literature, iron interventions are generally well accepted by caregivers and parents of infants and children, as caregivers recognise the importance of iron for their infant's and child's health and development and are willing to comply with supplementation and/or fortification of foods (327). In a recent study examining caregivers' acceptability of MMN supplementation in infants six to 23 months of age, perception and trust regarding the health benefits were critical elements for acceptability of MMNs (328). As supported by the qualitative work in this Thesis from both the systematic review and work in The Gambia, early nutrition interventions in infants and children under 24 months of age were generally well accepted by caregivers, as well as mothers understanding the benefits of giving young infants iron supplements (132, 307).

As noted in the qualitative systematic review, an important consideration to increase acceptability of early iron interventions is addressing potential side effects, such as gastrointestinal side effects like changes in stool colour with caregivers (132). Other trials have noted the importance of the sensory properties of interventions, such as the taste and smell of MMN interventions (328), and so ensuring these issues are considered and evaluated as part of programme design is imperative.

As supported from the findings of this Thesis around the importance of education around interventions, as well as involving family members being important (132, 307), the literature shows that providing adequate education and information to caregivers about the benefits of iron interventions, as well as potential side effects and their mitigation, can contribute to greater acceptability and adherence (329). The acceptability of interventions in infants and young children must be expanded beyond the immediate caregivers, such as to fathers, grandparents, and other care providers with influences.

Overall, iron interventions demonstrate benefits in improving iron status and are generally well accepted by caregivers, however, mitigating side effects and providing adequate education are important for acceptability and adherence to interventions.

9.4 CONTINUUM OF CARE

When combined, the research presented in this Thesis highlights the need for a continuum of care approach; rethinking how to best serve mothers and infants, by not separating ante- and post-natal programmes. This entails a more integrated approach across the life course for iron supplementation and prevention of ID, encompassing interventions that best suit the needs of women of reproductive age, infants, and children. The combined WHO, United Nations Childrens Fund (UNICEF), United Nations Population Fund (UNFPA) and The World Bank working document titled *"Accelerated implementation of maternal and newborn continuum of care as part of improving reproductive health"*

highlights the need for an approach integrating nutrition programmes into various sectors, across the life course (330).

The 2021 Lancet series on nutrition, spotlighting nutrition-related interventions in women of reproductive age, infants, and children, found strong evidence for reducing ID and IDA was limited to delayed cord clamping for PTB infants and breastfeeding promotion for infants under six months of age (325). For children under five years of age, there was moderate evidence for MNPs to reduce ID and IDA (325). Lastly, for reducing ID and IDA in pregnant women, there was strong evidence for IFA supplementation or, in at risk populations, MMN supplements (325). However, delivering these interventions individually is not sufficient and may miss vulnerable infants under six months of age, so a focus on interventions to reduce ID and IDA across the life course must be taken (326). Although iron therapy in pregnancy is effective (16), there are several barriers such as access to antenatal care, ensuring a continuous supply of IFA across gestation, and compliance to daily supplementation when side effects are common. These barriers inhibit optimal coverage, which would otherwise ensure all infants are born with an endowment sufficient to last through the duration of exclusive breastfeeding. Such barriers also impact on a mothers' own status and if supplementation stops at delivery, which it often does, women with low iron status who experience blood loss during labour enter lactation with low stores, impacting their own health and wellbeing. Programmes that extend maternal supplementation beyond postpartum and consider direct supplementation to infants may offer benefit beyond the existing siloed programmes. By considering the interconnectedness of these life stages and their individual needs, interventions can be tailored to promote optimal nutrition and overall health across different life stages. By starting with iron interventions at least in pregnancy, and continuing them post-partum and into early life, later deficiencies, and negative health outcomes due to early life deficiencies, may be prevented.

9.4.1 Integrating nutrition programmes

A further strategy to improve iron status among women of reproductive age and young infants, beyond existing programmes, is the integration of programmes that focus on both nutrition-specific and nutrition-sensitive approaches. Integrated approaches, beyond just iron supplementation in isolation, to address multiple factors contributing to ID and to ensure a sustained impact across different life stages are relevant. For example, recent trials have shown limited effects of other single nutrition-specific interventions, such as lipid-based nutrient supplements, on growth and development outcomes (331-333). ID has multiple aetiologies, with factors such as infection control improving the response to iron supplementation (334). Therefore, in environments where nutritional intakes are sub-optimal, nutrition interventions alone are unlikely to yield positive growth results and multiple stressors should be targeted at once (335-337). In addition to supplementation, combining

dietary diversification and/or fortification has been recommended as a comprehensive ID prevention strategy (338). Likewise, nutrition education and counselling alongside supplementation are essential components of an integrated approach, aiming to improve dietary diversity, enhance iron absorption through dietary strategies, and promote healthy lifestyles (339). Nutrition education and counselling can empower individuals to make informed choices about their diet and iron intake.

As highlighted by the qualitative research included in this Thesis (Chapter 4 and 8), education and community involvement are key to acceptability of an intervention (132, 307). In line with this, the aforementioned working document highlights the need for support, development, and strengthening of human resources in line with the national needs for women of reproductive age, and maternal and newborn health, through the updating of curricula, and enhancing capacity to train, deploy, support, and maintain skilled workers (330). As mentioned in both the qualitative components of this Thesis, there is a need for considering cost implications when scaling up and feasibility in a real-world setting (132, 307). The working document notes a need for continued advocacy for removing financial barriers to access of services, increasing health budget allocation, and ensuring sufficient financial support to reproductive health (330). Programmes focused on ameliorating the burden of IDA need to consider these wider issues.

The continuum of care model can be further strengthened by additional integrated approaches to improve iron status, like those mentioned above. Overall, an integrated approach encompassing iron interventions alongside other nutrition-specific and nutrition-sensitive interventions from infancy to pregnancy, along with nutrition education and counselling, is relevant for preventing ID and promoting optimal health outcomes across the life course.

9.5 REMAINING QUESTIONS

Several questions remain, necessitating further research around the importance of iron in infants, children, and women of reproductive age. These questions focus on aspects of iron metabolism, supplementation, and the long-term effects of ID on growth and development, and are addressed in turn, below.

9.5.1 Infants under six months of age

This Thesis has tackled many of the concerns around early iron supplementation, however it also raises other broader points. As detailed in Section 2.4 of this Thesis, there are multiple diagnostic criteria for iron depletion, ID, and anaemia, and exact cut-offs in infants should be further defined due to unclear definitions of anaemia, and markers such as Hb changing rapidly in this age group.

The effects of iron supplementation during this critical period for growth and development beyond the well understood short-term effects of haematology need further consideration. More research is needed around the longer-term outcomes of altered iron homeostasis during critical phases of development (such as brain and associated neurocognitive outcomes). Evidence is lacking in this area as highlighted through the quantitative systematic review presented in this Thesis. Only two studies had long-term follow ups in older children looking at impacts of early interventions on later neurobehavioral outcomes (at six to nine years of age) (340, 341).

Due to the low iron content in breastmilk, specifically in populations where women enter pregnancy and lactation with poor micronutrient status, the adequacy of breastmilk micronutrient status for the duration of EBF is still lacking robust evidence (342). Given trace elements compete for uptake mechanisms and excess of one trace element may inhibit absorption of another, excess iron has been shown to decrease absorption of zinc and copper in adults (343). Two studies of high versus low iron containing formula in infants under six months of age, found that infants receiving the high iron containing formula had lower copper absorption (344). A study examining iron absorption from breastmilk in iron supplemented versus placebo infants found no difference in breastmilk iron absorption at six months of age, while infants were still exclusively breastfed (36). However, at nine months of age, when infants were only partially breastfed since six months of age, iron absorption from breastmilk was lower in the iron supplemented group (36).

While Iron Babies found no differences in morbidity reports or adverse events between treatment groups and no instances of mortality, these findings need to be supported from larger trials where effectiveness is also assessed.

Addressing these concerns is important for guiding evidence-based recommendations and ensuring the safety and efficacy of iron interventions in infants under six months of age. As outlined in the limitations, Iron Babies was a small pilot trial conducted in a controlled environment and underpowered to look at morbidity, so while no risks were identified in this Thesis, further research is needed to provide more comprehensive insights into any potential risks associated with iron supplementation during this critical early life period.

9.5.2 Acceptability of iron interventions in young infants

The research presented in this Thesis has highlighted several issues regarding the acceptability of iron interventions in infants under six months of age from the perspective of caregivers. Such issues include understanding caregivers' attitudes, beliefs, and experiences around early iron interventions across various geographical, social, religious, and cultural settings for successful implementation of future interventions. The global context of caregivers' knowledge, perceptions, and awareness regarding IDA

and the benefits of iron interventions in infants under six months of age is vital for future implementation. Both the Iron Babies qualitative study and the qualitative systematic review found that dynamics among family members and proper dissemination of information were important for acceptability (132, 301, 307). Likewise, caregiver preference and usability of different delivery formats of iron interventions, such as drops, syrups, powders, or fortified foods, as well as factors like ease of administration, palatability, and convenience need further understanding. Understanding how interventions be integrated into existing practices and routines to improve acceptability and adherence remains outstanding for this age group. In the maternal IDIs from the Iron Babies qualitative study, feasibility and scale up, such as affordability and remembering daily supplementation, were raised as concerns (307). Additionally, what are the further global caregiver perceptions around changes in their infants' growth, behaviour, and overall well-being after initiating iron interventions? While many positive changes were seen through mothers' perspectives in the Iron Babies qualitative study, such as improved health, some negative outcomes were perceived from the general literature in the systematic review such as the infant being hyperactive, the infant getting sick, a decrease in overall health, general negative effect, and in one Peruvian peri-urban community, a mother cited impaired mental development as a concern (132, 307). Lastly, what are the most effective communication strategies to educate caregivers about the importance of iron interventions and address their concerns or misconceptions, as well as how can healthcare providers, community health workers, and other stakeholders effectively engage and communicate with caregivers to improve their acceptance and adherence to interventions? ID in this group of infants should be addressed, with the research presented suggesting no major barriers from the perspective of caregivers around acceptability, however, additional understanding in varied contexts is needed to confirm these findings.

9.5.3 Across the life course

While several areas for further research remain in terms of iron interventions in infants under six months of age as well as caregiver acceptability, it is also important to consider outstanding questions regarding iron interventions at other critical times during the life course, such as women of reproductive age and during pregnancy, as well as general knowledge gaps around iron interventions. How can interventions be effectively integrated into existing healthcare systems, nutrition programmes, and community-based initiatives to ensure an integrated approach to nutrition interventions? Additionally, what barriers, facilitators, and best practices for collaboration and coordination between various sectors, including healthcare, nutrition, agriculture, and education, exist? How can policies and interventions to optimize iron status and prevent deficiencies throughout the lifespan be integrated into current health systems? The aforementioned combined WHO, UNICEF,

UNFPA and The World Bank working document notes the need for continued and enhanced support to programmes that address the root causes of maternal mortality, such as adolescent education, income generation, gender equity, and nutrition (330). A willingness at global, regional, and country levels for collaborative work on health across the life course, for women or reproductive age, infants, and children was noted (330). Overall, addressing these outstanding issues and questions of an integrated approach to iron supplementation throughout the life course will contribute to the development of comprehensive and evidence-based strategies for improving iron status and preventing deficiencies in women of reproductive age, pregnancy, infants, and children.

9.6 STRENGTHS AND LIMITATIONS

A discussion of the strengths and limitations of each published manuscript are detailed within each Chapter (Chapter 3, 4, 7 and 8). However, below is a summary of the overall strengths and limitations from the work within this Thesis.

A key strength to the research presented was the inclusion of both quantitative and qualitative research from the start. Systematic reviews and meta-analyses commonly focus on quantitative data only, without considering key qualitative or implementation aspects. The balance of the two systematic reviews provided key background context by laying the foundation of existing literature around the topics of interest. The quantitative systematic review on iron and/or MMN interventions in infants under six months of age and the qualitative systematic review of caregiver perceptions of nutrition interventions in infants and children under 24 months of age allowed for a wider view as well as a more concise studying of the topics of interest, given that the full scope of published literature was detailed. Additionally, the data from the double blind, RCT design of the Iron Babies study that it took place in such a young population, the first iron supplementation trial in this age group in The Gambia, was a strength. This study demonstrated that iron supplementation in this young age group improved iron status, was safe, and had no adverse effects on feeding or early growth. Likewise, the qualitative adjunct study added key insights into important factors for implementation and scale up of an intervention like in Iron Babies into other real world settings. Implementation factors such as acceptability are important for further scale up of such interventions. These studies add key information to an age group that is understudied.

The main limitations of this Thesis were Iron Babies' small sample size (which was not powered to look at secondary outcomes such as morbidity, growth and feeding), the specificity of the context, the controlled environment in which the study took place, and no long-term follow up of infants. While this was intended as a proof of principle trial, larger scale RCTs, with long-term follow up of infant and childhood health and developmental outcomes are needed to confirm the Iron Babies findings. This

limited sample size restricted some of the secondary analyses and reduced power in others (345). It was a small study compared to others of infant growth and nutrition and, therefore, had a small absolute number of infants who were stunted, wasted, or underweight (7). This small sample size with limited follow up was likewise seen from the iron and/or MMN systematic review in which most studies had limited sample sizes and only two had longer term follow ups looking at neuro-development in later childhood (105). Long-term growth and developmental outcomes are important to examine in a larger trial. Lastly, The Gambia is a highly specific setting where clinical trials are welcomed due to the long standing relationship between the MRCG@LSHTM and local communities, so different settings must be considered (346). Overall, these limitations offer opportunities for further research with larger RCTs in various settings.

9.7 RECOMMENDATIONS FOR PROGRAMMES, POLICY, AND RESEARCH

The research presented in this Thesis supports that iron supplementation in infants under six months of age may be an important intervention to prevent IDA.

The results of the Iron Babies trial adds to previous evidence supporting that supplementary iron to young, breastfed infants is both haematologically effective and safe (105). While the evidence from the literature was inconclusive around the impacts of early iron interventions on growth, morbidity and/or mortality, and neuro-developmental outcomes (105), if larger scale RCTs across various populations find similar positive results to Iron Babies, universal iron supplementation programmes for infants under six months of age in populations with a high prevalence of anaemia may be appropriate. Education of supplementation and distribution of supplements in more rural contexts could be done through routine infant welfare visits, such as the EPI vaccine roll out visits used during Iron Babies, to ensure effective reach. Accessibility, education, and respecting community hierarchies was continuously noted as important for implementation both in the Iron Babies qualitative study and wider literature (132, 307). Iron supplementation in the form of drops or syrups, as used in Iron Babies, while ensuring the product's quality, safety, and bioavailability, may be most feasible for administration to young infants, but conclusive findings in this area still lack within the literature (132). Cost implications were often cited as a potential barrier when considering scale up of interventions, so programmes would be further supported by additional research on cost-effectiveness and scalability of different iron supplementation strategies in diverse settings (307). As evident through the skewed burden that ID has in LMICs from the quantitative systemic review, programmes and policies should consider strategies for scaling up iron supplementation interventions to reach the most vulnerable population (105). Overall, specific recommendations may vary based on the local context and prevalence of IDA, therefore, policymakers and healthcare professionals should consider local

evidence and guidelines when implementing iron supplementation programmes for infants under six months of age. Formative research must be conducted, and caregivers need to be provided with clear and comprehensive information about the benefits and potential side effects of iron supplementation, as well as support through counselling, education, and reminders to improve compliance and adherence to the supplementation regimen. In summary, a focus on conducting well-powered RCTs, mitigating side effects, providing caregiver education, considering caregiver perceptions, effective dissemination of information, and conducting effectiveness trials for scaling up interventions will contribute to future programmes and policy.

9.8 CONCLUSION

The research presented in this Thesis highlights a missed opportunity for intervening to improve infant iron status in young infants in contexts where, because of poor maternal status and rapid use of iron stores during periods of breastfeeding, infant status may be precarious. Iron supplementation during pregnancy is a cost-effective intervention that contributes to the overall health and wellbeing of both the mother and infant, shown to improve maternal iron status, reduce her risk of anaemia, and improve birth outcomes, especially in settings where dietary iron intakes are limited (16). However, infants globally still suffer from ID and the impacts of being born with low iron status, therefore, the early opportunity of nutrition interventions in young infants is an important window to address. While routine supplementation is only recommended for infants from six to 23 months of age in areas with an anaemia prevalence of greater than 40% (24), early iron interventions are an area of research that is lacking in the literature due to the appropriate but strong focus on the promotion of EBF during the first six months of life. Breastmilk concentrations alone cannot meet infant iron requirements at any stage of lactation, highlighting the importance of the foetal iron endowment (347). Overall, ensuring adequate iron intake in early infancy is important for supporting optimal growth, neurodevelopment, and immune function as it helps prevent IDA and its associated adverse effects on cognitive and physical development (18, 20, 348, 349). The proof of concept trial presented showed that daily oral iron could be introduced safely from six to ten weeks of age for 14 weeks. Additionally, qualitative data suggested that mothers were not hesitant about supplementing their infants at this age but scale up into other populations requires further research. Implementation factors, such as maternal acceptability, and community education and involvement are key considerations for sustainability and effectiveness of future interventions. This Thesis shows how iron supplementation before six months of age in breastfed infants may be required, safe, and well accepted in areas where maternal diets are habitually low in iron leading to low iron endowments and EBF not meeting infant nutritional needs for this vital phase of growth and development.

APPENDICES

APPENDIX 1 ADDITIONAL FILES FROM PUBLISHED PAPER 1 (CHAPTER 3): ACKNOWLEDGING THE GAP: A SYSTEMATIC REVIEW OF MICRONUTRIENT SUPPLEMENTATION IN INFANTS UNDER SIX MONTHS OF AGE

EXTENDED DATAFILE 1: PRISMA Checklist

Section/topic	#	Checklist item	Reported in
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Title
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Abstract
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	Introduction
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	Objectives
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	Methods
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Methods
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Methods
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Extended Datafile
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Methods
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Methods
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Methods
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Methods
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Methods
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	Methods

Section/topic	#	Checklist item	Reported in
Risk of bias across	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective	Methods

studies		reporting within studies).	
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	NA
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Results
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Results
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Extended Datafile
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Results
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	NA
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Results
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression)	NA
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	Summary of main results
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	Strengths and limitations
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Comparison to the literature
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	Data availability

EXTENDED DATAFILE 2: Search Terms

MEDLINE

1.	Iron/ or Iron compounds/ or Anemia, Iron Deficiency/ or Iron, Dietary/ or Anemia/	146171
2.	Folic Acid/ or Ferric Compounds/ or Ferrous Compounds/	54603
3.	(ferrous* or ferric* or fe or IFA or iron*).tw	263841
4.	Micronutrients/ or Trace Elements/	22065
5.	(micronutrient* or multinutrient* or multimicronutrient* or multivitamin* or multimineral* or MMN or MMS).tw	25532
6.	Dietary Supplements/	54993
7.	(trace adj (element* or mineral* or nutrient*)).tw	19260
8.	(diet* adj3 supplement*).tw	42294
9.	or/1-8	468769
10.	Drug Administration Schedule/ or Dose Response Relationship, Drug/ or Time Factors/	1577764
11.	(day* or daily* or week* or biweek* or intermittent* or alternat*).mp	3688844
12.	10 or 11	4825062
13.	Infant/ or Infants/ or Infancy/	778154
14.	(infan* or newborn* or neonat* or baby or babies).tw	723054
15.	13 or 14	1284243
16.	exp clinical trial/	850082
17.	(randomized controlled trial or controlled clinical trial).pt	587968
18.	(randomi?ed or placebo* or randomly or trial or groups).ab	2770906
19.	drug therapy.fs	2175511
20.	or/16-19	4756890
21.	exp animals/ not humans.sh	4666856
22.	20 not 21	4137030
23.	9 and 12 and 15 and 22	3702
24.	limit 23 to yr="1980-2019"	3631

Embase

1.	iron/ or iron intake/	179436
2.	iron deficiency/ or iron deficiency anemia/ or iron deficiency anaemia/ or anemia/ or anaemia/	234130
3.	folic acid/ or ferric iron/ or ferrous iron/ or iron derivative/ or iron compounds/	96828
4.	exp trace element/	40926
5.	diet supplement/	11082
6.	(iron* or ferric* or ferrous* or fe).tw	329816
7.	(diet* adj3 supplement*).tw	52954
8.	(micronutrient* or multinutrient* or multimicronutrient* or multivitamin* or multimineral* or MMN or MMS).tw	34632
9.	(trace adj (element* or mineral* or nutrient*)).tw	26014
10.	or/1-9	714105

11.	drug administration/ or drug dose regimen/ or time/	485828
12.	(day* or daily* or week* or biweek* or intermittent* or alternat*).tw	5389362
13.	11 or 12	5770364
14.	infants/ or infancy/ or infant/	705765
15.	(baby or babies or newborn* or neonat* or infan*).tw	975780
16.	14 or 15	1361001
17.	exp clinical trial/	1482504
18.	randomization/ or placebo/ or prospective trial/	1002000
19.	single blind procedure/ or double blind procedure/ or crossover procedure/	246856
20.	(randomi?ed or RCT or placebo* or crossover or prospective trial).tw	1063242
21.	(random* adj3 (allocat* or assign*)).tw	184101
22.	(randomly or groups or trial).ab	3607408
23.	((singl* or doubl* or trebl* or tripl*) adj3 (blind* or mask*)).tw	241717
24.	or/17-23	5178898
25.	10 and 13 and 16 and 24	4654
26.	limit 25 to yr="1980-2019"	4480

CENTRAL

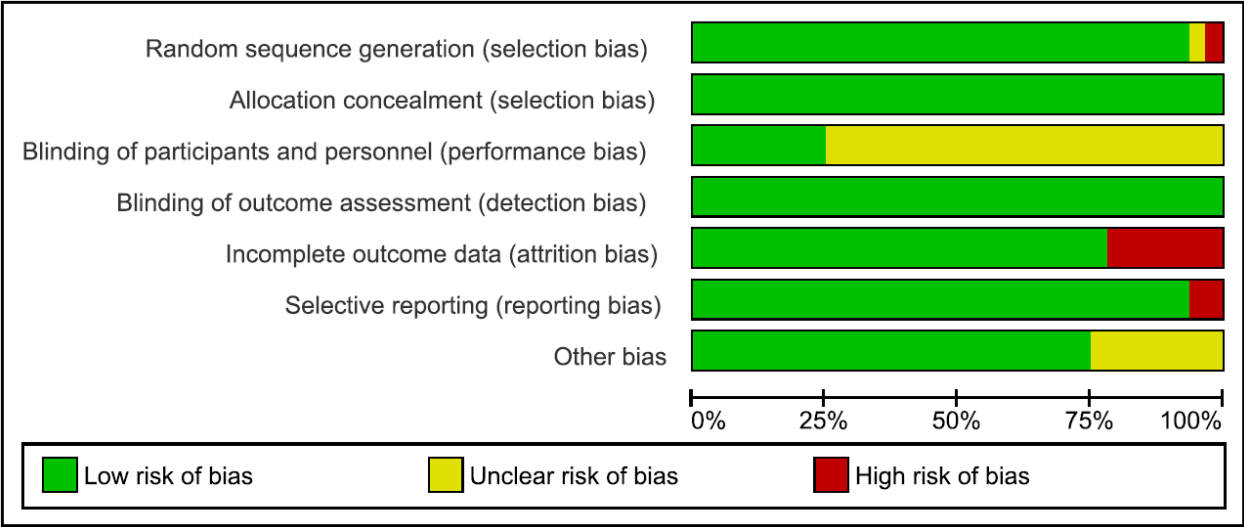
1.	#1MeSH descriptor Iron, this term only	9101
2.	#2MeSH descriptor Iron, Dietary, this term only	2007
3.	#3MeSH descriptor Anemia, Iron-Deficiency, this term only	2813
4.	#4MeSH descriptor Folic Acid, this term only	4649
5.	#5MeSH descriptor Dietary Supplements, this term only	12450
6.	#6MeSH descriptor Trace Elements, this term only	945
7.	#7 trace NEXT (element* or mineral* or nutrient*)	1783
8.	#8MeSH descriptor Micronutrients, this term only	1919
9.	#9diet* NEAR/3 supplement*	18789
10.	#10micro-nutrient* or micronutrient* or multi-nutrient* or multinutrient* or multimicronutrient* or multimicro-nutrient* or multivitamin* or multi-vitamin* or multimineral* or multi-mineral*	4292
11.	#11MeSH descriptor Ferric Compounds, this term only	696
12.	#12MeSH descriptor Ferrous Compounds, this term only	602
13.	#13ferrous* or ferric* or fe or iron*	15105
14.	#14 (#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13)	36923
15.	#15MeSH descriptor Drug Administration Schedule, this term only	28373
16.	#16MeSH descriptor Time Factors, this term only	96609
17.	#17(day* or daily* or week* or biweek* or bi NEXT week* or intermittent* or alternat*)	680274
18.	#18(#15 OR #16 OR #17)	707004
19.	#19(#14 AND #18)	24728
20.	#20(baby or babies or newborn* or neonat* or infan*)	77588
21.	#21(#19 AND #20)	2998

EXTENDED DATAFILE 3: Registered and Ongoing Trials & Reviews

Of the 13 additional materials identified, five registered or ongoing trials were excluded, because they assessed low-birth-weight or pre-term infants. The remaining were made up of six registered trials, one protocol registered on Cochrane, and one published paper describing a trial protocol. The Cochrane Protocol is titled: *Daily iron supplementation for prevention or treatment of iron deficiency anaemia in infants, children, and adolescents*(350). The protocol, however, does not specify if this will include infants under six months of age. The published paper describing an RCT protocol will supplement 220 Polish infants at four months of age until nine months of age with a daily iron powder or placebo and then assess neurodevelopment at 12, 24 and 36 months of age(351). Details of the six remaining registered trials can be found in the table below.

Registration Year	Title	Trial Type	Age Group	Length of Intervention	Intervention	Outcomes
2006(352)	Intermittent malaria treatment and iron supplementation for the control of malaria and anaemia in infants in the Forest Belt of rural Ghana: a double-blind randomised controlled trial	RCT	10 weeks	12 months	1. Iron supplementation (2mg/kg/day) + sulphadoxine pyrimethamine (SP) 2. Iron + placebo 3. Placebo + SP	Prevalence of anaemia
2011(353)	Intervention trial to reduce the level of anaemia among infants	Non-randomised controlled intervention	4 or 6 months	6 months, follow up: 12 months	1. Iron starting at 4 months of age 2. Iron starting at 6 months of age. Both at 2mg/kg/day	Haemoglobin
2015(354)	Effectiveness of daily and weekly iron supplementation	RCT	5-7 months	1 year of age	1. Weekly ferrous fumarate 2. Daily ferrous fumarate	Prevalence of anaemia
2016(355)	A clinical trial to evaluate the effects of iron therapy in term new-borns	RCT	Recruited at birth	6 months of age	1. Ferrous ascorbate (2mg/kg/day) 2. None	Haemoglobin, serum ferritin, neurodevelopment
2017(356)	To Assess the Effect of Early vs Routine Iron Supplementation on Iron Store and Growth in Term Infants	RCT	6 weeks or 6 months	1 year	1. Iron starting at 6 weeks of age 2. Iron starting at 6 months of age. Both at 2mg/kg/day	Iron deficiency anaemia, iron stores, growth
2019(357)	Iron supplementation in early infancy	Randomised parallel group trial	90-120 days	6 months of age	Iron prophylaxis at dose of 20 mg biweekly	Haemoglobin, ferritin and transferrin saturation

EXTENDED DATAFILE 4: Risk of Bias Assessment Graph and Summary.



	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Angulo-Barroso, 2016	+	+	+	+	+	+	+
Aydin, 2017	+	+	+	+	-	+	?
Berger, 2006	+	+	+	+	-	-	+
Bhandari, 2007	+	+	?	+	+	+	+
Bora, 2019	+	+	+	+	+	+	+
Carter, 2018	+	+	?	+	+	+	+
Desai, 2002	+	+	+	+	+	+	+
Dijkhuizen, 2001	+	+	?	+	+	+	?
Domellof, 2001	?	+	?	+	+	+	+
Duggan, 2012	+	+	?	+	+	+	+
Ermis, 2002	+	+	?	+	+	+	+
Fahmida, 2007	+	+	+	+	+	+	+
Friel, 2003	+	+	?	+	+	+	?
Lauer, 2019	+	+	?	+	+	+	+
Liu, 2013	+	+	?	+	+	+	+
Locks, 2016	+	+	?	+	+	+	+
Lozoff, 2016	+	+	?	+	-	+	+
Manji, 2014	+	+	?	+	+	+	+
Massaga, 2003	+	+	?	+	-	+	+
McDonald, 2015	+	+	?	+	+	+	+
Menendez, 1997	+	+	?	+	+	+	+
Menendez, 2004	+	+	?	+	+	+	+
Nagpal, 2004	+	+	?	+	-	+	?
Pongcharoen, 2011	+	+	+	+	+	+	+
Rahimy, 2007	-	+	?	+	+	+	+
Schellenberg, 2004	+	+	?	+	-	+	+
Sudfeld, 2019	+	+	+	+	+	+	+
Tielsch, 2006	+	+	?	+	-	-	?
Wasantwisut, 2006	+	+	?	+	+	+	+
Wieringa, 2003	+	+	?	+	+	+	?
Yurdakok, 2004	+	+	?	+	+	+	?
Ziegler, 2009	+	+	?	+	+	+	?

EXTENDED DATAFILE 5: Table: Results by outcome.

First Author, Publication Year	Trial Location	Sample Size and Age Group	Trial Design	Intervention	Results
BIOCHEMICAL					
Aydin, 2017(358)	Istanbul, Turkey	112 infants at 4 until 9 months of age	2-arm RCT	2mg/kg/day of 1. Ferrous sulphate (FS) drops or 2. Ferric polymaltose (FP) drops.	Significant increase in iron markers (Hb*, haematocrit, serum iron and TS*) at nine months of age for both groups were seen, with the FS group being significantly higher than the FP group (117 ± 6 g/L, 34.6 ± 1.7 %, 55.5 ± 1.8 mcg, 20.8 ± 3.9 g/L vs 113 ± 5 g/L, 33.5 ± 1.5 %, 42.5 ± 1.8 mcg, 14.1 ± 7.5 g/L, respectively). ID* (Hb ≥ 110 g/L + TS < 16 %) and IDA* (Hb < 110 g/L + TS < 16 %) was seen in 30.3% and 8.9% of infants receiving FS and 41% and 12% of infants receiving FP.
Berger, 2006(359)	Que Vo, in the Red River Delta, Vietnam	915 infants at 4 - 7 months of age for 6 months	4-arm RCT	Daily 1. Iron (10mg), 2. Zinc (10mg), 3. Iron + zinc, or 4. Placebo. // A single dose of vitamin A was given to infants at the start of the trial (100,000IU).	Changes in Hb and SF* were higher in iron and iron + zinc verses zinc and placebo groups (22.6 and 20.6 vs 6.4 and 9.8 g/L; 36.0 and 24.8 vs -18.2 and -16.9 µg/L, for iron and iron + zinc verses zinc and placebo groups, respectively; p < 0.0001).
Bhandari, 2007(360)	New Delhi, India	94,359 infants at 1 - 23 months of age for 12 months	2-arm RCT	Daily 1. IFA* (12.5mg of iron + 50µg of folic acid) or 2. IFA + zinc (10mg). // Infants under 6 months of age were administered 1/2 tablet (crushed) into a powder and then 1 tablet (powdered) from 6 months of age onwards.	% SF < 20 µg/L was marginally lower in the IFA group verses the IFA + zinc group (36.9 vs 40.8 %, respectively).
Bora, 2019(361)	Northeast India	200 infants 36 hours after birth until 6 months of age (1/2 born to anaemic mothers)	2-arm RCT	Daily 1. Ferrous ascorbate drops (2mg/kg) or 2. Standard of care.	ID at birth (cord SF < 75 µg/L) was seen in 40% of infants born to anaemic and 13.3% to non-anaemic mothers (p < 0.01). Significantly higher Hb and SF were seen in supplemented verses standard of care infants at 6 months of age (103.7 ± 9.3 vs 97.0 ± 9.4 g/L and 133.93 ± 52.41 vs 78.09 ± 42.03 µg/L, respectively), with comparable benefits of iron in infants born to anaemic and non-anaemic mothers.
Carter, 2018(362) (Sub-analysis of McDonald, 2015(363))	Dar es Salaam, Tanzania	2,006 infants at 6 weeks of age for 18 months	4-arm RCT	Daily 1. Zinc (5mg) or 2. Multivitamins (60mg vitamin C, 8mg vitamin E, 0.5mg thiamine, 0.6mg riboflavin, 4mg niacin, 0.6mg vitamin B6, 130µg folate, and 1µg vitamin B12) or 3. Zinc + multivitamins or 4. Placebo. // Infants under 6 months of age were administered 1 tablet (crushed into a powder) and then 2 tablets from 6 months of age onwards (powdered).	Infants given multivitamins had higher mean Hb (99 [97, 101] g/L) than those given placebo (96 [94, 98] g/L) or zinc alone (96 [94, 97] g/L) at 18 months. Multivitamins were associated with lower odds of ID (OR*: 0.49 [0.3, 0.9]) at 6 months of age and a 28% reduction in risk of severe anaemia (Hb < 85 g/L) (HR*: 0.72 [0.56, 0.94]) through 18 months, whereas zinc was associated with greater odds of ID (OR: 1.8 [1.0, 3.3]) at 6 months of age and had no positive effect on severe anaemia through 18 months.

Desai, 2003(364)	Asembo, West Kenya	546 mildly anaemic infants (Hb 70 – 109 g/L) at 2 - 36 months of age for 12 weeks	4-arm RCT	1. Intermittent preventive treatment (IPT) with sulfadoxine-pyrimethamine (SP) at 4 and 8 weeks (25mg/kg of sulfadoxine + 1.25mg/kg of pyrimethamine; infants ≤ 10kg received half a tablet (crushed into a powder), and those > 10kg received a tablet (powdered)) + 3–6mg/kg/day of ferrous sulphate drops for 12 weeks or 2. Placebo IPT SP + iron or 3. IPT SP + placebo iron or 4. Double placebo. // Bed nets + single SP dose was given to all participants upon enrolment.	Mean Hb at 12 weeks was higher for all three supplemented groups versus placebo (110.5, 107, 108 vs 99.1 g/L; for IPT + iron, iron only, IPT only vs placebo groups, respectively). Daily iron + malaria prophylaxis was most effective in treating mild anaemia, while the prevalence of severe anaemia (Hb < 70 g/L) was 1.9, 4.5, 8.9 and 7.4 % for the IPT + iron, iron only, IPT only and placebo groups, respectively.
Dijkhuizen, 2001(365)	West Java, Indonesia	478 infants at 4 until 10 months of age	4-arm RCT	5 days/week, with 10mg/day each of 1. Iron syrup or 2. Zinc syrup or 3. Iron + zinc or 4. Placebo.	Iron alone was more effective than iron + zinc in increasing Hb (115 ± 10 vs 110 ± 11 g/L) and SF (36.8 vs 26.6 µg/L) and reducing the prevalence of anaemia (Hb < 110 g/L) (38 vs 20 % reduction) (p < 0.01 for all). IDA prevalence (Hb < 110 g/L + SF < 12 µg/L) was significantly lower in iron and iron + zinc vs zinc only or placebo groups (3 and 8 vs 35 and 30 %, respectively).
Domellof, 2001 ⁽⁸⁰⁾	Honduras and Sweden	232 infants at 4 until 9 months of age	6-arm RCT (3-arms per country)	Daily 1. Ferrous sulphate (1mg/kg) (early intervention) or 2. Placebo (4 - 6 months of age) + ferrous sulphate (1mg/kg) (6 - 9 months of age) (late intervention), or 3. Placebo.	At 6 months of age, both countries' early intervention groups had significantly higher Hb and SF (Honduras: 114.8 ± 9.7 vs 107.8 ± 7.6 and 110.5 ± 7.2 g/L; 55.0 [32, 92] vs 30.5 [12, 67] and 32.5 [17, 54] µg/L; Sweden: 125.9 ± 7.0 vs 119.3 ± 7.1 and 117.7 ± 6.1 g/L; 90.6 [61, 131] vs 56.2 [28, 110] and 56.4 [25, 120] µg/L; for early vs late and placebo intervention groups, respectively; p < 0.05 for all). At 9 months of age, both countries' early intervention groups had significantly higher SF (Honduras: 42.9 [24, 74] and 31.9 [18, 57] vs 15.8 [8, 31] µg/L; Sweden: 66.9 [47, 33] and 47.3 [21, 81] vs 22.9 [12, 51] µg/L; for early vs late and placebo intervention groups, respectively; p < 0.001 for all). Hb differences were statistically significant in both Honduras' intervention groups versus placebo (115.9 ± 10.9 and 113.9 ± 8.3 vs 108.7 ± 8.8 g/L; for early and late intervention vs placebo groups, respectively; p < 0.001), along with a statistically significant decrease in IDA prevalence (Hb < 110 g/L + > 2 more abnormal iron indices) (9 vs 29 % for the supplemented vs placebo groups, p = 0.135), both at 9 months of age. In Sweden, iron caused no reduction in the already low IDA prevalence at 9 months of age (< 3 %).

Ermis, 2002(366)	Zonguldak, Turkey	113 infants at 5 until 9 months of age	4-arm RCT	Ferrous sulphate drops at 1. 1mg/kg/day or 2. 2mg/kg/day or 3. 2mg/kg/48hours or 4. Placebo.	There was no ID ($Hb \leq 105 \text{ g/L} + MCV^* \leq 70 \text{ fL} + SF \leq 12 \mu\text{g/L}$) in all three iron groups at nine months of age and there were significant increases verses baseline values for Hb, MCV and SF for all three iron groups ($p < 0.001$). MCV, SF and Hb were significantly higher in all three iron groups verses placebo ($80 \pm 1.9, 81.6 \pm 1.4, 80 \pm 1.5$ vs $74.3 \pm 2.9 \text{ fL}; 38.5 \pm 7.3, 52.4 \pm 8.3, 45 \pm 12.7$ vs $17.2 \pm 4.6 \mu\text{g/L}$, and $116 \pm 3.1, 117 \pm 2.9, 116 \pm 2.9$ vs $113 \pm 4.0 \text{ g/L}$, for the 1mg/kg/day, 2mg/kg/day, 2mg/kg/48hrs vs placebo groups, respectively; $p < 0.001$ for all). MCV was superior in the group receiving 2mg/kg/day verses the other iron groups ($p > 0.01$) and SF was also significantly higher at 9 verses 5 months of age in this group (52.4 ± 8.3 vs $29.2 \pm 7.4 \mu\text{g/L}$, respectively).
Fahmida, 2007(367)	East Lombok, Indonesia	800 infants at 3 - 6 months of age for 6 months	4-arm RCT	Daily 1. Zinc sulphate syrup (10 mg) or 2. Zinc + ferrous sulphate (10mg) syrup or 3. Zinc + iron + vitamin-A (1,000IU) syrup or 4. Placebo. // Vitamin A at a dose of 100,000IU was given to all infants at the start of the trial.	The MMN* group had an increase in Hb (95.4 ± 14.9 to $96.9 \pm 17.5 \text{ g/L}$), whereas the zinc only and placebo groups had a significant decrease in Hb (96.5 ± 15.4 to 90.2 ± 15.2 and 95.8 ± 15.9 to $90.8 \pm 15.2 \text{ g/L}$, respectively) during the trial. Hb in the MMN and zinc + iron groups ($96.3 \pm 16.0 \text{ g/L}$) were significantly higher than the zinc only and placebo groups after supplementation. After supplementation, anaemia prevalence ($Hb < 100 \text{ g/L}$) significantly increased in the zinc only (83 to 91.5 %) and placebo groups (79.3 to 92 %), with the MMN group being the only group to see a decrease (84.2 to 80.3 %).
Friel, 2003(368)	Newfoundland, Canada (low-income population)	77 infants at 1 until 6 months of age; followed until 12 months	2-arm RCT	Daily 1. Ferrous sulphate syrup (7.5mg) or 2. Placebo.	At 6 months of age, the iron verses placebo group had significantly higher Hb and MCV (124 ± 9.1 vs $116 \pm 6.6 \text{ g/L}$ and 81 ± 4 vs $77 \pm 3.8 \text{ fL}$, respectively) but the significance was no longer seen at 12 months of age ($p = 0.06$ for all). There was a decline in SF over time, but the decline was less in the iron group. At 6 months of age IDA ($Hb < 110 \text{ g/L} + SF < 12 \mu\text{g/L}$) and ID ($SF < 12 \mu\text{g/L}$) was seen in 0% and 7% of iron, and 14% and 33% of placebo infants.
Liu, 2013(369) (Sub-analysis of Duggan, 2012 ⁽³⁷⁰⁾)	Dar es Salaam, Tanzania	2,387 infants at 6 weeks of age for 24 months (born to HIV positive mothers)	2-arm RCT	Daily 1. Multivitamin (60mg vitamin C, 8mg vitamin E, 0.5mg thiamine, 0.6mg riboflavin, 4mg niacin, 0.6mg vitamin B6, 130µg folate and 1µg vitamin B12) or 2. Placebo. // Infants under 6 months of age were administered 1 capsule (crushed into a powder) and then 2 capsules (powdered) from 6 months of age onwards.	Hb concentrations were significantly higher in the treatment verses placebo group at 12 (97.7 vs 96.4 g/L , $p = 0.03$), 18 (97.6 vs 95.7 g/L , $p = 0.004$), and 24 months (99.3 vs 97.5 g/L , $p = 0.02$). Infants in the treatment group had a 12% lower risk of anaemia ($Hb < 110 \text{ g/L}$) (HR: 0.88 [0.79, 0.99], $p = 0.03$). The treatment was associated with a 28% reduced risk of severe anaemia ($Hb < 85 \text{ g/L}$) among infants born to women without anaemia (HR: 0.72 [0.56, 0.92], $p = 0.008$), but not among those

					born to women with anaemia (HR: 1.10 [0.79, 1.54], $p = 0.57$, p for interaction = 0.007).
Lozoff, 2016(371) (Linked to Angulo-Barroso, 2016(372))	Hebei, China	1,276 infant-mother pairs at infant age of 6 weeks until 9 months (mothers supplemented from 16 weeks gestation until birth)	4-arm RCT	Daily 1. Ferrous sulphate in pregnancy (300mg) + ferrous proteinsuccinylate drops in infancy (1mg/kg) or 2. Placebo in pregnancy + iron in infancy or 3. Iron in pregnancy + placebo in infancy or 4. Double placebo. // All pregnant women received 0.40mg folate from enrolment to delivery.	Iron in infancy, but not pregnancy, reduced ID risk ($\geq$ two abnormal iron measures, i.e. SF < 75 $\mu\text{g/L}$, Hb < 110 g/L etc) in infants at 9 months of age (RR*: placebo/iron: 0.89 [0.79, 0.998] vs placebo/placebo: 0.79 [0.63, 0.98]; for placebo/iron vs iron/placebo: 0.87 [0.77, 0.98]; for iron/iron: 0.86 [0.77, 0.97] vs placebo/placebo, and for iron/iron vs iron/placebo), but more than 60% of infants still had ID at this age. Effects of iron in pregnancy were observed when higher amounts of iron were distributed in infancy.
Massaga, 2003(373)	Muheza, Tanzania	291 infants (vulnerable to malaria) at 12 - 16 weeks of age for six months	4-arm RCT	1. Daily ferric ammonium citrate (7.5mg elemental iron) or 2. Amodiaquine (daily doses of 10, 10 and 5 mg/kg for three consecutive days in 60-day intervals) or 3. Iron + amodiaquine or 4. Double placebo.	Infants receiving malaria prophylaxis and malaria prophylaxis + iron were protected against anaemia (PCV* < 24%) (PE*: 71.2 [38.5, 87.0] and 74.4 [43.46, 88.4] %, respectively), but those receiving iron only were only partly protected against anaemia (PE: 59 [18.7, 79.3] %) ($p < 0.01$ for all).
Menendez, 1997(374)	Ifakara, Tanzania	832 infants at risk of malaria at 8 weeks until 6 months (for iron) and 12 months (for antimalarial) of age	4-arm RCT	1. 2mg/kg/day of ferrous glycine sulphate + weekly dapsone (3x-weekly 125mg pyrimethamine + 25mg dapsone) or 2. Iron + placebo dapsone or 3. Placebo iron + dapsone 4. Double placebo.	Iron supplementation and malaria prophylaxis had a protective effect against severe anaemia (PCV < 25%) (PE*: 28.8 [6.3, 45.8] and 57.3 [43.0, 67.9] % for iron + placebo dapsone and iron + dapsone, respectively) at the trial end point.
Menendez, 2004(375) (Sub-analysis of Menendez, 1997 ⁽³⁷⁴⁾)	Ifakara, Tanzania	833 infants at risk of malaria at 8 weeks until 6 months (for iron) and 12 months (for antimalarial) of age	4-arm RCT	1. 2mg/kg/day of ferrous glycine sulphate + weekly dapsone (3x-weekly 125mg pyrimethamine + 25mg dapsone) or 2. Iron + placebo dapsone or 3. Placebo iron + dapsone 4. Double placebo.	Iron supplemented infants had a significantly lower prevalence of ID (SF < 12 $\mu\text{g/L}$) at 5 ($p < 0.01$), 8 ($p < 0.001$) and 12 months of age ($p < 0.001$), but ID did not differ between those protected and un-protected against malaria at any time point (5 months: 1.1 [0.1, 3.9] vs 1.2 [0.1, 4.2] %; 8 months: 4.8 [2.1, 9.2] vs and 6.4 [3.0, 11.8] %; 12 months: 7.8 [4.1, 13.2] vs 17.6 [11.5, 25.2] %; for iron + dapsone vs iron + placebo dapsone, respectively).
Nagpal, 2004(376)	New Delhi, India (rural)	100 infants at 4 - 6 months of age for 8 weeks	2-arm RCT	Daily 1. Ferric ammonium citrate drops (2 mg/kg) or 2. Placebo.	Significantly higher Hb and SF were seen in the iron versus placebo group after supplementation (115 ± 8 vs 108 ± 9 g/L, $p = 0.009$ and 306 ± 22 vs 87 ± 32 $\mu\text{g/L}$, $p < 0.005$; respectively). The adjusted rise in Hb was higher in initially anaemic infants at the second follow-up by 10 g/L.

Rahimy, 2007(377)	Cotonou, Republic of Benin	612 infants at 4 OR 6 - 18 months of age, for 2 months	2-arm RCT by age	2x daily ferrous fumarate powder (15mg/kg/day) at 1. 4 months of age (early intervention) or 2. 6 - 18 months of age (late intervention).	After supplementation, there was a significant increase in mean values of Hb and mean corpuscular Hb but no significant change in MCV (101 ± 11 to 106 ± 9 and 101 ± 10 to 109 ± 11 g/L; 22.0 ± 2.0 to 23.4 ± 2.4 and 21.4 ± 2.9 to 22.4 ± 2.7 pg; 75.5 ± 6.0 to 74.4 ± 5.8 and 75.3 ± 7.8 to 76.0 ± 7.4 fL for the early and late intervention groups, respectively). Decreased anaemia prevalence (Hb < 100 g/L for infants under 6 months and Hb < 105 g/L for infants over 6 months of age) in both groups was seen (42.6 to 33.8 % and 62 to 30.2 %; in the early and late intervention groups, respectively). Initially anaemic children had higher increases in Hb versus the rest of the population (91 ± 6 to 100 ± 9 and 95 ± 7 to 106 ± 10 g/L; in early and late intervention groups, respectively).
Schellenberg, 2004(378)	Ifakara, Tanzania	311 infants at 2 months - 5 years of age for 14 days or 3 months	2-arm RCT	1. 14-days of daily ferrous sulphate tablets (30mg of elemental iron) + 1x dose of sulfadoxine-pyrimethamine (SP) at day 1 (125mg sulfadoxine + 6.25mg pyrimethamine) (short-term) or 2. 3-months of daily ferrous sulphate + 3x SP doses at the start of each month (long-term). // Infants < 10kg received half a tablet (crushed into a powder) of both SP and ferrous sulphate.	2 weeks after completing treatment, the prevalence of PCV < 33% was 57.8% in the short-term and 44.4% in the long-term groups ($p = 0.04$), with mean PCV significantly higher in the long-verses short- term groups (32.1 ± 4.5 vs 30.8 ± 4.9 %, respectively; $p = 0.031$) and no difference in the prevalence of PCV < 25%. Benefits of the extended therapy were only apparent 6 months after recruitment (OR: 0.16 [0, 1.86], $p = 0.06$).
Tielsch, 2006(379)	Southern Nepal, India	26,250 infants at 1 - 35 until 36 months of age	3-arm RCT	Daily 1. (IFA) (12.5mg iron + 50µg folic acid) or 2. IFA + zinc (10 mg), or 3. Placebo. // Infants under 1 year of age received 1/2 a tablet (crushed into a powder); all infants older than 6 months of age also received vitamin A as part of a national programme.	The IFA containing groups were stopped early on recommendation of the data and safety monitoring board. However, in a subsample, 12 months after the start, Hb was highest in the IFA group (11.11 ± 1.18 vs 10.96 ± 1.61 and 10.31 ± 1.71 g/L; for IFA vs IFA + zinc and placebo, respectively). Median SF was significantly higher in the IFA group than both other groups and significantly higher in the IFA + zinc group verses placebo (53.57, 35.92, 19.58 g/L; for IFA, IFA + zinc and placebo groups, respectively). The prevalence of IDA (Hb < 110 g/L + SF < 12 µg/L) was lowest in the IFA group (4 vs 12 and 26 %; for IFA vs IFA + zinc and placebo groups, respectively).

Wasantwisut, 2006(380)	Rural Northeast Thailand	609 infants at 4 - 6 months of age for 6 months	4-arm RCT	Daily 1. Ferrous sulphate syrup (10mg) or 2. Zinc sulphate syrup (10mg) or 3. Iron + zinc or 4. Placebo. // All supplements contained vitamin C (15g/L). Vitamin A drops at a dose of 1500mg retinol equivalent were given to each subject at the beginning of the trial.	Infants not receiving iron had a decrease in Hb of 11.0 g/L between baseline and 6 months ($p < 0.001$) with the overall effect of iron on end point Hb being an increase of 10.8 [8.0, 13.6] g/L, whereas the effect of zinc with or without iron was a decrease in Hb by 3.4 [-6.3, -0.6] g/L. After 6 months of supplementation, the groups receiving iron (iron or iron + zinc) had significantly higher SF than those receiving only zinc or placebo (69.6 and 50.7 vs 24.4 and 24.2 $\mu\text{g/L}$; for iron and iron + zinc vs zinc only and placebo, respectively; $p < 0.001$). Anaemia prevalence (Hb < 110 g/L) was significantly lower in infants receiving only iron than in those receiving iron + zinc, only zinc or placebo (16.9 vs 41.8, 69 and 60.6 %, respectively) and ID (SF < 12 $\mu\text{g/L}$) and IDA (Hb < 110 g/L + SF < 12 $\mu\text{g/L}$) prevalence were lower in infants receiving iron or iron + zinc verses zinc only and placebo (ID: 0 and 3 vs 15.5 and 6.1 %; IDA: 0 and 1.5 vs 15.5 and 6.2 %, respectively).
Wieringa, 2003(381)	West Java, Indonesia	387 infants at 4 months of age for 6 months	6-arm RCT	5days/week with daily 1. Ferrous sulphate syrup (10mg) or 2. Zinc sulphate syrup (10mg) or 3. Iron + zinc syrup or 4. 2.4mg beta-carotene syrup or 5. Zinc + beta-carotene syrup or 6. Placebo.	Hb was higher in infants receiving iron only (118 ± 10 g/L) verses zinc + iron (115 ± 10 g/L) and significantly higher than those receiving zinc only (110 ± 10 g/L), beta-carotene only (112 ± 9 g/L), zinc + beta-carotene (107 ± 10 g/L) or placebo (110 ± 11 g/L). SF was highest in the group receiving iron only and significantly higher in the iron + zinc supplemented group than in the placebo group ($p < 0.001$).
Yurdakok, 2004 ⁽³⁸²⁾	Ankara, Turkey	70 infants at 4 until 7 months of age	3-arm RCT	Ferrous sulphate drops at 1. 1mg/kg/day or 2. 7mg/kg/week (once a week, three divided doses every Tuesday) or 3. Standard of care.	Infants supplemented weekly with iron had the lowest rates of ID (SF < 10 $\mu\text{g/L}$) or IDA (SF < 10 $\mu\text{g/L}$ + Hb < 105 g/L) and infants whose mothers had ID (SF < 12 $\mu\text{g/L}$) or IDA (Hb < 120 g/L + two additional abnormal iron status indices) during the trial period were more likely to develop ID or IDA (31.8, 26.0, and 13.6 % of infants in the control, daily and weekly groups, respectively; $p > 0.05$ for all). SF levels decreased between 4 and 6 months of age in the control and daily groups, while the weekly group showed no such decrease ($p < 0.05$). In all groups, the mean levels of SF were significantly increased from 6 to 7 months of age during the weaning period.

Ziegler, 2009(383)	Iowa, USA	75 infants at 1 until 5.5 months of age; followed until 18 months of age.	2-arm RCT	Daily micronutrient supplementation (1059IU vitamin A, 270IU vitamin D and 24.5mg vitamin C) with 1. Ferrous sulphate (7mg) or 2. Without.	The difference in adjusted SF was statistically significant at 4 and 5.5 months for the with iron versus non-iron groups (143 ± 71 vs 109 ± 62 and 97 ± 61 vs 63 ± 41 $\mu\text{g/L}$, respectively), however, SF decreased continuously throughout the trial in both groups. Hb concentration showed no difference at any age, although it was higher for the group receiving iron than those not during supplementation, but not afterwards. At 5.5 months but not at other ages, plasma soluble transferrin receptor (sTfR) was significantly lower in the iron versus non-iron group ($p = 0.005$). MCV was significantly higher in the iron versus non-iron groups at 7.5 ($p = 0.046$) and 9 ($p = 0.01$) months of age.
GROWTH					
Aydin, 2017(358)	Istanbul, Turkey	112 infants at 4 until 9 months of age	2-arm RCT	2mg/kg/day of 1. Ferrous sulphate drops or 2. Ferric polymaltose drops.	Similar increases for weight, length and head circumference were seen for both groups at 9 months of age ($p < 0.07$).
Berger, 2006 ⁽³⁵⁹⁾	Que Vo, in the Red River Delta, Vietnam	915 infants at 4 - 7 months of age for 6 months	4-arm RCT	Daily 1. Iron (10mg), 2. Zinc (10mg), 3. Iron + zinc, or 4. Placebo. // A single dose of vitamin A was given to all infants at the start of the trial (100,000IU).	Weight gain was higher in the zinc (8.19 ± 0.92 kg) versus iron + zinc (8.05 ± 0.90 kg), placebo (8.05 ± 0.80 kg) and iron only (7.98 ± 0.90 kg) groups at 9 months of age. No significant effects of the different interventions on length at 9 months of age were seen (71.15 ± 2.53 , 71.01 ± 2.41 , 70.99 ± 2.51 and 70.84 ± 2.47 cm for zinc only, placebo, iron only and iron + zinc, respectively).
Dijkhuizen, 2001(365)	West Java, Indonesia	478 infants at 4 until 10 months of age	4-arm RCT	5 days/week, with 10mg/day each of 1. Iron syrup or 2. Zinc syrup or 3. Iron + zinc or 4. Placebo.	During the trial HAZ*, WAZ* and WHZ* decreased significantly in all groups ($p < 0.01$) and there were no group differences after supplementation ($p > 0.5$ for all).
Fahmida, 2007(367)	East Lombok, Indonesia	800 infants at 3 - 6 months of age for 6 months	4-arm RCT	Daily 1. Zinc sulphate syrup (10mg) or 2. Zinc + ferrous sulphate (10mg) syrup or 3. Zinc + iron + vitamin-A (1,000IU) syrup or 4. Placebo. // Vitamin A at a dose of 100,000IU was given to all infants at the start of the trial.	There was no beneficial effect on the prevalence of stunting (HAZ), wasting (WHZ) and underweight (WAZ) across all four groups after 6 months of supplementation ($p \leq 0.01$). Initially stunted infants mean HAZ decreased during the supplementation and this inter-group difference became significantly larger in the zinc + iron and MMN groups but not the placebo and zinc only groups, after 4 months of supplementation (zinc + iron: -2.58 to -2.08 , MMN: -2.51 to -2.14 vs placebo: -2.63 to -2.52 , and zinc: -2.53 to -2.51).
Friel, 2003(368)	Newfoundland, Canada (low-income population)	77 infants at 1 until 6 months of age; followed until 12 months	2-arm RCT	Daily 1. Ferrous sulphate syrup (7.5mg) or 2. Placebo.	There was no effect of iron therapy versus placebo on weight, length, or head circumference at any clinic visit (birth, 1, 3.5, 6 and 12 months of age) ($p < 0.05$).

Lauer, 2019(384) (Sub-analysis of McDonald, 2015(363))	Dar es Salaam, Tanzania	2,006 infants at 6 weeks of age for 18 months	4-arm RCT	Daily 1. Zinc (5mg) or 2. Multivitamins (60mg vitamin C, 8mg vitamin E, 0.5mg thiamine, 0.6mg riboflavin, 4mg niacin, 0.6mg vitamin B6, 130µg folate, and 1µg vitamin B12) or 3. Zinc + multivitamins or 4. Placebo. // Infants under 6 months of age were administered 1 tablet (crushed into a powder) and then 2 tablets from 6 months of age onwards (powdered).	There were no significant differences in any growth biomarkers of infants who received zinc verses those who did not or those who received multivitamins verses those who did not at any of the assessed time points (6 and 12 months of age, $p \geq 0.05$ for all).
Locks, 2016(385) (Sub-analysis of McDonald, 2015(363))	Dar es Salaam, Tanzania	2,006 infants at 6 weeks of age for 18 months	4-arm RCT	Daily 1. Zinc (5mg) or 2. Multivitamins (60mg vitamin C, 8mg vitamin E, 0.5mg thiamine, 0.6mg riboflavin, 4mg niacin, 0.6mg vitamin B6, 130µg folate, and 1µg vitamin B12) or 3. Zinc + multivitamins or 4. Placebo. // Infants under 6 months of age were administered 1 tablet (crushed into a powder) and then 2 tablets from 6 months of age onwards (powdered).	All infants experienced growth faltering and supplements did not have a significant effect on mean change in HAZ or on rates of stunting, wasting, or underweight. Changes in WAZ and WHZ were significantly different across the four groups ($p < 0.001$ for both), where mean decline in the WAZ from baseline to the end of follow-up in the zinc + multivitamin group was significantly less than in the placebo group (-0.36 ± 0.04 to -0.50 ± 0.04 , $p = 0.020$) and the decline in the WHZ was significantly greater in the zinc-only group than in the placebo group (-0.57 ± 0.07 to -0.35 ± 0.07 , $p = 0.021$).
Lozoff, 2016(371) (Linked to Angulo-Barroso, 2016(372))	Hebei, China	1,276 infant-mother pairs at infant age of 6 weeks until 9 months (mothers supplemented from 16 weeks gestation until birth)	4-arm RCT	Daily 1. Ferrous sulphate in pregnancy (300mg) + ferrous proteinsuccinylate drops in infancy (1mg/kg) or 2. Placebo in pregnancy + iron in infancy or 3. Iron in pregnancy + placebo in infancy or 4. Double placebo. // All pregnant women received 0.40mg folate from enrolment to delivery.	There were no statistically significant effects of supplementation on anthropometry as well as no group differences in length gain both at 9 months of age ($p < 0.05$ for all). No differences were observed when comparing infants who were iron sufficient verses deficient at birth. Between groups, weight gain was significantly lower in infants whose mothers received placebo in pregnancy (placebo/iron: 6161 [6039, 6283] vs iron/iron: 6356 [6235, 6477] and iron/placebo: 6354 [6231, 6477] g, $p < 0.05$).
Nagpal, 2004(376)	New Delhi, India (rural)	100 infants at 4 - 6 months of age for 8 weeks	2-arm RCT	Daily 1. Ferric ammonium citrate drops (2 mg/kg) or 2. Placebo.	No significant differences between groups anthropometry was seen after 8 weeks of supplementation (head circumference: 41.3 ± 1.1 and 41.2 ± 1.0 vs 43.3 ± 0.9 and 43.1 ± 0.9 cm; weight: $5,611 \pm 633$ and $5,806 \pm 660$ vs $6,337 \pm 644$ and $6,509 \pm 99$ g; length 61.5 ± 3.2 and 64.6 ± 3.3 vs 62.6 ± 2.7 and 65.8 ± 2.7 cm; for iron and placebo at baseline vs after supplementation, respectively).
Wasantwisut, 2006(380)	Rural Northeast Thailand	609 infants at 4 - 6 months of age for 6 months	4-arm RCT	Daily 1. Ferrous sulphate syrup (10mg) or 2. Zinc sulphate syrup (10mg) or 3. Iron + zinc or 4. Placebo. // All supplements contained vitamin C (15g/L). Vitamin A drops at a dose of 1500mg retinol equivalent were given to each subject at the beginning of the trial.	Supplementation did not differentiate group length (placebo: 70.6 ± 2.4 , iron: 70.6 ± 2.5 , zinc: 70.6 ± 2.5 , iron + zinc: 70.4 ± 2.2 cm), but that when controlled by gender and birth weight, iron supplementation improved WLZ*, whereas zinc did not (placebo: -0.7 ± 0.7 , iron: -0.5 ± 0.7 , iron + zinc: -0.6 ± 0.8 zinc: -0.7 ± 0.8 ; $p = 0.003$).

Ziegler, 2009(383)	Iowa, USA	75 infants at 1 until 5.5 months of age; followed until 18 months of age.	2-arm RCT	Daily micronutrient supplementation (1059IU vitamin A, 270IU vitamin D and 24.5mg vitamin C) with 1. Ferrous sulphate (7mg) or 2. Without.	At 5.5 months of age, iron in addition to the micronutrients had no significant effect on growth across the groups after supplementation (weight or length, $p < 0.05$ for all).
MORBIDITY AND/OR MORTALITY					
Aydin, 2017(358)	Istanbul, Turkey	112 infants at 4 until 9 months of age	2-arm RCT	2mg/kg/day of 1. Ferrous sulphate drops or 2. Ferric polymaltose drops.	The prevalence of reported adverse effects was 34.8%, with no statistical difference between groups. The most common side effect was gastrointestinal complaints (diarrhoea, constipation, and vomiting).
Berger, 2006(359)	Que Vo, in the Red River Delta, Vietnam	915 infants at 4 - 7 months of age for 6 months	4-arm RCT	Daily 1. Iron (10mg), 2. Zinc (10mg), 3. Iron + zinc, or 4. Placebo. // A single dose of vitamin A was given to all infants at the start of the trial (100,000IU).	There were no significant effects of supplementations on morbidity (number of cases: acute respiratory infection (102 - 112), cough (76 - 83), diarrhoea (46 - 52) or fever (22 - 30)).
Bhandari, 2007(360)	New Delhi, India	94,359 infants at 1 - 23 months of age for 12 months	2-arm RCT	Daily 1. IFA (12.5mg of iron + 50µg of folic acid) or 2. IFA + zinc (10mg). // Infants under 6 months of age were administered 1/2 tablet (crushed into a powder) and then 1 tablet (powdered) from 6 months of age onwards.	Overall death rates did not differ significantly between groups when adjusted for cluster randomization (HR = 1.02, [0.87, 1.19]) and that the addition of zinc to IFA did not affect adjusted hospitalizations rates overall or specific rate ratios for diarrhoea and pneumonia (overall rate ratio = 1.08 [0.98, 1.19]; diarrhoea-specific rate ratio = 1.15 [0.99, 1.34]; or pneumonia-specific rate ratio = 1.09 [0.94, 1.25]).
Bora, 2019(361)	Northeast India	200 infants 36 hours after birth until 6 months of age (1/2 born to anaemic mothers)	2-arm RCT	Daily 1. Ferrous ascorbate drops (2mg/kg) or 2. Standard of care.	No differences in the parental report of diarrhoea, vomiting or respiratory illnesses between the iron and standard of care groups ($p < 0.05$).
Desai, 2003(364)	Asembo, West Kenya	546 mildly anaemic infants (Hb 70 – 109 g/L) at 2 - 36 months of age for 12 weeks	4-arm RCT	1. Intermittent preventive treatment (IPT) with sulfadoxine-pyrimethamine (SP) at 4 and 8 weeks (25mg/kg of sulfadoxine + 1.25mg/kg of pyrimethamine; infants ≤ 10 kg received half a tablet (crushed into a powder), and those > 10 kg received a tablet (powdered) + 3–6mg/kg/day of ferrous sulphate drops for 12 weeks or 2. Placebo IPT SP + iron or 3. IPT SP + placebo iron or 4. Double placebo. // Bed nets + single SP dose was given to all participants upon enrolment.	There were no significant interactions between iron and malaria prophylaxis on the risk of malaria or non-malaria morbidity (HR: clinical malaria: 0.69 [0.39-1.22], $p = 0.21$; and nonmalaria morbidity: 1.16 [0.90-1.48], $p = 0.25$). Between 4 and 12 weeks after enrolment, IPT versus iron was associated with significant reductions in malaria parasitaemia and clinic visits and a non-significant reduction in clinical malaria ($p = 0.07$).
Duggan, 2012(370)	Dar es Salaam, Tanzania	2,397 infants at 6 weeks of age for 24 months (born to HIV positive mothers)	4-arm RCT	Daily 1. Multivitamin (60mg vitamin C, 8mg vitamin E, 0.5mg thiamine, 0.6mg riboflavin, 4mg niacin, 0.6mg vitamin B6, 130µg folate and 1µg vitamin B12) or 2. Placebo. // Infants under 6 months of age were administered 1 capsule (crushed into a powder) and then 2 capsules (powdered) from 6 months of age	Hospitalizations (RR: 0.83 [0.62, 1.13], $p = 0.23$), unscheduled clinic visits (RR: 0.97 [0.85, 1.10], $p = 0.59$), and maternal reports of diarrhoea (RR: 0.97 [0.87, 1.10], $p = 0.64$) were not significantly different between the groups, while fever ($p = 0.02$) and vomiting ($p = 0.007$) were significantly lower in the multivitamin group. Among 429 children whose mothers

				onwards. All mothers were provided with oral multivitamins and antiretroviral therapy from enrolment until end of follow-up. As part of standard medical care, all children received growth monitoring, immunizations, routine medical care for illnesses and large doses of vitamin A at 9, 15 and 21 months of age.	received antiretroviral therapy, multivitamin use had no effect on mortality but was associated with a significant reduction in hospitalizations ($p = 0.035$), episodes of fever ($p = 0.005$), and episodes of fever and cough ($p = 0.019$).
Lauer, 2019(384) (Sub-analysis of McDonald, 2015(363))	Dar es Salaam, Tanzania	2,400 infants in low malaria settings at 6 weeks of age for 18 months (born to HIV negative mothers)	4-arm RCT	Daily 1. Zinc (5mg) or 2. Multivitamins (60mg vitamin C, 8mg vitamin E, 0.5mg thiamine, 0.6mg riboflavin, 4mg niacin, 0.6mg vitamin B6, 130µg folate, and 1µg vitamin B12) or 3. Zinc + multivitamins or 4. Placebo. // Infants under 6 months of age were administered 1 tablet (crushed into a powder) and then 2 tablets (powdered) from 6 months of age onwards.	At 6 months of age, no significant treatment effects of zinc or multivitamins on environmental enteric dysfunction or systemic inflammation ($p \geq 0.05$) were seen.
Liu, 2013(369) (Sub-analysis of Duggan, 2012(370))	Dar es Salaam, Tanzania	2,387 infants at 6 weeks of age for 24 months (born to HIV positive mothers)	2-arm RCT	Daily 1. Multivitamin (60mg vitamin C, 8mg vitamin E, 0.5mg thiamine, 0.6mg riboflavin, 4mg niacin, 0.6mg vitamin B6, 130µg folate and 1µg vitamin B12) or 2. Placebo. // Infants under 6 months of age were administered 1 capsule (crushed into powder) and then 2 capsules (powdered) from 6 months of age onwards.	New cases of HIV infection during the follow-up were identified in 3.9% children in the placebo group and 4.7% in the multivitamin group. There was no effect of multivitamins on mother to child transmission (OR: 1.23 [0.77, 1.95], $p = 0.39$).
Lozoff, 2016(371) (Linked to Angulo-Barroso, 2016(372))	Hebei, China	1,276 infant-mother pairs at infant age of 6 weeks until 9 months (mothers supplemented from 16 weeks gestation until birth)	4-arm RCT	Daily 1. Ferrous sulphate in pregnancy (300mg) + ferrous proteinsuccinylate drops in infancy (1mg/kg) or 2. Placebo in pregnancy + iron in infancy or 3. Iron in pregnancy + placebo in infancy or 4. Double placebo. // All pregnant women received 0.40mg folate from enrolment to delivery.	No significant group differences in hospitalization (3–7 hospitalizations/group) or illnesses by 9 months of age were seen. Doctor visits were reported for upper respiratory symptoms (41–47%/group), gastrointestinal symptoms (16–21%/group) and pneumonia (3–5%/group).
Massaga, 2003(373)	Muheza, Tanzania	291 infants (vulnerable to malaria) at 12 - 16 weeks of age for six months	4-arm RCT	1. Daily ferric ammonium citrate mixture (7.5mg elemental iron) or 2. Amodiaquine (in a daily dose of 10, 10 and 5mg/kg/day for three consecutive days in intervals of 60 days) or 3. Iron + amodiaquine or 4. Double placebo.	Malaria prophylaxis was protective in the prevention of malaria fevers (PE: 64.7 [42.4, 77.2] and 63.9 [44.1, 76.7] %, $p < 0.001$, for amodiaquine and amodiaquine + iron, respectively; vs 13.0 [-22.2, 38.0] %, $p = 0.5$, for iron only). At the 4 months follow up, there was no rebound morbidity.

McDonald, 2015(363)	Dar es Salaam, Tanzania	2,400 infants in low malaria settings at 6 weeks of age for 18 months (born to HIV negative mothers)	4-arm RCT	Daily 1. Zinc (5mg) or 2. Multivitamins (60 mg vitamin C, 8mg vitamin E, 0.5mg thiamine, 0.6mg riboflavin, 4mg niacin, 0.6mg vitamin B6, 130µg folate, and 1µg vitamin B12) or 3. Zinc + multivitamins or 4. Placebo. // Infants under 6 months of age were administered 1 capsule (crushed into a powder) and then 2 capsules (powdered) from 6 months of age onwards. All mothers who enrolled during pregnancy received standard prenatal care: anthropometric assessment, intermittent prophylaxis for malaria, tetanus toxoid immunization, deworming using mebendazole, anaemia assessment, and iron-folic acid. As part of standard medical care, all children received growth monitoring, immunizations, routine medical care for illnesses and large doses of vitamin A at 9 (100,000IU) and 15 months of age (200,000IU).	There was a nonsignificant increase in all-cause mortality among infants who received zinc (HR: 1.80 [0.98, 3.31], $p = 0.06$) compared with those who. Multivitamins did not affect the occurrence of any of the morbidity symptoms such as hospitalizations (risk ratio (RR): 1.18 [0.67, 2.11], $p = 0.56$) or unscheduled outpatient visits (RR: 1.11 [0.92, 1.34], $p = 0.28$).
Menendez, 1997(374)	Ifakara, Tanzania	832 infants at risk of malaria at 8 weeks until 6 months (for iron) and 12 months (for antimalarial) of age	4-arm RCT	1. 2mg/kg/day of ferrous glycine sulphate + weekly dapsone (3x-weekly 125mg pyrimethamine + 25mg dapsone) or 2. Iron + placebo dapsone or 3. Placebo iron + dapsone 4. Double placebo.	Iron had no effect on the frequency of malaria (0.87 vs 1.00 cases per person-year; PE: 12.8 [-12.8, 32.5] %; $p = 0.30$) and groups receiving malaria prophylaxis had lower frequencies of malaria (0.53 vs 1.34 episodes per person-year; PE: 60.5 [48.2, 69.9] %, $p < 0.001$). During the follow up period from 12 - 24 months, infants who received malaria prophylaxis during the intervention had higher rates of malaria than those not receiving malaria prophylaxis during their first year of life (RR: 2.2 [1.3, 3.7], $p < 0.01$ vs 1.8 [1.3, 2.6], $p < 0.001$; respectively).
Nagpal, 2004(376)	New Delhi, India (rural)	100 infants at 4 - 6 months of age for 8 weeks	2-arm RCT	Daily 1. Ferric ammonium citrate drops (2 mg/kg) or 2. Placebo.	No statistical differences in reported interval morbidity was seen in the groups, but black colouring of the stools was significantly higher in the iron versus placebo group (90.7 vs 1.6 %; $p < 0.001$).
Schellenberg, 2004(378)	Ifakara, Tanzania	311 infants at 2 months - 5 years of age for 2 weeks or 3 months	2-arm RCT	1. 14-days of daily ferrous sulphate tablets (30mg of elemental iron) + 1x dose of sulfadoxine-pyrimethamine (SP) at day 1 (125mg sulfadoxine + 6.25mg pyrimethamine) (short-term) or 2. 3-months of daily ferrous sulphate + 3x SP doses at the start of each month (long-term). // Infants < 10kg received half a tablet (crushed into a powder) of both SP and ferrous sulphate.	No differences in the incidence of attendance to outpatients, admission, blood transfusion, or clinical malaria was seen between groups ($p > 0.2$ for all). The prevalence of <i>P. falciparum</i> parasitaemia was similar in both groups in both cross-sectional surveys ($p > 0.27$).

Tielsch, 2006(379)	Southern Nepal, India	26,250 infants at 1 - 35 until 36 months of age	3-arm RCT	Daily 1. (IFA) (12.5mg iron + 50µg folic acid) or 2. IFA + zinc (10 mg), or 3. Placebo. // Infants under 1 year of age received half a tablet (crushed into powder); all infants older than 6 months of age also received vitamin A as part of a national programme.	Although the IFA containing arms had to be stopped early, the all-cause mortality (RR: IFA: 1.03 [0.78, 1.37] and IFA + zinc: 1.00 [0.74, 1.34]) and reported morbidities (i.e. attack rates for diarrhoea, persistent diarrhoea, dysentery, or respiratory infections) between treatment groups did not differ.
Yurdakok, 2004(382)	Ankara, Turkey	70 infants at 4 until 7 months of age	3-arm RCT	Ferrous sulphate drops at 1. 1mg/kg/day or 2. 7mg/kg/week (once a week, three divided doses every Tuesday) or 3. Standard of care.	Side effects occurred in 44.4% of infants, with no significant difference between groups, but higher percentages in the weekly group (vomiting: 17.3 vs 27.2; decreased appetite: 21.7 vs 31.8; black stool: 21.7 vs 36.3; diarrhoea: 0 vs 4.5; constipation: 13.0 vs 9.0 %; for daily vs weekly, respectively).
NEURO-BEHAVIOURAL DEVELOPMENT					
Angulo - Barroso, 2016(372) (Linked to Lozoff, 2016 ⁽³⁷¹⁾)	Hebei, China	1,196 infant-mother pairs at infant age of 6 weeks until 9 months (mothers supplemented from 16 weeks gestation until birth)	4-arm RCT	Daily 1. Ferrous sulphate in pregnancy (300mg) + ferrous proteinsuccinylate drops in infancy (1mg/kg) or 2. Placebo in pregnancy + iron in infancy or 3. Iron in pregnancy + placebo in infancy or 4. Double placebo. // All pregnant women received 0.40mg folate from enrolment to delivery.	By nine months of age, iron supplementation in infancy but not pregnancy improved gross motor scores overall ($p < 0.001$; reflexes: $p = 0.03$; stationary: $p < 0.001$; and locomotion: $p < 0.001$), but there were no group differences in overall neurologic integrity (INFANIB total score, $p = 0.43$) of infants.
Bora, 2019(361)	Northeast India	200 infants 36 hours after birth until 6 months of age (1/2 born to anaemic mothers)	2-arm RCT	Daily 1. Ferrous ascorbate drops (2mg/kg) or 2. Standard of care.	At 6 months of age, in the iron group, motor development was closer to age appropriate norms (5.83 ± 0.69 vs 5.18 ± 1.35 for iron vs placebo, respectively) and active and passive tone milestones were significantly higher (94 vs 72 % for iron vs placebo, respectively) ($p < 0.01$ for both).
Friel, 2003 ⁽³⁶⁸⁾	Newfoundland, Canada (low-income population)	77 infants at 1 until 6 months of age; followed until 12 months	2-arm RCT	Daily 1. Ferrous sulphate syrup (7.5mg) or 2. Placebo.	The iron group scored significantly higher in Bayley psychomotor developmental indices (100 ± 12 vs 93 ± 9 for iron vs placebo, respectively) and tended toward improved visual acuity, which became significant when excluding noncompliers (1.54 ± 1.18 vs 0.87 ± 0.79 ; for iron vs placebo, respectively; $p = 0.07$) at 13 months of age.
Manji, 2014 ⁽³⁸⁶⁾ (Sub-analysis of Duggan, 2012 ⁽³⁷⁰⁾)	Dar es Salaam, Tanzania	192 infants, born to HIV infected mothers, with negative HIV status at 15 months, supplemented from 6 weeks of age for 24 months	2-arm RCT	1. Daily multivitamin (60 mg vitamin C, 8mg vitamin E, 0.5mg thiamine, 0.6mg riboflavin, 4mg niacin, 0.6mg vitamin B6, 130µg folate and 1µg vitamin B12) 2. Placebo. // Infants under 6 months of age were administered 1 capsule (crushed into a powder) and then 2 capsules (powdered) from 6 months of age onwards.	No effect of multivitamin supplementation was seen on any of the developmental domains at 15 months of age: (standardised mean differences: cognitive: 0.35 [-0.49, 1.18], $p = 0.42$; expressive language: -0.09 [-0.58, 0.39], $p = 0.7$; receptive language: 0.15 [-0.35, 0.64], $p = 0.56$; fine motor: 0.38 [-0.01, 0.78], $p = 0.06$); or gross motor: 0.27 [-0.26, 0.80], $p = 0.32$), but there was a trend toward improved fine motor skills among infants in the multivitamin group ($p = 0.06$).

Pongcharoen, 2011(340) (Follow up of Wasantwisut, 2006(380))	Rural Northeast Thailand	560 infants at 9 years of age, supplemented at 4 - 6 months of age for 6 months	4-arm RCT follow up	Daily 1. Ferrous sulphate syrup (10mg) or 2. Zinc sulphate syrup (10mg) or 3. Iron + zinc or 4. Placebo. // All supplements contained vitamin C (15g/L). Vitamin A drops at a dose of 1500mg retinol equivalent were given to each subject at the beginning of the trial.	No significant differences in any of the outcomes between the groups were seen at 9 years of age (mean intelligence quotients ranged from 92.9-93.7 for full scale, 93.9-95.4 for verbal, and 93.1-94.0 for performance; the Raven's Coloured Progressive Matrices score ranged from 21.4-22.4; school performance scores ranged from 70.7-72.3 for Thai, 67.2-69.8 for English, 69.2-70.7 for mathematics, and 69.9-72.0 for science).
Sudfeld, 2019(341) (Follow up of McDonald, 2015(363))	Dar es Salaam, Tanzania	365 infants followed up at 6 - 8 years of age, supplemented from 6 weeks of age for 18 months	4-arm RCT follow up	Daily 1. Zinc (5mg) or 2. Multivitamins (60 mg vitamin C, 8 mg vitamin E, 0.5 mg thiamine, 0.6 mg riboflavin, 4 mg niacin, 0.6 mg vitamin B6, 130µg folate, and 1µg vitamin B12) or 3. Zinc + multivitamins or 4. Placebo. // Infants under 6 months of age were administered 1 capsule (crushed into a powder) and then 2 capsules (powdered) from 6 months of age onwards.	There was no effect of zinc ($p = 0.54$) or multivitamin ($p = 0.41$) supplementation on any of the three development domains: (standardised mean differences (SMD): general intelligence: 0.02 [-0.09, 0.14] or 0.04 [-0.07, 0.16]); executive function: 0.03 [-0.10, 0.15] or 0.00 [-0.12, 0.13]; and mental health scores: 0.02 [-0.14, 0.19] or 0.07 [-0.10, 0.24]; for zinc or multivitamins, respectively).

* Abbreviations:

- Hb: haemoglobin
- ID: iron deficiency
- IDA: iron deficiency anaemia
- TS: transferrin saturation
- SF: serum ferritin
- IFA: iron folic acid
- OR: odds ratio
- MMN: multiple micronutrient
- PE: protective effect
- MCV: mean corpuscular volume
- HR: hazard ratio
- RR: risk ratio
- PCV: packed cell volume
- HAZ: height for age z-score
- WAZ: weight for age z-score
- WHZ: weight for height z-score
- WLZ: weight for length z-score

APPENDIX 2 ADDITIONAL FILES FROM PUBLISHED PAPER 2 (CHAPTER 4): CAREGIVER PERCEPTIONS OF NUTRITION INTERVENTIONS IN INFANTS AND CHILDREN UNDER 24 MONTHS OF AGE: A SYSTEMATIC REVIEW

Extended Datafile 1. PRISMA checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Title page
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	ABSTRACT
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Pg 2
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	Pg 2
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Table 3
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Pg 2-3
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Table 2
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Pg 3
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Pg 3
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Pg 3

Study risk of bias assessment	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Table 1
	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Pg 3
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	NA
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	Table 3
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	NA
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Pg 3
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Pg 3
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	NA
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	NA
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	Pg 3
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	Pg 3
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Figure 1
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Pg 4
Study characteristics	17	Cite each included study and present its characteristics.	Extended Datafile 3
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Extended Datafile 4

Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	NA
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	Extended Datafile 4
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	NA
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	NA
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	NA
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	Extended Datafile 4
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	Extended Datafile 4
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Pg 8-10
	23b	Discuss any limitations of the evidence included in the review.	Pg 10
	23c	Discuss any limitations of the review processes used.	Pg 10
	23d	Discuss implications of the results for practice, policy, and future research.	Pg 8-10
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	Pg 2
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	Pg 2
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	NA
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Title Page
Competing interests	26	Declare any competing interests of review authors.	Title Page
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	NA

Extended Datafile 2: 67 Excluded Full Texts

#22974 - Assibey-Mensah 2019

Effectiveness of Peer Counselor Support on Breastfeeding Outcomes in WIC-Enrolled Women

Assibey-Mensah, V; Suter, B; Thevenet-Morrison, K; Widanka, H; Edmunds, L; Sekhobo, J; Dozier, A

Journal of nutrition education and behavior // 2019;51(6):650-657

2019 //DOI: 10.1016/j.jneb.2019.03.005

No nutrition intervention

#23432 - Ahoya 2017

Baby friendly community initiative (BFCl): From guidelines to implementation-demonstrated results from western Kenya

Ahoya, B; Kavle, J; Gathi, C; Obade, M; Mwando, R; Akeyo, F; Straubinger, S

Annals of Nutrition and Metabolism // 2017;71(Supplement 2):527-528

B. Ahoya, Maternal Neonatal Child Health, Maternal and Child Survival Program/PATH, Kenya 2017

//DOI: 10.1159/000480486

No nutrition intervention

#21148 - Debpuur 2020

Supporting maternal and child nutrition: views from community members in rural Northern Ghana

Debpuur, C; Nonterah, E A; Chatio, S T; Adoctor, J K; Dambayi, E; Beeri, P; Nonterah, E W; Ayi-Bisah, D; Watson, D; Kehoe, S H; al., et

Public health nutrition // 2020;():1-8

2020 //DOI: 10.1017/S136898002000302X

No nutrition intervention

#21566 - deBarros 2016

Adherence to and acceptability of home fortification with vitamins and minerals in children aged 6 to 23 months: a systematic review

de Barros, S F; Cardoso, M A

BMC public health // 2016;16():299-299

2016 //DOI: 10.1186/s12889-016-2978-0

Wrong study design

#23577 - Abiona 2006

Acceptability, feasibility and affordability of infant feeding options for HIV-infected women: A qualitative study in south-west Nigeria

Abiona, T C; Onayade, A A; Ijadunola, K T; Obiajunwa, P O; Aina, O I; Thairu, L N

Maternal and Child Nutrition // 2006;2(3):135-144

T.C. Abiona, Obafemi Awolowo University, PO Box 1928, Ile-Ife 220005 Osun State, Nigeria. E-mail: titiabiona@yahoo.com 2006 //DOI: 10.1111/j.1740-8709.2006.00050.x

No nutrition intervention

#17762 - Lanou 2019

Micronutrient powder supplements combined with nutrition education marginally improve growth amongst children aged 6-23 months in rural Burkina Faso: a cluster randomized controlled trial

Lanou, H B; Osendarp, S J M; Argaw, A; De Polnay, K; Ouédraogo, C; Kouanda, S; Kolsteren, P

Maternal & child nutrition // 2019;15(4):e12820-e12820

2019 // DOI: 10.1111/mcn.12820

No caregiver perception

#22950 - Aubel 2004

Senegalese grandmothers promote improved maternal and child nutrition practices: the guardians of tradition are not averse to change

Aubel, J; Toure, I; Diagne, M

Social Science & Medicine // 2004;59(5):945-959

2004 //DOI: 10.1016/j.socscimed.2003.11.044

No nutrition intervention

#21505 - Cooper 2019

Perspectives on maternal, infant, and young child nutrition and family planning: Considerations for rollout of integrated services in Mara and Kagera, Tanzania

Cooper, C M; Kavle, J A; Nyoni, J; Drake, M; Lemwayi, R; Mabuga, L; Pfitzer, A

Maternal and Child Nutrition // 2019;15(Supplement 1):e12735-e12735

DOI: 10.1111/mcn.12735

No nutrition intervention

#13547 - Stewart 2013

Complementary feeding practices and acceptability of lipid based nutrient supplements among infants in rural western kenya

Stewart, C; Owino, V; Oiyee, S; Cheyde, R; Williams, A; Dentz, H; Null, C; Dewey, K

Annals of Nutrition and Metabolism // 2013;63(SUPPL. 1):443-443

2013 //DOI: 10.1159/000354245

No full text (ie conference proceeding, abstract etc)

#11977 - Zongrone 2017

Applying a feasibility lens to an integrated nutrition program in peri-urban and urban Senegal

Zongrone, A; Le Port, A; Becquey, E; Vanderkooy, A; Huybregts, L; Diatta, A D; Sessou, E; Leroy, J; Rawat, R; Ruel, M

Annals of Nutrition and Metabolism // 2017;71(Supplement 2):789-790

2017 //DOI: 10.1159/000480486

No full text (ie conference proceeding, abstract etc)

#23425 - Aidam 2017

Building on culture: A grandmother-inclusive strategy yields significant results on maternal child health and nutrition (MCHN) outcomes in southern Sierra Leone

Aidam, B; Webb Girard, A; MacDonald, C; Wee, R; Aubel, J; Simba, J; Gruenewald, C; Bangura, A

Annals of Nutrition and Metabolism // 2017;71(Supplement 2):1244-1244

2017 //

DOI: 10.1159/000480486

No full text (ie conference proceeding, abstract etc)

#14571 - Ruel-Bergeron 2017

Growing the evidence for nutrition programming: Perceptions and implementation of a stunting prevention program rural in Malawi

Ruel-Bergeron, J; Hurley, K; Oemcke, R; Buckland, A; Kapadia-Kundu, N; Kang, Y; Shu Fune Wu, L; Mitra, M; Phuka, J; Klemm, R; West, K; Christian, P

Annals of Nutrition and Metabolism // 2017;71(Supplement 2):86-86

2017 // DOI: 10.1159/000480486

No full text (ie conference proceeding, abstract etc)

#18036 - Kim 2015

Assessing implementation fidelity of a community-based infant and young child feeding intervention in Ethiopia identifies delivery challenges that limit reach to communities: a mixed-method process evaluation study

Kim, S S; Ali, D; Kennedy, A; Tesfaye, R; Tadesse, A W; Abrha, T H; Rawat, R; Menon, P

BMC public health // 2015;15():316-316

2015 //DOI: 10.1186/s12889-015-1650-4

No nutrition intervention

#22101 - Brannon 2017

Integrating themes, evidence gaps, and research needs identified by workshop on iron screening and supplementation in iron-replete pregnant women and young children

Brannon, Patsy M; Stover, Patrick J; Taylor, Christine L

American Journal of Clinical Nutrition // 2017;106():1703S-1712S

2017 //DOI: 10.3945/ajcn.117.156083

No caregiver perception

#18490 - Kingori 2010

'Rumours' and clinical trials: a retrospective examination of a paediatric malnutrition study in Zambia, southern Africa

Kingori, P; Muchimba, M; Sikateyo, B; Amadi, B; Kelly, P; Kingori, Patricia; Muchimba, Maureen; Sikateyo, Bornwell; Amadi, Beatrice; Kelly, Paul

BMC Public Health // 2010;10(1):556-556

2010 //DOI: 10.1186/1471-2458-10-556

Wrong age group

#12961 - Ware 2018

Perceptions and experiences of caregivers of severely malnourished children receiving inpatient care in Malawi: An exploratory study

Ware, S G; Daniel, A I; Bandawe, C; Mulaheya, Y P; Nkunica, S; Nkhoma, D; Kokota, D; Stewart, R C; Voskuil, W

Malawi medical journal : the journal of Medical Association of Malawi // 2018;30(3):167-173

2018 //DOI: 10.4314/mmj.v30i3.7

No nutrition intervention

#11845 - Sule 2009

Impact of nutritional education on nutritional status of under-five children in two rural communities of south-west Nigeria.

Sule, S S; Onayade, A A; Abiona, T C; Fatusi, A O; Ojofeitimi, E O; Esimai, O A; Ijadunola, K T

The Nigerian postgraduate medical journal // 2009;16(2):115-125

Nigeria 2009 //

No nutrition intervention

#20571 - Gonzalez 2017

Acceptability of unsweetened small quantity lipid-based nutrient supplement (SQ-LNS) in children aged 6 to 23 months: Formative research for the spoon project in Colombia, Guatemala and Mexico

Gonzalez, W; Bonvecchio, A; Duque, T; Bernal, I J; Montenegro-Bethancourt, G; Alvarado, R; Armendariz, G; Cosio, I; Vossenaar, M; Tumilowicz, A

Annals of Nutrition and Metabolism // 2017;71(Supplement 2):555-556

2017 // DOI: 10.1159/000480486

No full text (ie conference proceeding, abstract etc)

#21318 - Daly 2013

Formative research for the development of a home fortification programme for children in Zambia

Daly, Z; Suter, M; Aongola, A; McLean, J

Annals of Nutrition and Metabolism // 2013;63(SUPPL. 1):993-993

2013 //DOI: 10.1159/000354245

No full text (ie conference proceeding, abstract etc)

#23500 - Affleck 2012

Caregivers' responses to an intervention to improve young child feeding behaviors in rural Bangladesh: a mixed method study of the facilitators and barriers to change

Affleck, W; Peltó, G

Social science & medicine (1982) // 2012;75(4):651-658

2012 //DOI: 10.1016/j.socscimed.2012.03.030

No nutrition intervention

#19605 - Hellmann 2013

Withdrawal of artificial nutrition and hydration in the Neonatal Intensive Care Unit: parental perspectives

Hellmann, J; Williams, C; Ives-Baine, L; Shah, P S

Archives of Disease in Childhood -- Fetal & Neonatal Edition // 2013;98(1):F21-5

2013 //DOI: 10.1136/fetalneonatal-2012-301658

No nutrition intervention

#13389 - Suryantan 2013

Experiences from a comprehensive community nutrition program in two districts in Kenya. Lessons for nationwide mnp supplementation roll out

Suryantan, J; Wambua, F; Mutua, S; Tyler, D; Bloem, M A

Annals of Nutrition and Metabolism // 2013;63(SUPPL. 1):856-856

2013 // DOI: 10.1159/000354245

No full text (ie conference proceeding, abstract etc)

#23424 - Aidam 2020

An innovative grandmother-inclusive approach for addressing suboptimal infant and young child feeding practices in Sierra Leone

Aidam, B A; MacDonald, C A; Wee, R; Simba, J; Aubel, J; Reinsma, K R; Girard, A W

Current Developments in Nutrition // 2020;4(12):nzaa174-nzaa174

2020 // DOI: 10.1093/cdn/nzaa174

No nutrition intervention

#23078 - Armendariz 2017

Knowledge, attitudes and practices of first level health workers towards breastfeeding, complementary feeding and micronutrients supplements in Mexico: Formative research for the spoon project

Armendariz, G; Alvarado, R; Bonvecchio, A; Gonzalez, W

Annals of Nutrition and Metabolism // 2017;71(Supplement 2):555-555

2017 //DOI: 10.1159/000480486

No full text (ie conference proceeding, abstract etc)

#23079 - Armar-Klemesu 2018

Using Ethnography to Identify Barriers and Facilitators to Optimal Infant and Young Child Feeding in Rural Ghana: Implications for Programs

Armar-Klemesu, Margaret; Osei-Menya, Sarah; Zakariah-Akoto, Sawudatu; Tumilowicz, Alison; Lee, James; Hotz, Christine

Food & Nutrition Bulletin // 2018;39(2):231-245

2018 // DOI: 10.1177/0379572117742298

No nutrition intervention

#23101

Breastfeeding in Samoa: A Study to Explore Women's Knowledge and the Factors which Influence Infant Feeding Practices

Archer, Lucy E; Dunne, Thomas F; Lock, Lauren J; Price, Lucy A; Ahmed, Zubair

Hawai'i journal of medicine & public health: a journal of Asia Pacific Medicine & Public Health ;76(1):15-22

No nutrition intervention

#23386 - Alam 2020

"In the past, the seeds i planted often didn't grow." A mixed-methods feasibility assessment of integrating agriculture and nutrition behaviour change interventions with cash transfers in rural Bangladesh

Alam, A; Khatun, W; Khanam, M; Ara, G; Bokshi, A; Li, M; Dibley, M J

International Journal of Environmental Research and Public Health // 2020;17(11):1-15

2020 //DOI: 10.3390/ijerph17114153

Wrong age group

#22626

Mothers' Understanding of Infant Feeding Guidelines and Their Associated Practices: A Qualitative Analysis

Begley, Andrea; Ringrose, Kyla; Giglia, Roslyn; Scott, Jane

International journal of environmental research and public health ;16(7):

DOI: 10.3390/ijerph16071141

No nutrition intervention

#23503 - Adugna 2014

Women's perception and risk factors for delayed initiation of breastfeeding in Arba Minch Zuria, Southern Ethiopia

Adugna, Dessalegn Tamiru

International Breastfeeding Journal // 2014;9(1):1-14

Nutrition Unit, Public Health Department, Arba Minch University, Arba Minch, Ethiopia 2014 //

DOI: 10.1186/1746-4358-9-8

No nutrition intervention

#23144 - Andrews 2015

'I didn't know why you had to wait': an evaluation of NHS infant-feeding workshops amongst women living in areas of high deprivation

Andrews, E J; Symon, A; Anderson, A S

Journal of Human Nutrition & Dietetics // 2015;28(6):558-567

Centre for Public Health Nutrition Research, Ninewells Hospital & Medical School, University of Dundee, Dundee UK School of Nursing & Midwifery, University of Dundee, Dundee UK 2015 // DOI: 10.1111/jhn.12269

No nutrition intervention

#12284 - Balogun 2015

Factors influencing breastfeeding exclusivity during the first 6 months of life in developing countries: A quantitative and qualitative systematic review

Balogun, Olukunmi Omobolanle; Dagvadorj, Amarjagal; Anigo, Kola Mathew; Ota, Erika; Sasaki, Satoshi

Maternal and Child Nutrition // 2015;11(4):433-451

2015 // DOI: 10.1111/mcn.12180

Wrong study design

#23464 - Ahishakiye 2017

Stressors and coping strategies of infant and young child feeding practices in Rwanda: Perceptions of mothers, fathers, grandmothers and community health workers

Ahishakiye, J; Bouwman, L; Brouwer, I; Matsiko, E; Koelen, M

Annals of Nutrition and Metabolism // 2017;71(Supplement 2):400-401

J. Ahishakiye, Dietetics Department, University of Rwanda, Rwanda 2017 //DOI: 10.1159/000480486

No full text (ie conference proceeding, abstract etc)

#18426 - Kodish 2017

Formative research to understand household utilization of a lipid-based nutrient supplement in rural Malawi and Mozambique

Kodish, Stephen

Dissertation Abstracts International: Section B: The Sciences and Engineering // 2017;78(6-B(E)):No-Specified

2017 //

No full text (ie conference proceeding, abstract etc)

#19369 - Harding 2014

Caregiver perceptions, practices, and preferences relating to multiple micronutrient powders for children 6-23 mo of age in the northern highlands of Peru

Harding, K; Zavaleta, N; Roche, M; Neufeld, L

FASEB journal // 2014;28(1 SUPPL. 1):

2014 //

No full text (ie conference proceeding, abstract etc)

#23545 - Ackatia-Armah 2016

Fostering reflective trust between mothers and community health nurses to improve the effectiveness of health and nutrition efforts: An ethnographic study in Ghana, West Africa

Ackatia-Armah, Nana M; Addy, Nii Antiaye; Ghosh, Shibani; Dubé, Laurette

Social Science & Medicine // 2016;158():96-104

McGill Center for the Convergence in Health and Economics (MCCHE), Desautels Faculty of Management, McGill University, Montreal, QC, Canada Nevin Scrimshaw International Nutrition Foundation, Friedman School of Nutrition Science and Policy, Tufts University 2016 // DOI: 10.1016/j.socscimed.2016.03.038

No nutrition intervention

#23484 - Agbozo 2018

Understanding why child welfare clinic attendance and growth of children in the nutrition surveillance programme is below target: lessons learnt from a mixed methods study in Ghana

Agbozo, Faith; Colecraft, Esi; Jahn, Albrecht; Guetterman, Timothy

BMC Nursing // 2018;17(1):N.PAG-N.PAG

Department of Family and Community Health, School of Public Health University of Health and Allied Sciences PMG 31 Ho Ghana Institute of Public Health University of Heidelberg Medical Faculty Heidelberg Germany Department of Nutrition and Food Science Uni 2018 //DOI: 10.1186/s12912-018-0294-y

No nutrition intervention

#23460 - Ahluwalia 2000

Georgia's breastfeeding promotion program for low-income women

Ahluwalia, I B; Tessaro, I; Grummer-Strawn, L M; MacGowan, C; Benton-Davis, S

Pediatrics // 2000;105(6):E85-E85

I.B. Ahluwalia, Division of Reproductive Health, National Center for Chronic Disease Prevention and Health Promotion, Centers for Disease Control and Prevention, Atlanta, GA 30341-3724, USA. 2000

No nutrition intervention

#21451 - Creed-Kanashiro 2017

Acceptability of multi-micronutrient powders (MNP) in young children: Factors affecting caregivers' decisions - By family, health personnel and community actors in three regions of Peru

Creed-Kanashiro, H; Bartolini, R; Goya, C; Jimenez, M

Annals of Nutrition and Metabolism // 2017;71(Supplement 2):842-842

H. Creed-Kanashiro, Instituto de Investigacion Nutricional, Lima, Peru 2017 //DOI: 10.1159/000480486

No full text (ie conference proceeding, abstract etc)

#23328 - Almeida 2020

A Qualitative Study of Breastfeeding and Formula-Feeding Mothers' Perceptions of and Experiences in WIC

Almeida, Rebeca; Alvarez Gutierrez, Shawnee; Whaley, Shannon E; Ventura, Alison K

Journal of Nutrition Education & Behavior // 2020;52(6):615-625

Department of Kinesiology and Public Health, California Polytechnic State University, San Luis Obispo, CA Division of Research and Evaluation, Public Health Foundation Enterprises WIC, Irwindale, CA 2020 //DOI: 10.1016/j.jneb.2019.12.006

No nutrition intervention

#23463 - Ahishakiye 2019

Challenges and responses to infant and young child feeding in rural Rwanda: a qualitative study

Ahishakiye, Jeanine; Bouwman, Laura; Brouwer, Inge D; Matsiko, Eric; Armar-Klemesu, Margaret; Koelen, Maria

Journal of Health, Population & Nutrition // 2019;38(1):1-10

Health and Society Chair Group, Wageningen University, Wageningen, The Netherlands Human Nutrition and Dietetics Department, College of Medicine and Health Sciences, University of Rwanda, Kigali, Rwanda Nutrition and health over the life course chair group 2019 // DOI: 10.1186/s41043-019-0207-z

No nutrition intervention

#19155 - Ho 2013

Formative work on complementary feeding practices and anemia knowledge to guide the development of a home-fortification protocol in Rwanda

Ho, K; McLean, J; Omwega, A; Collison, D; Nyirahabineza, A; Ngabo, F

Annals of Nutrition and Metabolism // 2013;63(SUPPL. 1):654-654

K. Ho, Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, Canada 2013 //DOI: 10.1159/000354245

No full text (ie conference proceeding, abstract etc)

#23547 - Acheampong 2020

Breastfeeding and caring for children: A qualitative exploration of the experiences of mothers with physical impairments in Ghana

Acheampong, A K; Aziato, L; Marfo, M; Amedvor, P

BMC Pregnancy and Childbirth // 2020;20(1):331-331

L. Aziato, Department of Adult Health, School of Nursing and Midwifery, University of Ghana, Legon, Accra, Ghana. E-mail: aziatol@yahoo.com 2020 //DOI: 10.1186/s12884-020-03028-1

No nutrition intervention

#23602 - Abbeddou 2015

Comparison of methods to assess adherence to small-quantity lipid-based nutrient supplements (SQ-LNS) and dispersible tablets among young Burkinabé children participating in a community-based intervention trial

Abbeddou, Souheila; Hess, Sonja Y; Yakes Jimenez, Elizabeth; Somé, Jérôme W; Vosti, Stephen A; Guissou, Rosemonde M; Ouédraogo, Jean-Bosco; Brown, Kenneth H

Maternal & Child Nutrition // 2015;11():90-104

Program in International and Community Nutrition, Department of Nutrition, University of California, Davis California, USA Nutrition/Dietetics Program, Departments of Individual, Family and Community Education and Family and Community Medicine, University 2015 //DOI: 10.1111/mcn.12162

No caregiver perception

#23007 - Bentley 2013

The breastfeeding, antiretrovirals, and nutrition (BAN) study in Malawi: use of qualitative methods to guide study design and evaluation of a randomized controlled trial

Bentley, M; Corneli, A; Parker, M; Chasela, C; Kayira, D; Moses, A; Tembo, M; Van Der Horst, C; Jamieson, D; Adair, L

Annals of nutrition & metabolism // 2013;63():25-26

2013 //DOI: 10.1159/000354245

No full text (ie conference proceeding, abstract etc)

#16322 - Muraya 2017

"If it's issues to do with nutrition...I can decide...": gendered decision-making in joining community-based child nutrition interventions within rural coastal Kenya

Muraya, Kelly W; Jones, Caroline; Berkley, James A; Molyneux, Sassy

Health Policy & Planning // 2017;32():v31-v39

Health Systems & Research Ethics Department, P.O Box 230-80108, Kilifi, Kenya Centre for Tropical Medicine & Global Health, Nuffield Department of Medicine, University of Oxford, Old Road Campus, Headington, Oxford OX3 7BN, UK 2017 // DOI: 10.1093/heapol/czx032

No caregiver perception

#15783 - Pelto 2017

The mother-child dyad is a central factor in point-of-use fortification of complementary foods with micronutrient powders in Ethiopia and Mozambique

Pelto, G; Tumilowicz, A; Kjaer Pedersen, K; Schnefke, C H; Vetter sand, J; Possolo, E; Hagos, S

Annals of Nutrition and Metabolism // 2017;71(Supplement 2):533-533

G. Pelto, Division of Nutritional Sciences, Cornell University, Ithaca, NY, United States 2017 //DOI: 10.1159/000480486

No full text (ie conference proceeding, abstract etc)

#23592 - Abdollahi 2014

Oral zinc supplementation positively affects linear growth, but not weight, in children 6-24 months of age

Abdollahi, M; Abdollahi, Z; Fozouni, F; Bondarianzadeh, D

International Journal of Preventive Medicine // 2014;5(3):280-286

D. Bondarianzadeh, 7 Hafezi Street, Farahzadi Blvd, Shahrak-e-Qods, Tehran, Iran, Islamic Republic of. E-mail: d.bondarianzadeh@nnftri.ac.ir 2014 //

No caregiver perception

#23421 - Aiga 2017

Knowledge, attitude and practices: assessing maternal and child health care handbook intervention in Vietnam

Aiga, H; Nguyen, V D; Nguyen, C D; Nguyen, T T; Nguyen, L T

Tropical medicine & international health // 2017;22():329--329-

2017 // DOI: 10.1111/(ISSN)1365-3156

No nutrition intervention

#12121 - Young 2013

Community acceptability and utilization of micronutrient powders in Bihar, India

Young, M F; Kekre, P; Verma, P; Srikantiah, S; Majumdar, A; Trehan, S; Das, A K; Chaudhuri, I; Sheth, M; Webb-Girard, A; Ramakrishnan, U; Martorell, R

FASEB Journal // 2013;27(Meeting Abstracts):

M.F. Young, Global Health, Emory University, Atlanta, GA, United States 2013 //

No full text (ie conference proceeding, abstract etc)

#19160 - Hlaing 2016

Local food-based complementary feeding recommendations developed by the linear programming approach to improve the intake of problem nutrients among 12-23-month-old Myanmar children

Hlaing, L M; Fahmida, U; Htet, M K; Utomo, B; Firmansyah, A; Ferguson, E L

British Journal of Nutrition // 2016;116(S1):S16-S26

L.M. Hlaing, Department of Public Health, National Nutrition Center, Ministry of Health, Nay Pyi Taw 100604, Myanmar. E-mail: lmhlaing78@gmail.com 2016 //DOI: 10.1017/S000711451500481X

No nutrition intervention

#16326

Effect of soy flour addition and heat-processing method on nutritional quality and consumer acceptability of cassava complementary porridges

Muoki, Penina N; de Kock, Henriette L; Emmambux, Mohammad Naushad

Journal of the science of food and agriculture ;92(8):1771-1779

DOI: 10.1002/jsfa.5545

No caregiver perception

#14461 - Salasibew 2019

The fidelity and dose of message delivery on infant and young child feeding practice and nutrition sensitive agriculture in Ethiopia: a qualitative study from the Sustainable Undernutrition Reduction in Ethiopia (SURE) programme

Salasibew, M M; Moss, C; Ayana, G; Kuche, D; Eshetu, S; Dangour, A D

Journal of health, population, and nutrition // 2019;38(1):29-29

2019 // DOI: 10.1186/s41043-019-0187-z

No nutrition intervention

#22821 - Barros 2017

Designing culturally appropriate messages and approaches for maternal, infant, and young child nutrition in mozambique: Use and adaptation of trials of improved practices

Barros, I; Picolo, M; Victor, L; Kavle, J

Annals of Nutrition and Metabolism // 2017;71(Supplement 2):1267-1267

I. Barros, Maternal, Newborn, and Child Health and Nutrition Division, Maternal and Child Survival Program/ PATH, Mozambique 2017 //DOI: 10.1159/000480486

No full text (ie conference proceeding, abstract etc)

#23387 - Alam 2017

Designing an integrated agriculture and nutrition intervention to improve maternal and child nutrition in rural Bangladesh: A formative research

Alam, A; Bokshi, A; Ara, G; Li, M; McConchie, R; Khanam, M; Dibley, M

Annals of Nutrition and Metabolism // 2017;71(Supplement 2):404-404

A. Alam, Sydney School of Public Health, University of Sydney, Sydney, Australia 2017 //DOI: 10.1159/000480486

No full text (ie conference proceeding, abstract etc)

#22986 - Ashorn 2013

Long-term acceptability of LNS (lipid-based nutrient supplements) for infants in Malawi

Ashorn, U; Phiri, N; Maleta, K; Dewey, K; Arimond, M; Ashorn, P

Annals of Nutrition and Metabolism // 2013;63(SUPPL. 1):1011-1012

U. Ashorn, Department of International Health, School of Medicine, University of Tampere, Tampere, Finland 2013 //

DOI: 10.1159/000354245

No full text (ie conference proceeding, abstract etc)

#12123 - Young 2017

Caregiver perspectives and factors associated with utilization of a home fortification of complementary foods program

Young, M; Mehta, R; Gosdin, L; Kekre, P; Verma, P; Larson, L; Webb Girard, A; Ramakrishnan, U; Srikantiah, S; Martorell, R

Annals of Nutrition and Metabolism // 2017;71(Supplement 2):539-540

M. Young, Hubert Department of Global Health, Emory University, United States 2017 //DOI: 10.1159/000480486

No full text (ie conference proceeding, abstract etc)

#23440 - Ahmed 2013

Development and acceptability of locally developed ready-to-use complementary-food-supplements (RUCFS) in urban slum settings of dhaka, bangladesh

Ahmed, T; Choudhury, N; Hossain, M I; Islam, M M; Schumacher, B; De Pee, S; Tangsuphoom, N; Muiruri, J; Fuli, R; Parveen, M; Sarker, S; West Jr, K P; Christian, P

Annals of Nutrition and Metabolism // 2013;63(SUPPL. 1):582-582

T. Ahmed, ICDDR,B, Dhaka, Bangladesh 2013 // DOI: 10.1159/000354245

No full text (ie conference proceeding, abstract etc)

#20276 - Geletu 2019

Provision of low-iron micronutrient powders on alternate days is associated with lower prevalence of anaemia, stunting, and improved motor milestone acquisition in the first year of life: A retrospective cohort study in rural Ethiopia

Geletu, A; Lelisa, A; Baye, K

Maternal and Child Nutrition // 2019;15(3):e12785-e12785

K. Baye, Center for Food Science and Nutrition, College of Natural and Computational Sciences, Addis Ababa University, Addis Ababa, Ethiopia. E-mail: kaleabbaye@gmail.com 2019 // DOI: 10.1111/mcn.12785

No caregiver perception

#12295 - Adam 2019

The Philani MOVIE study: a cluster-randomized controlled trial of a mobile video entertainment-education intervention to promote exclusive breastfeeding in South Africa.

Adam, Maya; Tomlinson, Mark; Le Roux, Ingrid; LeFevre, Amnesty E; McMahon, Shannon A; Johnston, Jamie; Kirton, Angela; Mbewu, Nokwanele; Strydom, Stacy-Leigh; Prober, Charles; Barnighausen, Till

BMC health services research // 2019;19(1):211-211

England 2019 //DOI: 10.1186/s12913-019-4000-x

No nutrition intervention

#23550 - Acharya 2020

Exploring the use of mobile health to improve community-based health and nutrition service utilization in the hills of Nepal: Qualitative study

Acharya, A; Cunningham, K; Manandhar, S; Shrestha, N; Chen, M; Weissman, A

Journal of Medical Internet Research // 2020;22(9):e17659-e17659

A. Weissman, Asia Pacific Regional Office, Family Health International 360, Tower 3, Sindhorn Building, 130-132 Wireless Road, Lumpini, Phatumwan Bangkok 10330, Thailand. E-mail: aweissman@fhi360.org 2020 // DOI: 10.2196/17659

No nutrition intervention

#17448 - Mapesa 2020

Effect of community-based nutrition on infant nutrition and associated health practices in Narok, Kenya

Mapesa, J; Meme, J; Muthamia, O

African Health Sciences // 2020;20(2):724-734

J. Mapesa, Kenya Methodist University Nairobi Campus, Public Health Human Nutrition and Dietetics, Kenya. E-mail: job.mapesa@kemu.ac.ke 2020 // DOI: 10.4314/ahs.v20i2.24

No nutrition intervention

#18536 - Jordan 2018

Nutrition education improves dietary intake and growth of young children in Malawi and Cambodia- A randomised controlled trial

Jordan, I; Reinbott, A; Kuchenbecker, J; Heil, E; Phiri, G C; Krawinkel, M

Maternal & child nutrition // 2018;14():

2018 // DOI: 10.1111/mcn.12587

No full text (ie conference proceeding, abstract etc)

#22968 - Athavale 2020

A qualitative assessment of barriers and facilitators to implementing recommended infant nutrition practices in Mumbai, India

Athavale, P; Hoeft, K; Dalal, R M; Bondre, A P; Mukherjee, P; Sokal-Gutierrez, K

Journal of health, population, and nutrition // 2020;39(1):7-7

2020 // DOI: 10.1186/s41043-020-00215-w

No nutrition intervention

#23599

Assessing the Impact of a Community-Based Health and Nutrition Education on the Management of Diarrhea in an Urban District, Cairo, Egypt

Abdel-Aziz, Shaimaa B; Mowafy, Maha A; Galal, Yasmine S

Global journal of health science ;8(2):46-55

DOI: 10.5539/gjhs.v8n2p46

No nutrition intervention

#19147 - Hoddinott 2018

Randomized control trials demonstrate that nutrition-sensitive social protection interventions increase the use of multiple-micronutrient powders and iron supplements in rural pre-school Bangladeshi children

Hoddinott, J; Ahmed, A; Roy, S

Public health nutrition // 2018;21(9):1753-1761

2018 //DOI: 10.1017/S1368980017004232

No caregiver perception

#19030 - Jansen 2018

Acceptability and accessibility of child nutrition interventions: Fathers' perspectives from survey and interview studies

Jansen, E; Harris, H; Daniels, L; Thorpe, K; Rossi, T

International Journal of Behavioral Nutrition and Physical Activity // 2018;15(1):67-67

E. Jansen, Centre for Children's Health Research, 62 Graham Street (Level 6), South Brisbane, QLD 4101, Australia. E-mail: Elena.jansen@aau.at 2018 //DOI: 10.1186/s12966-018-0702-4

Wrong age group

#22752 - Bonvecchio 2007

Maternal knowledge and use of a micronutrient supplement was improved with a programmatically feasible intervention in Mexico

Bonvecchio, A; Pelto, G H; Escalante, E; Monterrubio, E; Habicht, J P; Nava, F; Villanueva, M A; Safdie, M; Rivera, J A

Journal of nutrition // 2007;137(2):440-446

2007 // DOI: 10.1093/jn/137.2.440

No caregiver perception

Extended Datafile 3a. Characteristics of studies included in this review

Reference	Country	Data Collection Methodology	Caregiver	Sample size	Nutrition intervention
Aaron et al, 2011	Senegal	Ratings	Caregiver	21	Complementary foods (CF) fortified with iron or the same level of iron + zinc from 12 months (mo) of age
Adams et al, 2018	Ghana	Surveys	Mother s and fathers	1,273	Small quantity lipid-based supplements (SQ-LNS) from six mo of age
Ahmed et al, 2014	Bangladesh	Questionnaire s and ratings	Mother s/ caregivers	90	Three ready-to-use supplementary food (RUSFs): rice-lentil based, chickpea based and standard RUSF (Pushti packet cereal-based) from six mo of age
Andersen et al, 2009	South Africa	Focus group discussions (FGDs)	Mother s	28	Nutrition Supplementation Programme: rehabilitation program for undernourished children under five years of age

Angdembe et al, 2015	Bangladesh	Questionnaires	Mothers	78	Multiple micronutrient powders (MMNPs) from six months of age
Ashorn et al, 2015	Malawi	Knowledge, attitudes, and practices (KAP) interviews and in-depth interviews (IDIs)	Mothers	1,098	Lipid-based nutrient supplements (LNS) from six months of age
Athavale et al, 2020	India	IDIs	Mothers and paternal grandmothers	33	Infant and young child feeding (IYCF) counselling from six mo of age
Bashir et al, 2016	Pakistan	Questionnaires	Mothers	60	Ready to use therapeutic food (RUTF) for malnourished children from three mo of age
Brewer et al, 2020	Peru	IDIs and FGDs	Mothers, fathers, grandparents, and aunts	129	Micronutrient powders (MNPs) from six mo of age
Campos et al, 2015	Brazil	Ratings	Mothers	50	Homemade chicken liver baby food versus ground beef baby food (control) from six mo of age
Cohuet et al, 2012	Niger	IDIs, FGDs, and questionnaires	Caregivers	1,842	RUSF from six mo of age for infants with moderate acute malnutrition (MAM) or at risk of malnutrition
Creed-Kanashiro et al, 2016	Peru	IDIs	Caregivers	64	MNPs for anaemic children from six mo of age
Creed-Kanashiro et al, 2018	Peru	IDIs	Mothers	108	Commercial infant food grinders where there is delayed/low consumption of thick CFs, including animal-source foods from six mo of age
Goyena et al, 2019	Philippines	IDIs	Mothers	141	MNPs from six mo of age
Gunaratna et al, 2015	Ethiopia	Questionnaires and ratings	Mothers	61	A biofortified crop, quality protein maize (QPM), as a CF versus conventional maize from six mo of age
Hess et al, 2011	Burkina Faso	Ratings	Mothers	36	LNS with or without zinc from nine mo of age (breastfed)
Horton et al, 2018	Senegal	FGDs	Mothers and grandmothers	~64-96	Government vitamin A distribution from six mo of age

Ip et al, 2009	Bangladesh	FGDs	Mother s	54	Sprinkles from six mo of age
Jefferds et al, 2010	Kenya	FGDs	Mother s and grandmothers	~30-54	Sprinkles from six mo of age
Kajjura et al, 2020	Uganda	FGDs and IDIs	Mother s	156	Supplementary food (SF) in children with MAM using either a malted sorghum-based porridge or fortified corn soy blend as a porridge from six mo of age
Kodish et al, 2017	Mozambique	IDIs	Male and female caregivers	31	SQ-LNS from six mo of age
Korenromp et al, 2015	Nigeria	Surveys and questionnaires	Caregivers (Mothers)	272	Health service MNPs from six mo of age
Kung'u et al, 2015	Nigeria	Semi-structured interviews	Caregivers	1,195	Nutrition training of oral rehydration solution (ORS) for use in diarrhoea followed by a take home diarrhoea management kit (zinc + ORS) for children under five years of age
Kwon et al, 2014	India	Semi-structured interviews and FGDs	Mother s and other caregivers	38	Nutrition education on the use of daily oral ferrous sulphate for children under five years of age
Locks et al, 2017	Madagascar	Surveys	Mother s	1,204	Integrated IYCF-MNP program aimed to reduce anemia among and improve IYCF from six mo of age
Loechl et al, 2009	Haiti	Interviews and FGDs	Caregivers (mothers)	254	Government Sprinkle distribution along with take-home food rations for two mo from six mo of age
McLean et al, 2018	Rwanda	FGDs, interviews and surveys	Mother s, grandmothers, and fathers	1,589	MNPs from six mo of age
Pelto et al, 2018	Ethiopia	IDIs	Mother s and one grandmother	45	MNPs from six mo of age
Phuka et al, 2011	Malawi	Ratings and FGDs	Mother s	67	Three new LNS vs Nutributter from eight mo of age

Roschnik et al, 2019	Malawi	FGDs and IDIs	Mother s and fathers	159	Community led MNP from six mo of age alongside childhood development interventions and malaria chemoprevention
Rothman et al, 2015	South Africa	Ratings and FGDs	Mother s	42	Two novel SQ-LNS from six mo of age
Ruel-Bergeron et al, 2018	Malawi	IDIs and FGDs	Mother s, fathers, and grandm others	49	A social and behaviour change communications campaign promoting optimal IYCF and hygiene practices, and monthly provision of daily SQ-LNS (Nutributter) from six mo of age
Samuel et al, 2020	Ethiopi a	Interviews	Mother s	11	MNPs from six mo of age
Shaari et al, 2019	Malays ia	Interviews	Caregiv er	25	Multiple micronutrient supplements from six mo of age
Tumilowicz et al, 2019	Ethiopi a	Survey	Mother s	1,9 16	MNP and IYCF behaviour change communication from six months of age
Uti et al, 2005	Nigeria	Interviews using a questionnaire	Mother s	100	Vitamin A supplementation programme from one mo of age
Young et al, 2018	India	Surveys and IDIs	Mother s	120	Iron folic acid syrup and MNPs from six mo of age

Extended Datafile 3b. Acceptability of studies included in this review

Reference	Low acceptability	High acceptability	Both cited	Inconclusive
Aaron et al, 2011		x		
Adams et al, 2018			x	
Ahmed et al, 2014			x	
Andersen et al, 2009			x	
Angdembe et al, 2015		x		
Ashorn et al, 2015			x	
Athavale et al, 2020			x	
Bashir et al, 2016		x		
Brewer et al, 2020			x	
Campos et al, 2015				
Cohuet et al, 2012		x		x
Creed-Kanashiro et al, 2016			x	
Creed-Kanashiro et al, 2018		x		
Goyena et al, 2019			x	
Gunaratna et al, 2015			x	

Hess et al, 2011		x		
Horton et al, 2018				x
Ip et al, 2009		x		
Jefferds et al, 2010			x	
Kajjura et al, 2020			x	
Kodish et al, 2017	x			
Korenromp et al, 2015			x	
Kung'u et al, 2015		x		
Kwon et al, 2014			x	
Locks et al, 2017			x	
Loechl et al, 2009			x	
McLean et al, 2018			x	
Pelto et al, 2018			x	
Phuka et al, 2011		x		
Roschnik et al, 2019		x		
Rothman et al, 2015		x		
Ruel-Bergeron et al, 2018			x	
Samuel et al, 2020			x	
Shaari et al, 2019		x		
Tumilowicz et al, 2019	x			
Uti et al, 2005		x		
Young et al, 2018		x		

Extended Datafile 4. Quality Assessment: Critical Appraisal Skills Programme (CASP) tool

Reference	1	2	3	4	5	6	7	8	9	10	Rating
Aaron et al, 2011	Y*	Y	Y	CD*	Y	N**	N	Y	Y	Y	Fair
Adams et al, 2018	Y	Y	Y	Y	CD	N	Y	Y	CD	Y	Fair
Ahmed et al, 2014	Y	Y	Y	CD	Y	N	Y	CD	Y	CD	Poor
Andersen et al, 2009	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Good
Angdembe et al, 2015	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Good
Ashorn et al, 2015	Y	Y	Y	CD	Y	Y	Y	Y	Y	Y	Good
Athavale et al, 2020	Y	Y	Y	Y	Y	Y	Y	Y	Y	CD	Good
Bashir et al, 2016	Y	Y	Y	Y	CD	N	Y	CD	Y	CD	Fair
Brewer et al, 2020	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Good
Campos et al, 2015	Y	Y	Y	CD	Y	N	Y	Y	Y	CD	Fair
Cohuet et al, 2012	Y	Y	Y	CD	Y	Y	Y	Y	Y	CD	Good
Creed-Kanashiro et al, 2016	Y	Y	Y	CD	Y	N	Y	Y	Y	Y	Good

Creed-Kanashiro et al, 2018	Y	Y	Y	Y	Y	Y	Y	Y	Y	CD	Good
Goyena et al, 2019	Y	Y	Y	CD	Y	Y	Y	Y	Y	Y	Good
Gunaratna et al, 2015	Y	Y	Y	CD	Y	Y	Y	Y	Y	Y	Good
Hess et al, 2011	Y	Y	Y	CD	Y	N	Y	Y	Y	CD	Fair
Horton et al, 2018	Y	Y	Y	CD	Y	Y	Y	Y	Y	CD	Good
Ip et al, 2009	Y	Y	Y	CD	CD	Y	Y	CD	Y	Y	Fair
Jefferds et al, 2010	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Good
Kajjura et al, 2020	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Good
Kodish et al, 2017	Y	Y	Y	CD	CD	N	Y	Y	Y	Y	Fair
Korenromp et al, 2015	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Good
Kung'u et al, 2015	Y	Y	Y	Y	Y	N	Y	Y	Y	CD	Good
Kwon et al, 2014	Y	Y	Y	Y	CD	N	Y	Y	Y	CD	Fair
Locks et al, 2017	Y	Y	Y	Y	Y	N	Y	CD	Y	Y	Good
Loechl et al, 2009	Y	Y	Y	CD	CD	Y	Y	Y	Y	Y	Good
McLean et al, 2018	Y	Y	Y	CD	Y	Y	Y	Y	Y	Y	Good
Pelto et al, 2018	Y	Y	Y	Y	CD	N	Y	Y	Y	Y	Good
Phuka et al, 2011	Y	Y	Y	Y	CD	N	Y	Y	Y	Y	Good
Roschnik et al, 2019	Y	Y	Y	CD	CD	N	Y	Y	Y	Y	Good
Rothman et al, 2015	Y	Y	Y	CD	Y	Y	Y	Y	Y	Y	Good
Ruel-Bergeron et al, 2018	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Good
Samuel et al, 2020	Y	Y	Y	Y	CD	N	Y	Y	Y	Y	Good
Shaari et al, 2019	Y	Y	Y	CD	CD	N	Y	Y	Y	CD	Fair
Tumilowicz et al, 2019	Y	Y	Y	CD	CD	N	Y	CD	Y	Y	Fair
Uti et al, 2005	Y	Y	Y	CD	CD	CD	Y	Y	Y	Y	Fair
Young et al, 2017	Y	Y	Y	CD	CD	N	Y	Y	Y	Y	Fair

*Y – Yes, **N – No, **CD – Cannot determine/ cannot tell

Reference: Aaron et al 2011	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	To assess the acceptability of zinc-fortified, cereal-based complementary foods and zinc-fortified wheat breads
2. Is a qualitative methodology appropriate?	Yes	Understanding caregiver and parents' acceptability of a nutrition intervention in young children
3. Was the research design appropriate to address the aims of the research?	Yes	Acceptability, triangle, and threshold tests
4. Was the recruitment strategy appropriate to the aims of the research?	Cannot tell	Does not specify how participants were recruited
5. Was the data collected in a way that addressed the research issue?	Yes	Conducted at health centres
6. Has the relationship between researcher and participants been adequately considered?	No	No mention
7. Have ethical issues been taken into consideration?	No	No mention

8. Was the data analysis sufficiently rigorous?	Yes	Probability of type I error/ $\alpha < 0.05$ and power ≥ 0.80 for all components of the study
9. Is there a clear statement of findings?	Yes	Zinc fortification of cereal flours in the ranges of fortification that were tested does not adversely affect the acceptability of complementary foods and breads prepared from these flours
10. How valuable is the research?	Yes	Summaries acceptability of fortified foods
Comments on key limitations	The study did summarise acceptability of fortified foods, however not in qualitative outcomes (IDIs/FGDs)	

Reference: Adams et al 2018	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	Retrospective and current parental perceptions of nutrient supplements generally and of small quantity lipid based nutrient supplements (SQ-LNS) and their effects compared with perceptions IFA and MMN capsules
2. Is a qualitative methodology appropriate?	Yes	Parental perceptions of a past nutrition intervention in young children
3. Was the research design appropriate to address the aims of the research?	Yes	Survey-based methods
4. Was the recruitment strategy appropriate to the aims of the research?	Yes	Pregnant women on a rolling basis due for routine visits
5. Was the data collected in a way that addressed the research issue?	Cannot tell	Does not specify where the follow up study took place
6. Has the relationship between researcher and participants been adequately considered?	No	No mention
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Cannot tell	No specification on qualitative data analysis
9. Is there a clear statement of findings?	Yes	Almost all ($\geq 90\%$) of mothers and fathers perceived that the assigned supplement positively impacted the child and expected continued positive impacts on the child's health and human capital into the future
10. How valuable is the research?	Yes	Investigates long term acceptability of a nutrition intervention in young infancy
Comments on key limitations	The study was survey based rather than through FGDs/IDIs. Likewise, while it is interesting to have longer term acceptability (4-6 years later), this may impact parents' memory	

Reference: Ahmed et al 2014	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	To develop RUSF using locally available food ingredients and test their acceptability
2. Is a qualitative methodology appropriate?	Yes	Mothers' acceptability of a nutrition intervention in young children

3. Was the research design appropriate to address the aims of the research?	Yes	Interviews with mothers as well as their grading on a hedonic scale
4. Was the recruitment strategy appropriate to the aims of the research?	Cannot tell	Does not specify
5. Was the data collected in a way that addressed the research issue?	Yes	The data was collected in a health centre
6. Has the relationship between researcher and participants been adequately considered?	No	Does not specify
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Cannot tell	Vague explanation of analysis
9. Is there a clear statement of findings?	Yes	Interviews with the caregivers/mothers revealed that 18/30 (60%) and 20/30 (66%) of children preferred the rice-lentil and chickpea-based RUSFs, respectively
10. How valuable is the research?	Cannot tell	Not specific enough differences
Comments on key limitations	The study did not give specifics on analysis and the results are too close to tell if the study was rigorous	

Reference: Andersen et al 2009	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	To study mothers' experience with the Nutrition Supplementation Programme (NSP), and assess this in relation to South Africa's emphasis on human rights
2. Is a qualitative methodology appropriate?	Yes	Mothers experience of a government nutrition program for children
3. Was the research design appropriate to address the aims of the research?	Yes	FGDs with mothers of infants under five included in the NSP
4. Was the recruitment strategy appropriate to the aims of the research?	Yes	Purposeful, criterion-based sampling method was used to select the participants for the FGDs
5. Was the data collected in a way that addressed the research issue?	Yes	The data was collected through primary health care clinics
6. Has the relationship between researcher and participants been adequately considered?	Yes	Hired moderators in the preferred language of the participants with some experience with moderating FGDs (trained by the main researcher and practised through pilot FGDs)
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Yes	Performed according to the Systematic Text Condensation method developed by Malterud (modified version of Giorgi's method) suited for the development of descriptions/notions related to human experience
9. Is there a clear statement of findings?	Yes	Mothers expressed satisfaction with receiving the supplements, which they perceived to be

		nutritious. They received little/no education and lacked knowledge/skills regarding how to help children gain weight. Many mothers experienced poor communication with staff members as well as unfavourable comments/ lack of respect
10. How valuable is the research?	Yes	Gives maternal perspective of a government funded nutrition program in young infants
Comments on key limitations	Only interviews mothers enrolled in program so acceptability may be skewed	
Reference: Angdembe et al 2015	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	To assess adherence to MMNP and associated factors among children aged 6–59 months in rural Bangladesh
2. Is a qualitative methodology appropriate?	Yes	For factors associated to a nutrition intervention in young children
3. Was the research design appropriate to address the aims of the research?	Yes	Semi structured questionnaires with mothers
4. Was the recruitment strategy appropriate to the aims of the research?	Yes	With a 95% confidence level and 10% margin of error, sample size was 59. Taking into account a nonresponse of 10%, final sample size was calculated to be 78. A one stage cluster sampling technique was used
5. Was the data collected in a way that addressed the research issue?	Yes	By trained interviewers using a standardized pretested semi-structured questionnaire, drafted in English, and translated into Bangla
6. Has the relationship between researcher and participants been adequately considered?	Yes	Trained interviewers in the local language
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Yes	Variables were fitted in the model in blocks: 1. Socio-demographic 2. Morbidity related 3. Mother's perception related 4. Program related 5. Knowledge related 6. Acceptability related 7. Female health worker grade variable
9. Is there a clear statement of findings?	Yes	MMNPs had high acceptability and adherence among mothers, with daily supplementation being preferred (to alternate days)
10. How valuable is the research?	Yes	Maternal acceptability of MMNP in young infants
Comments on key limitations	Only used a questionnaire rather than IDI/FGD so more quantitative	

Reference: Ashorn et al 2015	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	To determine the sustained acceptability of LNSs among 6- to 18-mo-old children in Malawi
2. Is a qualitative methodology appropriate?	Yes	Acceptability of a nutrition intervention in young children
3. Was the research design appropriate to address the aims of the research?	Yes	Knowledge, attitude, and practices (KAP) interviews at month 12 and 18 of the intervention and IDIs with a subset of mothers

4. Was the recruitment strategy appropriate to the aims of the research?	CD	Does not specify
5. Was the data collected in a way that addressed the research issue?	Yes	At homes of participants. The guides were translated into the local languages.
6. Has the relationship between researcher and participants been adequately considered?	Yes	A Malawian female researcher visited the homes of the participants. Also carried out IDIs with participants who later dropped out
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Yes	Framework analysis; thematic analysis
9. Is there a clear statement of findings?	Yes	The acceptability of LNS products was good but sharing of the products with family members and deviation from other feeding recommendations were frequent so individually targeted children were likely to receive less than the intended dose of the LNS
10. How valuable is the research?	Yes	Maternal acceptability of LNS, but sharing is common
Comments on key limitations	Recruitment strategy not specified	

Reference: Athavale et al 2020	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	In-depth qualitative assessment of family barriers and facilitators to implementing recommended child nutrition practices
2. Is a qualitative methodology appropriate?	Yes	Barriers and facilitators to government nutrition recommendations
3. Was the research design appropriate to address the aims of the research?	Yes	Maternal and maternal grandmothers' perceptions to government nutrition interventions in young children
4. Was the recruitment strategy appropriate to the aims of the research?	Yes	Purposively sampled
5. Was the data collected in a way that addressed the research issue?	Yes	In participants homes
6. Has the relationship between researcher and participants been adequately considered?	Yes	The interviews were conducted in the participant's home, in the participant's preferred language (Hindi or Marathi)
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Yes	Thematic analysis using inductive coding along with co-authors until saturation reached
9. Is there a clear statement of findings?	Yes	Key barriers included: lack of nutrition knowledge and experience, receiving conflicting messages from different sources, limited social support, and poor self-efficacy for maternal decision-making. Key facilitators Ethics statement of approval through committee included: professional nutrition guidance, personal self-efficacy and empowerment, and family support

10. How valuable is the research?	Cannot tell	Focuses more on facilitators/barriers than acceptability per se
Comments on key limitations	Greater focus on overall facilitators/barriers than acceptability	

Reference: Bashir et al 2016	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	To evaluate the effectiveness and acceptability of ready to use therapeutic food (RUTF) among malnourished children in a tertiary care hospital
2. Is a qualitative methodology appropriate?	Yes	To assess maternal acceptability of a nutrition intervention in young infants
3. Was the research design appropriate to address the aims of the research?	Yes	Well structured questionnaires with mothers
4. Was the recruitment strategy appropriate to the aims of the research?	Yes	Conducted at a tertiary care hospital
5. Was the data collected in a way that addressed the research issue?	Cannot tell	Does not specify
6. Has the relationship between researcher and participants been adequately considered?	No	No mention
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Cannot tell	No detail given
9. Is there a clear statement of findings?	Yes	All mothers were satisfied from ready to use therapeutic foods (100%)
10. How valuable is the research?	Cannot tell	Focuses more on quantitative acceptability outcomes (amount consumed)
Comments on key limitations	More quantitative, lacking FGDs/IDIs	

Reference: Brewer et al 2020	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	To explore factors at all levels of the Social-Ecological Model that affect MNP use and adherence in a Peruvian city with childhood anaemia rates higher than the national average
2. Is a qualitative methodology appropriate?	Yes	To explore caregivers KAP towards MNPs and anaemia
3. Was the research design appropriate to address the aims of the research?	Yes	IDIs and FGDs with caregivers
4. Was the recruitment strategy appropriate to the aims of the research?		Convenience sampling from within/around health establishments selected by choosing one health centre and one health post per district, favouring those establishments with the highest percentage of children with anaemia
5. Was the data collected in a way that addressed the research issue?	Yes	Health establishments

6. Has the relationship between researcher and participants been adequately considered?	Yes	Local health personnel
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Yes	Thematic analysis with structural coding, cross coding, and blind double coding
9. Is there a clear statement of findings?	Yes	Barriers: negative side effects (constipation, vomiting, and diarrhoea), poor taste of MNP, lack of familial and peer support for its use, insufficient informational resources provided by the health system, and limited human resources. Facilitators: concern about the long-term effects of anaemia, support from organizations external to the health system, well-coordinated care within the health system, and provision of resources by the MoH
10. How valuable is the research?	Yes	Barriers and facilitators for anemia prevention and control
Comments on key limitations	More focus on barriers and facilitators than specific care giver acceptability of intervention	

Reference: Campos et al 2015	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	to develop a homemade chicken liver baby food (CLBF) that meets infant's nutritional requirements for energy, macronutrients, sodium, and iron and to test its acceptance by infants and their mothers in a blind sensory test (ST)
2. Is a qualitative methodology appropriate?	Yes	Maternal preference for a complementary food
3. Was the research design appropriate to address the aims of the research?	Yes	Blind sensory test and hedonic scale
4. Was the recruitment strategy appropriate to the aims of the research?	Cannot tell	Does not specify
5. Was the data collected in a way that addressed the research issue?	Yes	In a basic health unit
6. Has the relationship between researcher and participants been adequately considered?	No	Does not specify
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Yes	The Kappa index was used to measure the agreement between mothers' like or dislike of liver and beef, answered in the questionnaire, and their score of the CLBF and ground beef baby food (GBBF) in the blind ST
9. Is there a clear statement of findings?	Yes	Infants tried and liked the CLBF that match dietary recommendations and could help prevent iron deficiency. Mothers, on the other hand,

		demonstrated an ‘I don’t like it; I never tried it’ attitude
10. How valuable is the research?	Cannot tell	Does not explore qualitative acceptability through IDIs/FGDs
Comments on key limitations	More quantitative outcomes	

Reference: Cohuet et al 2012	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	Acceptability of RUSF within households in four districts of Niger during large scale preventive distributions with RUSF targeted at children 6–35 months of age
2. Is a qualitative methodology appropriate?	Yes	To explore caregiver acceptability of a nutrition intervention targeted at young children
3. Was the research design appropriate to address the aims of the research?	Yes	Cross sectional survey, IDIs and FGDs
4. Was the recruitment strategy appropriate to the aims of the research?	Yes	Stratified villages into two strata based on accessibility defined as presence or absence of a health centre, market, water point, or main road within a 10 km radius. Villages within each stratum were selected using cluster-based sampling, proportional to population size, within the four districts with RUSF distributions. All households in the selected villages were eligible for inclusion in the study
5. Was the data collected in a way that addressed the research issue?	Cannot tell	Does not specify
6. Has the relationship between researcher and participants been adequately considered?	Yes	Community health workers within the same range as the caregivers
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Yes	The content of the FGDs and IDIs were analyzed using a list of codes defined before the start of data collection to include all themes related to the study objectives. To provide a richer analysis, we added additional codes for emerging themes that were not in the initial list. Both inductive and deductive codes were used
9. Is there a clear statement of findings?	Yes	IDIs and FGDs confirmed the overall positive perception of RUSF among caregivers. Mothers also described improvements of children’s health and vitality after RUSF consumption
10. How valuable is the research?	Cannot tell	Clear findings but more on practices rather than acceptability
Comments on key limitations	Does not specify where data was collected and focuses more on practices than acceptability per se	

Reference: Creed-Kanashiro et al 2016	Rating	Description

1. Was there a clear statement of the aims of the research?	Yes	To explore caregiver acceptability of MNP and the role of health personnel (HP) in three regions of Peru piloting a MNP programme
2. Is a qualitative methodology appropriate?	Yes	Yes, caregiver acceptability of a nutrition intervention in young children
3. Was the research design appropriate to address the aims of the research?	Yes	Interviews and observations of caregivers
4. Was the recruitment strategy appropriate to the aims of the research?	Cannot tell	Does not specify
5. Was the data collected in a way that addressed the research issue?	Yes	At caregiver homes
6. Has the relationship between researcher and participants been adequately considered?	No	Does not specify
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Yes	IDIs were transcribed and coded by themes, organized into thematic matrixes by region, type of participant and by peri-urban and rural areas. The researchers read all the matrixes, noting coincidences and differences within each region and between regions for each theme. The findings and comprehension of the data were discussed and consolidated
9. Is there a clear statement of findings?	Yes	Caregivers expressed preference for MNP to ferrous sulfate, not only for the taste and ease to give to the child but also to keep/transport
10. How valuable is the research?	Yes	Caregiver perception of MNP vs standard intervention
Comments on key limitations	Does not specify recruitment strategy or relationship between researchers and participants.	

Reference: Creed-Kanashiro et al 2018	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	To explore, through systematic formative research, the acceptability, use, and feasibility of a simple technology, commercial infant food grinders, in two rural Peruvian settings where there is delayed and low consumption of complementary foods of a thick consistency, including animal source foods (ASF)
2. Is a qualitative methodology appropriate?	Yes	Maternal acceptability of a new nutrition technology in young children
3. Was the research design appropriate to address the aims of the research?	Yes	FGDs, IDIs and home observations with mothers
4. Was the recruitment strategy appropriate to the aims of the research?	Yes	Purposive sampling

5. Was the data collected in a way that addressed the research issue?	Yes	In participant homes
6. Has the relationship between researcher and participants been adequately considered?	Yes	Data were collected by trained IIN anthropologists and nutritionists. A local Quechua speaking interviewer conducted the data collection with Quechua speaking women in Incahuasi
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Yes	Team members including the senior investigators separately reviewed the transcripts and the detailed observational field notes. Themes and sub-themes were coded, and thematic matrixes were constructed for everyone interviewed. Team members discussed emergent themes
9. Is there a clear statement of findings?	Yes	The technology was highly acceptable, used by most mothers (87.8%), and led to changes in cultural perceptions, facilitating increased feeding of appropriate textures (thick purees), ASF, and MNPs
10. How valuable is the research?	Cannot tell	Unclear if considered a nutrition intervention
Comments on key limitations	This is a technology rather than intervention	

Reference: Goyena et al 2019	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	To assess and explore the acceptability and compliance to MNP and Bigas-Mongo (BigMo) CF blend among mothers/caregivers of children aged 6 to 23 months and the promotion of these products by community health workers (CHWs)
2. Is a qualitative methodology appropriate?	Yes	Maternal/caregiver acceptability of a nutrition intervention in young children
3. Was the research design appropriate to address the aims of the research?	Yes	IDIs with mothers
4. Was the recruitment strategy appropriate to the aims of the research?	Cannot tell	Not enough detail given
5. Was the data collected in a way that addressed the research issue?	Yes	Participants homes
6. Has the relationship between researcher and participants been adequately considered?	Yes	CHWs
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Yes	The data were summarized by constructing frequency and percentage distributions. The contents were transcribed, coded by theme, consolidated, and discussed
9. Is there a clear statement of findings?	Yes	Maternal adaptive strategies in preparing and feeding MNP to their children could affect MNP use, while non-receptiveness of mothers to

		integrating MNP into the child's feeding routine, perceived side effects, and perceived unfavorable taste and smell were key factors considered to limit MNP use
10. How valuable is the research?	Yes	Maternal acceptability of a MNP intervention in young children
Comments on key limitations	Does not give sufficient detail on recruitment	

Reference: Gunatatna et al 2015	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	To assess women's and children's acceptance of a biofortified crop, quality protein maize (QPM), for CF in rural Ethiopia
2. Is a qualitative methodology appropriate?	Yes	Maternal acceptability of a nutrition intervention targeted to young children
3. Was the research design appropriate to address the aims of the research?	Yes	Questionnaires and hedonic scale
4. Was the recruitment strategy appropriate to the aims of the research?	Cannot tell	Not enough detail given
5. Was the data collected in a way that addressed the research issue?	Yes	In participants home
6. Has the relationship between researcher and participants been adequately considered?	Yes	Local residents trained in all aspects of the study
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Yes	Scores for sensory characteristics and overall acceptance, measured using an ordinal Likert scale, were analyzed using mixed effects ordinal logistic regression
9. Is there a clear statement of findings?	Yes	Women were more than twice as likely to prefer QPM over conventional maize
10. How valuable is the research?	Yes	Novel nutrition intervention for young children well accepted
Comments on key limitations	Does not give detail on recruitment/sampling	

Reference: Hess et al 2011	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	Acceptability of LNS containing varying zinc per daily LNS dose among Burkinabe children 9–15 months old and their mothers
2. Is a qualitative methodology appropriate?	Yes	Maternal acceptability of a nutrition intervention in young children
3. Was the research design appropriate to address the aims of the research?	Yes	Maternal sensory reaction using a hedonic scale, triangle tests and maternal reports
4. Was the recruitment strategy appropriate to the aims of the research?	Cannot tell	Does not specify
5. Was the data collected in a way that addressed the research issue?	Yes	At the study unit and participants homes

6. Has the relationship between researcher and participants been adequately considered?	No	Does not specify
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Yes	Data from the triangle tests were analysed using a binomial probability model at a significance level of $P = 0.05$ and 80% power. The maximum allowable false positive rate (α) was set at 0.05 and the maximum allowable false negative rate was set at 0.1. Using these criteria, at least 44 of 108 subjects (40.7%) would have to have identified the odd sample correctly to reject null hypothesis at a 5% level of significance
9. Is there a clear statement of findings?	Yes	Study participation was a positive experience for mothers, and the consumption of LNS was perceived as very beneficial for their children
10. How valuable is the research?	Cannot tell	More focus on perception than acceptability
Comments on key limitations	Does not specify recruitment strategy or relationship between participants and researchers. Does not focus on maternal qual outcomes (IDIs/FGDs)	

Reference: Horton et al 2018	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	To compare cost, coverage, and user satisfaction between twice-yearly campaigns and routine delivery of vitamin A supplements in Senegal
2. Is a qualitative methodology appropriate?	Yes	Caregiver perception of a nutrition intervention in young children
3. Was the research design appropriate to address the aims of the research?	Yes	FGDs with caregivers
4. Was the recruitment strategy appropriate to the aims of the research?	Cannot tell	Does not specify
5. Was the data collected in a way that addressed the research issue?	Yes	In a private work site
6. Has the relationship between researcher and participants been adequately considered?	Yes	The field staff had relevant degrees/ previous experience in undertaking qualitative studies
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?		Qualitative analysis was undertaken by the lead qualitative researcher, using well-accepted methodological principles. Transcripts were coded and reviewed to develop different coding systems for campaign and routine delivery. Content analysis was used to identify trends of concepts in and across individual codes
9. Is there a clear statement of findings?	Yes	Some mothers prefer the administration of supplements at a health facility as it is perceived as more hygienic and involving professional

		health workers, but others, especially those living further away, prefer house-to-house delivery, which was the norm for the campaign mode, citing difficulty with travelling due to housework
10. How valuable is the research?	Cannot tell	Only briefly touches on preference for administration in health facility verses home but no other maternal perceptions.
Comments on key limitations	Does not specify recruitment strategy	

Reference: Ip et al 2009	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	To compare the effectiveness of daily and flexible administration of micronutrient Sprinkles on adherence and acceptability among young children in rural Bangladesh
2. Is a qualitative methodology appropriate?	Yes	Maternal acceptability to a nutrition intervention in young infants
3. Was the research design appropriate to address the aims of the research?	Yes	FGDs with mothers
4. Was the recruitment strategy appropriate to the aims of the research?	Cannot tell	Does not specify
5. Was the data collected in a way that addressed the research issue?	Cannot tell	Does not specify
6. Has the relationship between researcher and participants been adequately considered?	Yes	Village based volunteer women health workers
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Cannot tell	Vague: All forms and questionnaires were manually checked for completeness, consistency, and range. Data were coded and processed
9. Is there a clear statement of findings?	Yes	The adherence and acceptability to flexible administration over 4 months were found preferable to daily
10. How valuable is the research?	Yes	Maternal acceptability of a nutrition intervention
Comments on key limitations	Does not specify how mothers were recruited for FGDs, where they took place or if the data analysis was rigorous	

Reference: Jefferds et al 2010	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	To describe community members' reactions to and experiences using Sprinkles, with an emphasis on acceptability, utilization, and promotion
2. Is a qualitative methodology appropriate?	Yes	Caregiver acceptability of a nutrition intervention in young children
3. Was the research design appropriate to address the aims of the research?	Yes	FGDs with mothers and grandmothers

4. Was the recruitment strategy appropriate to the aims of the research?	Yes	Purposeful sampling
5. Was the data collected in a way that addressed the research issue?	Yes	In participant's homes
6. Has the relationship between researcher and participants been adequately considered?	Yes	Data were collected in Dholuo by Luo research assistants
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Yes	Content analysis: coded for concepts, dominant themes, and variability
9. Is there a clear statement of findings?	Yes	Sprinkles were highly acceptable to adults and most children; some children thought Sprinkles were sugar. All families reported positive effects, particularly increased appetite, and recommended Sprinkles; none experienced major problems. Potential barriers identified were lack of knowledge of and experience with Sprinkles, availability of Sprinkles, and cost
10. How valuable is the research?	Yes	Caregiver acceptability of Sprinkles
Comments on key limitations	NA	

Reference: Kajjura et al 2020	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	Maternal perceptions and barriers experienced during the management of children aged 6 to 23 months diagnosed with moderate acute malnutrition (MAM) using either a malted sorghum-based porridge (MSBP) or fortified corn soy blend (CSB+) as a supplementary porridge
2. Is a qualitative methodology appropriate?	Yes	Maternal acceptability of a nutrition intervention
3. Was the research design appropriate to address the aims of the research?	Yes	Maternal FGDs and IDIs
4. Was the recruitment strategy appropriate to the aims of the research?	Yes	Purposively sampled
5. Was the data collected in a way that addressed the research issue?	Yes	In a mutually chosen location
6. Has the relationship between researcher and participants been adequately considered?	Yes	Conducted by trained research assistants in Lugbarati, the local language spoken by mothers. The research assistants were fluent in Lugbarati and resided in the Arua district, from where the mother-child pairs were recruited
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Yes	Independent manual coding transcripts twice to generate a preliminary coding framework, then classified into categories from which themes Emerged. This was followed by content analysis

9. Is there a clear statement of findings?	Yes	Mothers reported that they were satisfied with the use of MSBP or CSB+ to feed their children
10. How valuable is the research?	Yes	Maternal perception of nutrition intervention in young infants
Comments on key limitations	NA	

Reference: Kodish et al 2017	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	Patterns and determinants of SQ-LNS utilization among children 6–23months and potential sharing practices of other household members prior to intervention development
2. Is a qualitative methodology appropriate?	Yes	Caregiver perception towards nutrition intervention in young infants
3. Was the research design appropriate to address the aims of the research?	Yes	Caregiver IDIs
4. Was the recruitment strategy appropriate to the aims of the research?	Cannot tell	Does not specify
5. Was the data collected in a way that addressed the research issue?	Cannot tell	Does not specify
6. Has the relationship between researcher and participants been adequately considered?	No	Does not specify
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Yes	Atlas.ti v7.0 was used for coding and analysis of the transcripts. Grounded theory: line by line coding, focused coding, analytic notes
9. Is there a clear statement of findings?	Yes	IDIs revealed that caregivers did not provide the SQ-LNS in most cases during the full-day observations because they ran out of supply too early
10. How valuable is the research?	Yes	Both maternal and paternal perception of a nutrition intervention in young infants
Comments on key limitations	Does not specify recruitment strategy, where data collection took place or relationship between interviewer and participants	

Reference: Korenromp et al 2015	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	To determine the feasibility of distributing MNPs for home fortification during biannual Maternal, Neonatal and Child Health Week (MNCHW) events, as a strategy to improve young child nutrition
2. Is a qualitative methodology appropriate?	Yes	Caregiver acceptability of a nutrition intervention in young children
3. Was the research design appropriate to address the aims of the research?	Yes	Caregiver surveys

4. Was the recruitment strategy appropriate to the aims of the research?	Yes	Stratified sample of caregivers at the facilities, longitudinal cohort
5. Was the data collected in a way that addressed the research issue?	Yes	At health facilities or participants homes
6. Has the relationship between researcher and participants been adequately considered?	No	Does not specify
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Yes	Survey data were analysed using SPSS. Differences in responses between the first and second home visits were analysed using the independent Student t test for continuous variables, the χ^2 test for categorical variables and the normal distribution test of difference in proportions for proportional outcomes
9. Is there a clear statement of findings?	Yes	Among caregivers who received MNP, acceptance and use among targeted children was high
10. How valuable is the research?	Yes	Caregiver acceptability of a nutrition intervention in young children
Comments on key limitations	Does not specify relationship between researcher and participant	

Reference: Kung'u et al 2015	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	whether the Maternal, Newborn and Child Health Weeks (MNCHW) would present an opportunity to raise awareness of and demand for the use of zinc and ORS in the treatment for diarrhoea
2. Is a qualitative methodology appropriate?	Yes	Caregiver perception of a nutrition intervention in young infants
3. Was the research design appropriate to address the aims of the research?	Yes	Caregiver interviews
4. Was the recruitment strategy appropriate to the aims of the research?	Yes	Randomly selected by systematic sampling in which every kth caregiver was selected, where $k = \text{number of caregivers visiting the health clinics} \div 10$ (the number of caregivers interviewed at each health clinic)
5. Was the data collected in a way that addressed the research issue?	Yes	At health facilities or participant's homes
6. Has the relationship between researcher and participants been adequately considered?	No	Does not specify
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Yes	Thematically coded

9. Is there a clear statement of findings?	Yes	Attitudes regarding the use of zinc in the treatment for diarrhoea and practices, measured by care-seeking behaviour, also improved between the pre-MNCHW interviews
10. How valuable is the research?	Cannot tell	More focus on change in attitude between health visits
Comments on key limitations	This study focuses on change in attitude towards a health intervention rather than acceptability	

Reference: Kwon et al 2014	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	Mothers' KAP of iron deficiency anaemia and supplementation
2. Is a qualitative methodology appropriate?	Yes	Maternal KAP of a nutritional deficiency and intervention in young infants
3. Was the research design appropriate to address the aims of the research?	Yes	Maternal semi-structured interviews and FGDs
4. Was the recruitment strategy appropriate to the aims of the research?	Yes	Purposive sampling framework
5. Was the data collected in a way that addressed the research issue?	Cannot tell	Does not specify
6. Has the relationship between researcher and participants been adequately considered?	No	Does not specify
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Yes	both thematic analysis ²³ and individual interview synthesis
9. Is there a clear statement of findings?	Yes	Increasing community awareness of mild anemia, simplifying dosage instructions, and further strengthening the supportive environment for health workers would help in reducing the prevalence of IDA
10. How valuable is the research?	Cannot tell	The research more focuses on barriers to successful interventions rather than maternal acceptability
Comments on key limitations	Does not specify who conducted the interviews or where	

Reference: Locks et al 2017	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	The impact of an integrated infant and young child feeding (IYCF) and MNP intervention on children's risk of anemia and IYCF practices
2. Is a qualitative methodology appropriate?	Yes	Maternal acceptability of a nutrition intervention in young infants
3. Was the research design appropriate to address the aims of the research?	Yes	Maternal surveys
4. Was the recruitment strategy appropriate to the aims of the research?	Yes	Stratified multi-staged sampling

5. Was the data collected in a way that addressed the research issue?	Yes	At participant's homes
6. Has the relationship between researcher and participants been adequately considered?	No	Does not specify
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Cannot tell	Does not specify the qual analysis
9. Is there a clear statement of findings?	Yes	The addition of MNPs was well received and may improve IYCF
10. How valuable is the research?	Yes	Maternal perception of a nutrition intervention in young children
Comments on key limitations	Does not specify the researcher and participant relationship or qual data analysis	

Reference: Loechl et al 2009	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	The feasibility and acceptability of distributing micronutrient Sprinkles through a food-assisted maternal and child health and nutrition programme
2. Is a qualitative methodology appropriate?	Yes	Maternal acceptability of a nutrition intervention in young children
3. Was the research design appropriate to address the aims of the research?	Yes	Maternal interviews and FGDs
4. Was the recruitment strategy appropriate to the aims of the research?	Cannot tell	Does not specify
5. Was the data collected in a way that addressed the research issue?	Cannot tell	Does not specify
6. Has the relationship between researcher and participants been adequately considered?	Yes	Female village programme staff for FGDs and interviews were conducted by a consultant (external to the programme) who had ample prior experience with the use of qualitative research methods
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Yes	Transcripts were analysed separately. These data were supplemented with interviewer's field notes, which provided information on the participants, the context of the interview and the interviewer's own reflections about the interview
9. Is there a clear statement of findings?	Yes	Mothers understood instructions about use of Sprinkles, acceptance was high, and no selling of the product was reported or observed
10. How valuable is the research?	Yes	Maternal acceptability of a nutrition intervention in young children
Comments on key limitations	Does not specify how mothers were selected or where the interviews took place	

Reference: McLean et al 2018	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	To develop and test an implementation strategy and tools for an effective MNP programme adapted to the local context and health system
2. Is a qualitative methodology appropriate?	Yes	Caregiver acceptability of a nutrition intervention in young infants
3. Was the research design appropriate to address the aims of the research?	Yes	Caregiver FDs, IDIs and an acceptability survey
4. Was the recruitment strategy appropriate to the aims of the research?	Yes	Three-stage cluster sampling with purposive and systematic sampling
5. Was the data collected in a way that addressed the research issue?	Cannot tell	Does not specify
6. Has the relationship between researcher and participants been adequately considered?	Yes	The cultural context, food availability, and local capacity were evaluated to guide operational components of the programme with communication messages and tools developed from the results
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Yes	Framework analysis: hand-coding for smaller data sets. The data were examined with multiple iterative processes, and visualizations were used to describe dominant themes
9. Is there a clear statement of findings?	Yes	Appropriate food vehicles for MNP in the local context were identified, as well as preferred communication methods, and trusted sources of information in the community. Implementation of the programme continued to pose challenges during scale-up with issues pertaining to stock-outs, delivery, and reporting among partners
10. How valuable is the research?	Yes	Caregiver acceptability of a nutrition intervention in young children
Comments on key limitations	Did not specify where the IDIs and FGDs occurred	

Reference: Pelto et al 2018	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	household responses to MNP interventions, with an emphasis on caregivers' perspectives
2. Is a qualitative methodology appropriate?	Yes	Caregiver acceptability of a nutrition intervention in young children
3. Was the research design appropriate to address the aims of the research?	Yes	IDIs with mothers and one grandmother
4. Was the recruitment strategy appropriate to the aims of the research?	Yes	Purposive sampling
5. Was the data collected in a way that addressed the research issue?	Cannot tell	Does not specify

6. Has the relationship between researcher and participants been adequately considered?	No	Does not specify
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Yes	Thematic analysis with multiple levels and iterations of coding
9. Is there a clear statement of findings?	Yes	Attention to caregivers' perspectives reflected in their narratives offers opportunities to improve MNP utilization in Ethiopia, with potential application in other social and cultural settings
10. How valuable is the research?	Yes	Maternal acceptability of a MNP intervention in young infants
Comments on key limitations	Does not specify relationship between researcher and participant or where data was collected	

Reference: Phuka et al 2011	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	Maternal acceptability of three new LNSs in 8–12-month-old healthy children and their caregivers
2. Is a qualitative methodology appropriate?	Yes	Maternal acceptability of a nutrition intervention in young infants
3. Was the research design appropriate to address the aims of the research?	Yes	Consumption and hedonic rating as well as maternal FGD
4. Was the recruitment strategy appropriate to the aims of the research?	Yes	Purposively selected
5. Was the data collected in a way that addressed the research issue?	Cannot tell	Does not specify
6. Has the relationship between researcher and participants been adequately considered?	No	Does not specify
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Yes	Qualitative analysis using Atlas.ti for emerging themes
9. Is there a clear statement of findings?	Yes	The novel LNS was largely accepted
10. How valuable is the research?	Yes	Maternal acceptability of a novel LNS in young children
Comments on key limitations	Does not specify the relationship between researcher and participant or where FGDs took place	

Reference: Roschnik et al 2019	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	Adherence and acceptability of a community-led MNP intervention targeting children aged 6–59 months
2. Is a qualitative methodology appropriate?	Yes	Caregiver acceptability of a nutrition intervention in young children

3. Was the research design appropriate to address the aims of the research?	Yes	Caregiver FGDs and IDIs and direct observations
4. Was the recruitment strategy appropriate to the aims of the research?	Cannot tell	Does not specify
5. Was the data collected in a way that addressed the research issue?	Cannot tell	Does not specify
6. Has the relationship between researcher and participants been adequately considered?	No	Does not specify
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Yes	Iterative content analysis
9. Is there a clear statement of findings?	Yes	Likely contributors to uptake include perceived positive changes in the children following MNP use, the selection of a food vehicle that was already commonly given to children and the community driven, decentralized, and integrated delivery approach
10. How valuable is the research?	Yes	Caregiver acceptability of a MNP intervention in young children
Comments on key limitations	Recruitment strategy for the qualitative data was not outlined, nor was relationship between researcher and participant or where FGDs/IDIs were conducted	

Reference: Rothman et al 2015	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	The acceptability of 2 novel, SQ-LNS (A and B) for supplementing complementary foods among infants aged 6 to 12 months
2. Is a qualitative methodology appropriate?	Yes	Maternal acceptability of a nutrition intervention in young infants
3. Was the research design appropriate to address the aims of the research?	Yes	Maternal FGDs and exit interviews and hedonic rating
4. Was the recruitment strategy appropriate to the aims of the research?	Cannot tell	Does not specify
5. Was the data collected in a way that addressed the research issue?	Yes	Participant's home and central field station (FGDs)
6. Has the relationship between researcher and participants been adequately considered?	Yes	Fieldworkers and FGD facilitated by a trained facilitator all in the local languages
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Yes	A thematic framework was compiled by identifying themes (key words); tabulating was done by managing, cleaning, and simplifying the data; and interpretation was done by identifying main expressions

9. Is there a clear statement of findings?	Yes	More than 70% of mothers reported a score ≥ 4 on sensory attributes for both SQ-LNSs indicating that both supplements were well perceived. FGDs confirmed a positive attitude toward the supplements in the study population
10. How valuable is the research?	Yes	Maternal acceptability of novel SQ-LNS in young children
Comments on key limitations	Does not specify how participants were recruited for FGDs	

Reference: Ruel-Bergeron et al 2018	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	To understand the facilitators and barriers to participation in a SQ-LNS program using IDIs, and direct observations with caretakers
2. Is a qualitative methodology appropriate?	Yes	Caregiver perception of a nutrition intervention in young infants
3. Was the research design appropriate to address the aims of the research?	Yes	Caregiver IDIs and direct observations
4. Was the recruitment strategy appropriate to the aims of the research?	Yes	Purposive sampling for mothers and convenience for grandmothers/fathers
5. Was the data collected in a way that addressed the research issue?	Yes	In participants homes
6. Has the relationship between researcher and participants been adequately considered?	Yes	Village leaders and program volunteers. The purpose of separating community members into subgroups for FGDs was meant to elicit and categorize themes that might arise based on their common status and responsibilities in the community
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Yes	Grounded theory. Key themes from IDI and FGD were used to inductively create a formal codebook with definitions for each code. Line-by-line coding was conducted by a team of four standardized researchers whose consistency in coding was ensured using an interrater reliability test
9. Is there a clear statement of findings?	Yes	Perceptions of the SQ-LNS were positive, and visible changes in child health contributed to program participation. Conflicting priorities that prevented monthly collection of SQ-LNS and limited knowledge of child feeding messages constituted barriers to program participation
10. How valuable is the research?	Yes	Caregiver (not only maternal but also paternal and grandmother) acceptability of a SQ-LNS intervention in young children
Comments on key limitations	NA	

Reference: Samuel et al 2020	Rating	Description

1. Was there a clear statement of the aims of the research?	Yes	To assess factors associated with intake adherence and drivers for correct MNP use over time to inform scale-up of MNP interventions
2. Is a qualitative methodology appropriate?	Yes	Maternal factors for use of MNP supplementation in young children
3. Was the research design appropriate to address the aims of the research?	Yes	Maternal interviews
4. Was the recruitment strategy appropriate to the aims of the research?	Yes	Mothers who were at the health post for the monthly follow up were selected randomly, irrespective of adherence or non-adherence
5. Was the data collected in a way that addressed the research issue?	Cannot tell	Does not specify
6. Has the relationship between researcher and participants been adequately considered?	No	Does not specify
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Yes	The responses were coded and categorized according to the theoretical framework of the TPB
9. Is there a clear statement of findings?	Yes	Free MNP provision, trust in the government and field staff played a role in successful implementation. MNP is promising to be scaled up, by considering factors that positively and negatively determine adherence
10. How valuable is the research?	Yes	Maternal acceptability of a MNP intervention in young children
Comments on key limitations	Does not specify where data was collected or relationship between interviewer and participant	

Reference: Shaari et al 2019	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	To assess the feasibility of using MMS among Malaysian children
2. Is a qualitative methodology appropriate?	Yes	Caregiver feasibility of a nutrition intervention in young children
3. Was the research design appropriate to address the aims of the research?	Yes	Interviews with caregivers
4. Was the recruitment strategy appropriate to the aims of the research?	Cannot tell	Does not specify
5. Was the data collected in a way that addressed the research issue?	Cannot tell	Does not specify
6. Has the relationship between researcher and participants been adequately considered?	No	Does not specify
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Yes	Data on compliance, acceptance, child preference and the adverse effect of MMS were expressed as frequencies and percentages

9. Is there a clear statement of findings?	Yes	Caregivers had high acceptance of MMS with no adverse effects reported
10. How valuable is the research?	Cannot tell	Very limited results on acceptability
Comments on key limitations	Does not specify how participants were recruited, where data was collected or relationship between interviewer and participant	

Reference: Uti et al 2005	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	To assess performance of a trial to deliver MNP in 6–23-month-old children
2. Is a qualitative methodology appropriate?	Yes	Maternal acceptability of a nutrition in young children
3. Was the research design appropriate to address the aims of the research?	Yes	Population based cross-sectional survey
4. Was the recruitment strategy appropriate to the aims of the research?	Cannot tell	Does not specify
5. Was the data collected in a way that addressed the research issue?	Cannot tell	Does not specify
6. Has the relationship between researcher and participants been adequately considered?	No	Does not specify
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Cannot tell	Does not specify for qual outcomes
9. Is there a clear statement of findings?	Yes	MNP was accepted as most positive but with the highest negative side effects in the rural populations. Urban populations found the feeding the of MNP most challenging
10. How valuable is the research?	Yes	Maternal acceptability of a MNP intervention in young children
Comments on key limitations	Does not specify qual data analysis, how participants were recruited, where the data was collected or relationship between interviewer and participant	

Reference: Uti et al 2011	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	To determine the KAPs of mothers attending the centre towards vitamin A-rich foods and oral vitamin A supplements
2. Is a qualitative methodology appropriate?	Yes	Maternal KAP of a nutrition intervention in young children
3. Was the research design appropriate to address the aims of the research?	Yes	Interviews with a questionnaire to determine maternal KAPs
4. Was the recruitment strategy appropriate to the aims of the research?	Cannot tell	All mothers who attended clinic in a two-week window were included

5. Was the data collected in a way that addressed the research issue?	Cannot tell	Does not specify
6. Has the relationship between researcher and participants been adequately considered?	Cannot tell	Researcher and two trained assistants
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Yes	After checking and coding, data were transferred from the questionnaire to the spreadsheet, summary, frequencies; percentages of relative scores were calculated as appropriate, tables were also created. The data were analysed using descriptive statistics
9. Is there a clear statement of findings?	Yes	Vitamin A supplements were well accepted, and mothers noted positive change in their children's health
10. How valuable is the research?	Yes	Maternal acceptability of vitamin A supplements in young children
Comments on key limitations	Does not specify where data was collected and the recruitment strategy and relationship between participant and researcher both do not appear rigorous	

Reference: Young et al 2017	Rating	Description
1. Was there a clear statement of the aims of the research?	Yes	Examine the acceptability of IFAS versus MNPs for children 6–23 months old
2. Is a qualitative methodology appropriate?	Yes	Household/maternal acceptability of a nutrition intervention in young children
3. Was the research design appropriate to address the aims of the research?	Yes	Household surveys and maternal IDIs
4. Was the recruitment strategy appropriate to the aims of the research?	Cannot tell	Does not specify for the qualitative
5. Was the data collected in a way that addressed the research issue?	Cannot tell	Does not specify
6. Has the relationship between researcher and participants been adequately considered?	No	Does not specify
7. Have ethical issues been taken into consideration?	Yes	Ethics statement of approval through committee
8. Was the data analysis sufficiently rigorous?	Yes	Interviews were analysed using MaxQDA and Excel employing a thematic analysis approach. Deductive themes defined a priori were coded and described, and inductive themes emerging from the data were also noted and coded
9. Is there a clear statement of findings?	Yes	Mothers indicated that the direct administration of IFAS ensured that children consumed the full dose, and MNPs intake depended on the quantity of food consumed, especially among younger children, which emphasizes the need to integrate

		supplementation with the promotion of optimal IYCF practices
10. How valuable is the research?	Yes	Maternal and household acceptability of a MNP intervention in young children
Comments on key limitations	Does not specify how qualitative data was collected, how participants were chosen for IDIs or the relationship between the researcher and participant	

APPENDIX 3 ADDITIONAL FILES FROM PUBLISHED PAPER 3 (CHAPTER 6): IRON SUPPLEMENTATION
OF BREASTFED GAMBIAN INFANTS FROM 6 WEEKS TO 6 MONTHS OF AGE: PROTOCOL FOR A
RANDOMISED CONTROLLED TRIAL

EXTENDED DATAFILE 1

PARTICIPANT INFORMATION SHEET

Version 2.0 Date 08 June 2021

Study Title: Enhancing brain development by early iron supplementation of African infants:
An enabling pilot study

SCC/Protocol No:	19092

Sponsor & Funder:

What is informed consent?

You are invited to let your infant take part in a research study. Before you decide, you need to understand why the study is being done and what will happen in it. Please take time to read the following information or get the information explained to you in your language. Listen carefully. You can ask questions if there is anything that is not clear, or you do not understand. You may also wish to speak to your spouse, family members, friends or others before deciding to let your infant take part in the study.

If you decide to allow your infant to join the study, you will need to sign or put a thumbprint on a consent form saying you agree for your infant to be in the study. You will receive a copy of the consent form.

Why is this study being done?

As you know many children have “anaemia” (sickness that makes someone have less blood), and the number of children with this sickness is very high in Gambia. We know that giving children a medicine called “iron” treats and prevents someone from “anaemia”. There is information from people living in Europe and America telling us that breastfed infants should be given “iron” drops in order to prevent them from getting “anaemia”. This is the first time we are doing this study in Gambia. There is strong evidence to suggest that “anaemia” can impair the physical and developmental potential of children, especially in early childhood.

We hope to discover that breastfed infants in The Gambia will not develop “anaemia” if they are given “iron” drops starting at 6 weeks of age. We hope that one day we will know how to prevent “anaemia” in Gambian children.

Please ask us any questions that you do not understand. If you would like more information, we are happy to explain this to you more than once.

We will tell the results of this study to your community.

What is the new vaccine/drug?

“Iron” drops in the form of Ferrous Sulphate. This is an iron supplement commonly used in older children in The Gambia.

What does this study involve?

The following will be required from your infant if you agree to take part:

Your infant’s health will be examined and his/her height and weight will be measured. If your infant is enrolled in the study, he/she will be randomly allocated to one of the two study groups:

Group A: Iron drops

Group B: Placebo drops

Your infant will then take one of the vitamins every day for 14 weeks.

We will draw a small amount of blood from your infant at enrolment and then again after 14 weeks. At the enrolment visit and at the final visit we will draw 3 mls of blood.

We will also collect a stool sample from your infant at enrolment and baseline.

One of our study staff will visit you daily and to give your infant the drops and to ask questions regarding the health of your infant. Once per week, our study staff will ask you questions about what liquids and food your infant is drinking and eating.

One of our study staff will call you weekly after the end of the 14 weeks of supplementation to check on the health of your infant. We will administer two questionnaires, one about the infant’s health and one about what the infant is drinking.

If we find out that your infant is sick and decide that he/she cannot join the study because of that, he/she will receive the care routinely available in The Gambia. Your infant may be treated at the study site and if necessary, referred to a health facility that can manage the condition better.

If the research study needs to be stopped, we will tell you and your infant will have the normal medical care if needed.

What will happen to the samples taken in this study?

The collected blood and stool samples will be transported to the MRCG laboratories. There we will test the levels of iron and other vitamins in your infant’s blood and stool. In addition, we will assess markers in the blood that may indicate that your infant has an infection. We will also store the samples for further analysis of other markers that may indicate effects of iron supplements. Some of the samples may be transferred to laboratories overseas for analysis because we don’t have the equipment required for measuring all the factors we are investigating in the Gambia.

What harm or discomfort can you expect in the study?

Collection of blood can cause discomfort, but it will not cause any harm to your child. There is a chance that your infant may get a bruise from where we took the blood.

There is a small risk that iron drops can cause diarrhoea or other tummy discomfort. This will be closely monitored by our daily visits.

What benefits can you expect in the study?

By participating in this study your infant will receive medical care at a level greater than that usually given within the study area. We will closely monitor your child's health and give you regular feedback.

Will you be compensated for your child's/ward's participation in the study?

You and your infant will not get paid by the study, but MRC will provide transport or give you back the money for your transport.

Are there other products or treatment?

No.

What happens if you refuse to participate in the study or change your mind later?

You are free to let your infant join or not in the study and you are free to stop taking part at any time without giving a reason. You and your infant will still get the normal medical care.

If you do not want your infant to continue in the study, we will use only the samples and information already collected from your infant.

The study doctor may ask to do some tests if needed for your infant's safety.

If we find any new information during the study that may change if your infant can be in the study, we will inform you as soon as possible.

What compensation will be available if your child is injured during the study?

We will provide medical care if your infant gets any problems from the study through the MRC indemnity arrangements or insurance.

If it is an emergency, please go to your nearest health centre or clinic and call immediately the field worker who gave his/her telephone number to you.

How your child's information will be kept and who will be allowed to see it?

All information that is collected about your child in the study will be kept strictly confidential. Your child's personal information will only be seen by the study team members, the sponsor and if necessary, the Ethics Committee and Government authorities.

We will need to use information from you and your infant for this research project. All information collected about you will be kept private. Only the study staff and authorities who check that the study is being carried out properly will be allowed to look at information about you. Information will include your infant's initials/name/contact details/other identifiers used for the research project. We will keep all information about you safe and secure.

Data may be sent to other study staff in MRC Unit The Gambia at LSHTM, but this will be anonymised. This means that any information about you which leaves the clinic will have your name and address removed so that you cannot be recognised and your data will have a code number instead.

Some of your information will be sent to the United Kingdom. They must follow our rules about keeping your information safe.

Your personal details, meaning your name and other identifiable information, will be kept in a different safe place to the other study information and will be destroyed within 10 years of the end of the study.

At the end of the project, the study data will be archived at MRC Unit The Gambia at LSHTM. The data will be made available to other researchers worldwide for research and to improve medical knowledge and patient care. Your personal information will not be included and there is no way that you can be identified.

You can find out more about how we use your information

- At <https://www.lshtm.ac.uk/files/research-participant-privacy-notice.pdf>
- by asking one of the research team
- by sending an email to DPO@lshtm.ac.uk

Who should you contact if you have questions?

If you have any questions or are worried you can call Mr Mamadou Bah on +220 761 4091 and you can always also call the personal numbers of the study staff given to you. If you have any concerns, you can also contact staff at your health centre or hospital.

Please feel free to ask any question you might have about the study.

WHO HAS REVIEWED THIS STUDY?

The study has been checked by scientists at the Medical Research Council and by The Gambia Government/MRC Joint Ethics Committee. The Ethics Committee protects your rights and wellbeing and has given permission for it to take place. Additionally, it has been reviewed by the Ethics Committees at The London School of Hygiene and Tropical Medicine and at King's College London.

CONSENT FORM

Participant's Name _____

Participant's Identification Number: |_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|

OR

(Printed name of parent)

(Printed name of guardian)

☐ I have read the written information **OR**

☐ I have had the information explained to me by study personnel in a language that I understand and I

- confirm that my choice to let my child participate is entirely voluntarily,
- confirm that I have had the opportunity to ask questions about this study and I am happy with the answers that have been provided,
- understand that I allow access to the information about my child by the persons described in the information sheet,
- had enough time to think about whether I want my child take part in this study
- agree to allow my child take part in this study.

Tick as appropriate

I agree for my child's samples to be shipped outside The Gambia.

Yes ☐ No ☐

I agree to further research on my child's samples including genetic testing

Yes ☐ No ☐

Participant's parent/guardian
signature/thumbprint*

Date Time

Printed name of impartial
witness*

Signature of impartial witness* _____

Date Time

Printed Name of Person
obtaining consent _____

I attest that I have explained the study information accurately in _____ and was understood to the best of my knowledge by, the participant/parent/guardian and that he/she has freely given consent to participate *in the presence of the above named impartial witness (where applicable).
A copy of this ICF has been provided to the participant.

Signature of Person obtaining
consent _____

Date (dd/mm/yyyy) Time (24hr)

**Only required if the participant is unable to read or write.*

EXTENDED DATAFILE 2

R code for randomization

The following R syntax was used to randomly produce 24 permuted blocks with fixed block size of 6 (n=144). Results are reproducible by using the seed number specified.

```
setwd("D:/")

# install.packages("randomizeR")

library("randomizeR")

# Generate the code:

bc <- rep(6, 144/6)

groups <- c("1", "2", "3", "4", "5", "6")

pbr <- pbrPar(bc, K=6, groups = groups)

R<- genSeq(pbr, seed = 789023456)

code <- getRandList(R)

codet <- t(code)# transpose the data

test <- data.frame(codet)

names(test)[1] <- "code" # Give a variable name

# To check the output:

test

# Produce .csv file with output:

write.csv(test, "Randomisationcode.csv")
```



STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	<u>Title page</u>
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	<u>Trial registration</u>
	2b	All items from the World Health Organization Trial Registration Data Set	<u>NA</u>
Protocol version	3	Date and version identifier	<u>Title page</u>
Funding	4	Sources and types of financial, material, and other support	<u>Grant information</u>
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	<u>Title page</u>
	5b	Name and contact information for the trial sponsor	<u>Grant information</u>

5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	<u>Data & safety monitoring</u>
5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	<u>Data & safety monitoring</u>

Introduction

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	<u>Evaluation of risk & benefits</u>
	6b	Explanation for choice of comparators	<u>Interventions</u>
Objectives	7	Specific objectives or hypotheses	<u>Study objectives</u>
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	<u>Study design & site</u>

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	<u>Study design & site</u>
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	<u>Eligibility criteria</u>

Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	<u>Interventions</u>
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	<u>Withdrawal of participants</u>
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	<u>Interventions</u>
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	<u>Exclusion criteria</u>
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	<u>Outcomes</u>
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	<u>Figure 2</u>
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	<u>Sample size</u>
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	<u>Sample size</u>

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	<u>Randomisation & blinding</u>
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	<u>Interventions</u>
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	<u>Randomisation & blinding</u>
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	<u>Randomisation & blinding</u>
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	<u>Data & safety monitoring</u>

Methods: Data collection, management, and analysis

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	<u>Data collection, management & processing</u>
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	<u>Withdrawal of participants</u>

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	<u>Data & safety monitoring</u>
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	<u>Statistical analysis</u>
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	<u>NA</u>
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	<u>Statistical analysis</u>
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	<u>Data & safety monitoring</u>
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	<u>Data & safety monitoring</u>
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	<u>Data & safety monitoring</u>
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	<u>Data & safety monitoring</u>

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	<u>Ethics</u>
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	<u>Ethics</u>
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	<u>Informed consent</u>
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	<u>Future use of stored specimens</u>
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	<u>Participant confidentiality</u>
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	<u>Competing interests</u>
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	<u>Data availability</u>
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	<u>NA</u>
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	<u>Dissemination and data access</u>

31b	Authorship eligibility guidelines and any intended use of professional writers	<u>NA</u>
31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	<u>Extended Data</u>

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	<u>Extended Data</u>
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	<u>Future use of stored specimens</u>

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "[Attribution-NonCommercial-NoDerivs 3.0 Unported](#)" license.

APPENDIX 4 ADDITIONAL FILES FROM PUBLISHED PAPER 4 (CHAPTER 8): MATERNAL
ACCEPTABILITY OF IRON SUPPLEMENTATION IN YOUNG BREASTFED RURAL GAMBIAN INFANTS.

Appendix 1a. Focus group discussion guide with members of the National Nutrition Agency (NaNA).

INSTRUCTIONS TO FOCUS GROUP FACILITATOR(S): The idea of this focus group is to inform what we might ask in the interviews with mothers. Try to involve all participants in the discussion, if you have a quiet participant, address them, and ask their thoughts/ideas.

Note: Questions can be re-arranged to facilitate conversation flow. You can add questions or probe where needed. Likewise, follow up anything confusing or that may raise additional interesting findings. Each section is a topic we would like to cover, you do not have to cover the specific questions, instead we want to facilitate a conversation around each topic (in bold) below.

Welcome the participants and initially re-describe the purpose of conducting this study focus group, why they are participating and what will be covered. After describing the background of the study, give some details of the focus group. This is when the consent form will be signed, and you verify audio recording is acceptable. While it is recorded, some notes may be taken throughout (but do not forget to look at your participants).

Date:

Place:

Name of investigator:

Start time:

End time:

Number of participants:

Observations (moods, interruptions...):

PREAMBLE:

Thank you for agreeing to be here today. This focus group will last about an hour. We will cover questions on general health care in The Gambia and your perceptions. Likewise, we want to assess the acceptability of the intervention we are studying in our clinical trial. Please do not feel like you must answer anything you do not want to and ask for more information on any questions that do not make sense. Remember there are no wrong answers. Please try and respect when others are talking and give each person their turn. Please keep what is discussed in this session private to respect other members.

Are you happy for me to use the machine to record what we say? I may also take some notes.

Great, please stop me with any questions you may have or if anything does not make sense.

I will now start the recorder.

BACKGROUND

1. If everyone could go around in a circle and introduce themselves, say where they are from, their role at NaNA and how long they have been working here.
2. How would you put NaNA's role into context in The Gambia?
(PROBE: in terms of work in the field of health and nutrition)
(PROBE: where does NaNA receive its funding from, resources, etc)
(PROBE: how does NaNA convey/ advertise new health messages; are there ample communication channels?)

HEALTH CARE

3. How would you describe the current situation around iron deficiency, anaemia, and iron supplementation in The Gambia?
(PROBE: challenges, improvements to be made, current strategies, their beliefs, the future)
4. What is your attitude towards such an intervention as iron supplementation for all infants under 6 months of age?
(PROBE: plausible? Affordability, accessibility (transport?), availability (supply?))
5. What could be done to improve the implementation of health interventions?

SUPPLEMENTATION

6. How do you think families might react to iron supplementation in infancy?
(PROBE: religious, cultural factors etc)
(PROBE: would you foresee the community members as able to continue interventions such as supplementation of infants under 6 months of age with iron?)
(PROBE: do you think mothers would have a problem with supplementing your infant before 6 months of age? Why? What would the problems be?)
7. Within households who hold the decision making power in terms of the children's permission to be supplemented as part of a clinical trial?
(PROBE: Husbands, mothers in laws, grandmothers, grandfathers, etc.)
8. Could you think of any concerns there might be from the medical community about iron supplementation?"
(PROBE: gut microbiome development, infection rates)

EBF

9. Do you believe iron supplementation may be misunderstood by mothers as suggesting they should not breast feed?
(PROBE: understand that it is not instead of?)

POSTAMBLE:

Is there anything we did not talk about that you would like to add to our discussion?

Thank you for your time and answers.

Appendix 1b. Focus group discussion guide with MRCG health workers.

INSTRUCTIONS TO FOCUS GROUP FACILITATOR(S): The idea of this focus group is to touch on the topics that will make up the individual interviews with mothers. It will help guide further questions or those that may be missing for the individual interviews. Try to involve all participants in the discussion, if you have a quiet participant, address them, and ask their thoughts/ideas.

Note: Questions can be re-arranged to facilitate conversation flow. You can add questions or probe where needed. Likewise, follow up anything confusing or that may raise additional interesting findings. Each section is a topic we would like to cover, you do not have to cover the specific questions, instead we want to facilitate a conversation around each topic (in bold) below.

Welcome the participants and initially re-describe the purpose of conducting this study and interview, why they are participating and what will be covered. After describing the background of the study, give some details of the focus group. This is when the consent form will be signed, and you verify audio recording is acceptable. While it is recorded, some notes may be taken throughout (but do not forget to look at your participants).

Date:

Place:

Name of investigator:

Start time:

End time:

Number of Participants:

Observations (moods, interruptions...):

PREAMBLE:

Thank you for agreeing to be here today. This focus group will last about an hour. We will cover questions on general health care in The Gambia and your perceptions. Likewise, we want to assess the acceptability of the intervention we are studying in our clinical trial. Please do not feel like you must answer anything you do not want to and ask for more information on any questions that do not make sense. Remember there are no wrong answers. Please try and respect when others are talking and give each person their turn. Please keep what is discussed in this session private to respect other members.

Are you happy for me to use the machine to record what we say? I may also take some notes.

Great, please stop me with any questions you may have or if anything does not make sense.

I will now start the recorder.

BACKGROUND

1. If everyone could go around in a circle and introduce themselves, say where they are from, their role as a health worker and how long they have been working in the field.
2. Where does most nutritional information in communities come from?
(PROBE: schooling, religion, cultural beliefs/traditions, etc)

HEALTH CARE

3. How would you describe the current situation around iron deficiency, anaemia, and iron supplementation in The Gambia?
(PROBE: challenges, improvements to be made, current strategies, their beliefs, the future)
4. What is your attitude towards such an intervention as iron supplementation for all infants under 6 months of age?
(PROBE: plausible? Affordability, accessibility (transport?), availability (supply?))
5. What could be done to improve the implementation of health interventions?
(PROBE: generally, is available care in your country enough for the children's and mother's health?)

SUPPLEMENTATION

6. How do you think families might react to iron supplementation in infancy?
(PROBE: religious, cultural factors etc)
(PROBE: would you foresee the community members as able to continue interventions such as supplementation of infants under 6 months of age with iron?)
(PROBE: do you think mothers would have a problem with supplementing your infant before 6 months of age? Why? What would the problems be?)
7. Within households who hold the decision making power in terms of the children's permission to be supplemented as part of a clinical trial?
(PROBE: Husbands, mothers in laws, grandmothers, grandfathers, etc.)
8. Could you think of any concerns there might be from the medical community about iron supplementation?
(PROBE: gut microbiome development, infection rates)

EBF

9. Do you think there will be confusion for mothers as to why we are supplementing infants with iron while they are being breastfed?
(PROBE: understand that it is not instead of?)

POSTAMBLE:

Is there anything we did not talk about that you would like to?

Thank you for your time and answers.

Appendix 1c. Focus group discussion guide with mothers from surrounding villages whose infants are not enrolled in Iron Babies.

INSTRUCTIONS TO FOCUS GROUP FACILITATOR(S): The idea of this focus group is to touch on the topics that will make up the individual interviews with mothers. It will help guide further questions or those that may be missing for the individual interviews. Try to involve all participants in the discussion, if you have a quiet participant, address them, and ask their thoughts/ideas.

Note: Questions can be re-arranged to facilitate conversation flow. You can add questions or probe where needed. Likewise, follow up anything confusing or that may raise additional interesting findings. Each section is a topic we would like to cover, you do not have to cover the specific questions, instead we want to facilitate a conversation around each topic (in bold) below.

Welcome the participants and initially re-describe the purpose of conducting this study and interview, why they are participating and what will be covered. After describing the background of the study, give some details of the focus group. This is when the consent form will be signed, and you verify audio recording is acceptable. While it is recorded, some notes may be taken throughout (but do not forget to look at your participants).

Date:

Place:

Name of investigator:

Start time:

End time:

Number of Participants:

Observations (moods, interruptions...):

PREAMBLE:

We are conducting a study at MRCG to help young babies that may lack a nutrient. If they do not get the nutrient during pregnancy, they may not have enough during breastfeeding. This is the first time we are doing this study in The Gambia. Alongside the study we want mothers' opinions of giving this supplement to their infant. We will interview mothers from the study, but before doing so we want your ideas on some things and if you think it is acceptable.

This focus group will last about an hour. We will cover questions on what you think about supplements, breastfeeding and other questions regarding health. Please do not feel like you must answer anything you do not want to and ask for more information on any questions that do not make sense. Remember there are no wrong answers. Please try and respect when others are talking and give each

person their turn. Please keep what is discussed in this session private to respect other members.

Are you happy for me to use the machine to record what we say? I may also take some notes.

Great, please stop me with any questions you may have or if anything does not make sense.

I will now start the recorder.

BACKGROUND

1. If everyone could go around in a circle and introduce themselves, say their age and tribe.

HEALTH CARE

2. Did you take supplements while pregnant?
(PROBE: what?)
(PROBE: acceptability?)
(PROBE: if so where did you get them from?)
(PROBE: did you notice a difference in your health?)
3. How many children do you have and where were they born?
(PROBE: home, health centre etc)
4. After you have a newborn child who do you get advice and help with the infants from?
(PROBE: husband, mother, mother in law, other family members, friends, village leader etc)
(PROBE: who do you seek for advice on feeding, care of newborn child etc)
(PROBE: Who makes decisions about your child's health care? *See first probe*)
5. When you child needs health care, where do you go to?
(PROBE: do you find the care enough?)
(PROBE: do you struggle with transport? ie: getting it, costs etc)
(PROBE: do you struggle with getting help in time?)
6. Who makes decisions in terms of your child's health care? Such as giving the permission to enrol your infant in a trial or give them supplements?
(PROBE: Within the household - husbands, mothers in laws, grandmothers, grandfathers, etc.)

IRON & SUPPLEMENTATION

7. Have you ever heard about iron?
(PROBE: what?)
8. Has your child ever taken supplements?
(PROBE: if they had heard about iron, was iron ever one of them?)

(PROBE: if so where did they come from? Did you have to pay? And in what form? ie: pill, drops, powder)?

(PROBE: who gave the child the supplements?)

(PROBE: if so, did they have side effects? What?)

(PROBE: did you find them helpful for improving their health?)

(PROBE: if supplementation stopped, why?)

9. Would you be capable of supplementing the child yourself daily?

(PROBE: would they need a health worker to assist/ remind them?)

10. Would you have a problem with supplementing your infant before 6 months of age? (PROBE: why? What would the problems be?)

(PROBE: beliefs? Traditions?)

EBF

11. Do you breastfeed your children?

(PROBE: why or why not?)

(PROBE: until what age?)

(PROBE: when they start eating food what do they mainly eat?)

(PROBE: do you eat from a communal dish?)

12. Do you think supplementation from an early age will affect your choice to breastfeed?

(PROBE: understand that it is not instead of, but in addition to?)

MRCG STUDIES

13. Has MRCG done studies in your villages before?

(PROBE: opinions of their participation – pleased, un-happy etc?)

(PROBE: If yes, why do you chose to be part of the studies? i.e. access to health care, transport, food provision etc?)

POSTAMBLE:

Is there anything we did not talk about that you would like to?

Thank you for your time and answers. Please remember to keep todays discussion private.

Appendix 2. Interview schedules with mothers.

Note: Questions can be re-arranged to facilitate conversation flow. You can add questions or probe where needed. Likewise, follow-up anything confusing or that may raise additional interesting findings. If the interviewee wants to give you additional information, they may but try to keep the interview in the scope of the below topics. To facilitate a comfortable environment, do not sit head to head with your participant, take a comfortable body position. And do not be afraid of silence, give your participants the space to speak.

Initially re-describe the purpose of conducting this study and interview, why they are participating and what will be covered. This is when the consent form will be signed, and you verify audio recording is acceptable. While it is recorded, some notes may be taken throughout (but do not forget to look at your participant).

Date:

Place:

Participant ID (infant's Iron Babies ID):

Name of investigator:

Start time:

End time:

Interview Number:

Observations (moods, interruptions...):

PREAMBLE:

As you know this study is supplementing infants with iron with daily syrup drops. We will cover questions on what you think about iron, breastfeeding and other questions regarding health to understand if you find the drops acceptable. Please do not feel like you must answer anything you do not want to and ask for more information on any questions that do not make sense. Remember there are no wrong answers. This discussion will last about an hour.

Are you happy for me to use the machine to record what we say? I may also take some notes.

Great, please stop me with any questions you may have or if anything does not make sense.

I will now start the recorder. Are there any questions you have for me before we start?

BACKGROUND

1. What is your name, age and tribe?
2. How long have you lived here?
(PROBE: i.e. moved from another village, after marriage etc)

EDUCATION

3. Did you attend school? If so, until you were how old?
(PROBE: do you learn about the importance of good health in school?)

WORK

4. Do you work?
(PROBE: fields?)
(PROBE: if so, who looks after your child?)
(PROBE: what hours are you working in the field? /times of year?)

HEALTH CARE

5. How many children do you have?
Where were they born?
(PROBE: home, health centre etc)
6. When you have a newborn child, who supports and helps you?
(PROBE: family members – husband, mother, mother in law -, friends, village leaders etc)
(PROBE: who do you seek for advice on feeding, care of newborn child etc)
(PROBE: who makes decisions about your child's health care? i.e. them, husband, village leader etc)
7. If your child needs health care, where do you go?
(PROBE: do you find the care enough?)
(PROBE: do you struggle with transport? ie: getting it, costs etc)
(PROBE: do you struggle with getting help in time?)
8. Who makes decisions in terms of your child's health care? Such as giving the permission to enrol your infant in this trial or give them supplements?
(PROBE: Within the household - husbands, mothers in laws, grandmothers, grandfathers, etc.)

EBF

9. Can you tell me about your experience of breastfeeding?
(PROBE: where do you get information about BF?)
(PROBE: until what age do you BF the child?)
(PROBE: when they start eating food what do they mainly eat?)
(PROBE: do the children and family eat from a communal dish?)
10. What is your understanding of why we are giving your breastfed baby this medication?
(PROBE: i.e. that it is in addition, not instead of)

(PROBE: would supplementation from an early age affect if you chose to breast feed?)

IRON & SUPPLEMENTATION

11. Did you ever hear about this medication (iron) before this study?
(PROBE: what?)
12. When you hear about this study, why did you want your infant to join?
(PROBE: were you willing or did someone else in your family encourage you?)
(PROBE: what factors encouraged you? ie access to health care.)
13. How has your experience of your infant being supplemented been?
(PROBE: overall happy, unhappy?)
(PROBE: did the infant dislike taking the supplement? Spit them up etc?)
(PROBE: did the infant have side effects, i.e. diarrhoea, vomiting, etc?)
(PROBE: did you see a difference in their health? Good? Bad?)
(PROBE: if supplementation stopped, why?)
14. Has the experience of your infant being supplemented changed at all?
(PROBE: were you happy or unhappy at the start and is that now different?)
(PROBE: did you ever consider stopping the supplementation? Why?)
15. Have there been any major challenges in having your infant enrolled in this study?
(PROBE: side effects? Infant refusal? Pressure to stop from others/family members?)
16. Have there been any major benefits in having your infant enrolled in this study?
(PROBE: access to health care? Visible improvements in the infant? Less worry about infant's health? Etc)
17. Would you be capable of supplementing the infant yourself daily?
(PROBE: would they need a health worker to assist/ remind them?)
(PROBE: would procuring the supplement be a problem? ie transport etc)
18. Have any of your other children ever taken iron supplements in the past?
(PROBE: if so where did they come from? Did you have to pay? And in what form? ie: pill, drops, powder)?
(PROBE: who gave the child the supplements?)
(PROBE: if so, did they have side effects? What?)
(PROBE: did you find them helpful for improving their health?)
(PROBE: if supplementation stopped, why?)
19. Did you take any supplements while pregnant?
(PROBE: what were they? Who recommended them? Where did you get them from?)

(PROBE: were they okay – did you mind taking them?)

(PROBE: how did they make you feel? Did you notice anything different after taking them?)

POSTAMBLE:

Is there anything we did not talk about that you would like to add?

Thank you for your time and answers.

Acknowledging the gap: a systematic review of micronutrient supplementation in infants under six months of age

Isabella Stelle¹, Sruthi Venkatesan¹, Karen M. Edmond^{1,2} and Sophie E. Moore^{1,3}

¹ Department of Women and Children's Health, King's College London, London, UK; ² ; ³ MRC Unit The Gambia at the London School of Hygiene and Tropical Medicine, The Gambia

KING'S
College
LONDON

Introduction

- Iron deficiency anaemia is the largest nutritional deficiency disorder worldwide and one of the five leading contributors to the global disease burden
- There are policies and guidelines around iron supplementation in women of reproductive age and young children, however, in infants under six months of age, exclusive breast feeding (EBF) is the only practice recommended to prevent anaemia
- Further, deficiencies of other micronutrients are common worldwide with implications for infant nutritional status with undernutrition during the first 1000 days from conception until two years of age causing irreversible damage to growth and cognitive development
- This is especially relevant in low- and middle- income countries (LMICs), where 1/3 of children fail to reach their developmental milestones by school age
- Consequences to growth and development in early infancy (under six months of age) are not fully understood

Methods

- We searched the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE (Ovid) and Embase (Ovid) from January 1980 through December 2019
- Interventions included randomised controlled trials (RCTs) supplementing infants under six months of age with iron or multiple micronutrients (MMNs; > two micronutrients) with or without iron

Results

- Of 11,109 records identified, 32 publications from 23 trials were included (18 iron and five MMN supplementation trials) (Figure 1)
- All trials evaluated the effect of supplementation on biochemical outcomes; 10 reported on growth; 14 on morbidity and/or mortality; and 6 on neuro-behavioural development (Table 1)
- LMICs made up 88% (21/24) of the total trial locations (Figure 2)

Discussion

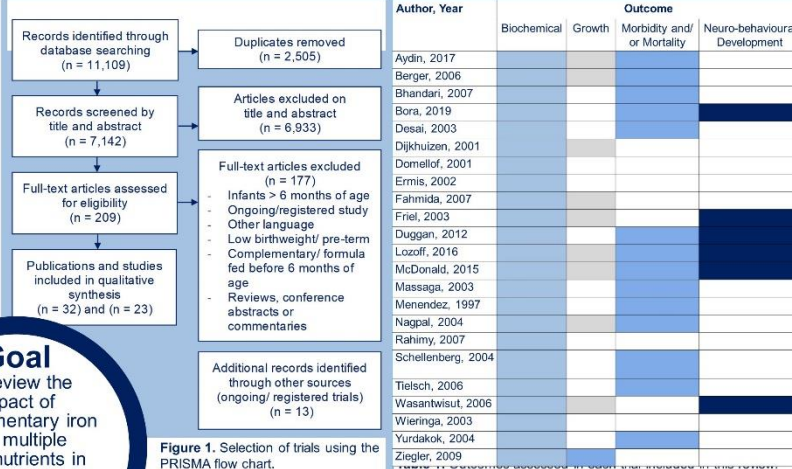
In infants less than six months of age, iron (alone or in combination with other nutrients) is effective at reducing the prevalence of anaemia. Positive impacts of supplementation were seen in infants from low resource settings in a high-income country (HIC) and in LMICs; however, in resource secure HICs the impact was not as significant. While biochemical status was improved with MMNs not containing iron, the effect is greater when MMNs contain iron. For growth, no consistent beneficial effects of supplementation in early infancy were observed. While there were no significant increases in morbidity and/or mortality, gastrointestinal side effects were reported for infants receiving iron. Finally, it was consistently observed that supplementation must continue past six months of age, because any beneficial impacts seen across all outcomes and supplementations disappear when supplementation ceases. There is a lack of policy addressing additional iron needs in infants under six months of age and likewise, more data is a needed to understand both the short and longer-term impacts of supplementation with iron or MMNs in this age group.

Conclusions

- Infants less than six months of age benefit biochemically from early supplementation with iron, but the effect of additional nutrients or MMNs, along with the impacts on growth, morbidity and/or mortality, and neuro-behavioural outcomes remains unclear
- Well-powered RCTs are required to determine whether routine supplementation with iron or MMNs containing iron should commence before six months of life in exclusively breast-fed infants in low-resource settings

References

- Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet* 2017;390(10100):1211-59.
- World Health Organization. Nutritional anaemias: tools for effective prevention and control. In: Development NHa, ed. G. Geneva, Switzerland 2017.
- McCoy DC, Peet ED, Ezzati M, et al. Early Childhood Developmental Status in Low- and Middle-income Countries: National, Regional, and Global



Goal
To review the impact of supplementary iron and multiple micronutrients in infants under 6 months of age on infant development



Figure 2. Distribution of countries included in this review.

- Prevalence Estimates Using Predictive Modeling. *PLoS Med* 2016;13(6):e1002034. doi: 10.1371/journal.pmed.1002034 [published Online First: 2016/06/09].
- Widdowson EM. Trace elements in foetal and early postnatal development. *Proc Nutr Soc* 1974;33(3):275-84. doi: 10.1079/pns19740050 [published Online First: 1974/12/01].

Caregiver perception of nutrition interventions in young infants: a systematic review

Isabella Stelle; supervised by Dr Sophie Moore and Prof Karen Edmond
Department: Women and Children's Health

KING'S
College
LONDON

Introduction

- In 2019 an estimated 5.2 million children under five years of age died mostly from preventable and treatable causes (nutrition-related factors contributed to approximately 45% of these deaths) (1)
- Undernutrition during the first 1000 days of life (from conception until two years of age) can cause irreversible damage to growth and cognitive development (2)
- Efficacy studies have shown that early nutrition interventions improve infant nutrition status (3-5)
- To examine whether such interventions can be effectively taken to scale, a better understanding of caregiver acceptability is required

Aim Here we present a systematic review of caregiver perception of nutrition interventions in their young infants.

Methods

- We searched the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE (Ovid), Embase (Ovid), CINAHL and PsychINFO from date of online journal inception through December 2020
- Eligibility criteria is laid out in Table 1
- Quality assessment was performed for all included studies using the Critical Appraisal Skills Programme (CASP) tool (6)
- Studies underwent narrative synthesis using inductive thematic analysis of the caregiver perception of the intervention
- The Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) guidelines are used (7). The review protocol was registered on PROSPERO (registration number CRD42021238050, 8th March 2021)

Selection Criteria	Key concept or facet	Inclusion	Exclusion
Population	Caregivers of infants <24 months of age	Caregivers; infants < 24 months of age	Infants > 24 months of age
Intervention	Nutrition interventions in infants <24 months of age	Oral (powder, liquid, tablet) and/or intravenous supplementation, food fortification, dietary/ lactation counselling	No nutrition intervention
Comparison	NA, placebo, no intervention/therapy, additional nutrition interventions	Placebo, no intervention/therapy, oral (powder, liquid, tablet) and/or intravenous supplementation, food fortification, dietary/ lactation counselling	
Outcome	Perception of intervention (secondary: impact on exclusive breast feeding, adverse events)	Caregiver perception of intervention	No caregiver perception of intervention
Study type	Experimental studies (controlled trials) and observational studies (cohort, case controlled, cross-sectional, qualitative)	Published experimental and observational studies including randomized or nonrandomized controlled trials, cohort, case controlled, cross-sectional survey, facility evaluations and qualitative studies	Studies without primary data collection, such as reviews and study protocols, studies not in English, and those not demonstrating a clear research methodology
Language		Studies published in the English language	Excluded if not written in English
Other		From conception of the journal through 2020	Published after 2020

Table 1. Eligibility criteria

Results

- Of 11,798 records identified, 37 publications were included
- This review explored the negative and positive caregiver perception of various nutrition interventions in young infants
- The scope of caregivers included mothers, fathers, maternal and paternal grandparents, and aunts, with mothers being the most common caregiver
- Perceptions were gathered through individual interviews, focus group discussions, questionnaires, surveys, and ratings
- 89% of studies noted high acceptability (N = 33), with six mentioning desires for continuation of the intervention. Just over half of the studies (N = 21) cited low acceptability. Misuse of supplements were seen in seven studies through sharing with other children and giving more to the infant when requested. Side effects were noted in 12 studies. While they were often cited as a reason for non-use (N = 7), they were also noted as, when mild, a sign of the intervention being effective (Figure 1)

Conclusions

- While negative perceptions were reported, overall positive perceptions and desire for continuation of the interventions were more frequent
- Side effects were noted, but not always with negative connotations
- Taking into consideration caregiver perception will strengthen sustainability of future nutrition interventions in young infants

Acknowledgements

I would like to thank Maggie Woo Kinshella, the second reviewer of this systematic review.

References

- World Health Organisation. 2020 [Available from: <https://www.who.int/news-room/fact-sheets/detail/children-reducing-mortality>].
- Martorell R. 2017; 29(2) doi: 10.1002/ajhb.22952
- Bhutta ZA et al. 2008; 37(9610): 417-40. doi: 10.1016/S0140-6736(07)61693-6.
- Christian P et al. 2015; 39(5): 361-72. doi: 10.1053/j.sempn.2015.06.009.
- Goudar SM et al. 2019;6:CD011695. doi: 10.1002/14651858.CD011695.
- Programme CAS. CASP Qualitative Checklist [online] 2018 [Available from: <https://casp-uk.net/wp-content/uploads/2018/01/CASP-Qualitative-Checklist-2018.pdf>].
- Moher D et al. 2009; 151(4):264-9, w64. doi: 10.7326/0003-4819-151-4-200908180-00135.

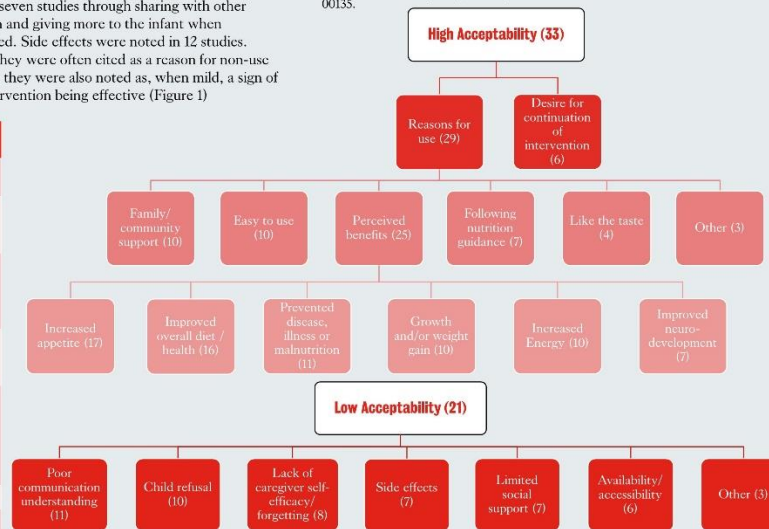


Figure 1. Themes and sub-themes of caregiver perception of nutrition interventions in young infants

APPENDIX 7 MEDRXIV IRON BABIES MAIN OUTCOMES MANUSCRIPT (PRE-PRINT)

1 Early iron supplementation of exclusively breastfed African infants: a proof-of-principle, placebo-
2 controlled, randomised, double-blinded efficacy trial

3 Mamadou Bah, MSc^{1,2}, Isabella Stelle, MSc³, Hans Verhoef, PhD², Alasana Saidykhan BSc¹, Prof Sophie
4 E. Moore, PhD³, Babucarr Susso, MD, DES¹, Prof Andrew M. Prentice, PhD¹, Carla Cerami, MD PhD^{1*}

5 ¹Medical Research Council (MRC) Unit The Gambia at London School of Hygiene & Tropical Medicine
6 (LSHTM), PO Box 273, Fajara, The Gambia

7 ²Division of Human Nutrition and Health, Wageningen University, PO Box 17, 6700 AA Wageningen,
8 The Netherlands

9 ³Department of Women and Children's Health, King's College London, 10th Floor North Wing, St.
10 Thomas' Hospital, Westminster Bridge Road, London, SE1 7EH, UK

11 *Correspondence to: Dr Carla Cerami, MRC Unit The Gambia @ LSHTM, Atlantic Boulevard, Fajara,
12 Banjul, The Gambia Email: Carla.Cerami@lshtm.ac.uk Tel: +220-7875756

Research in context

Evidence before the study

The World Health Organisation (WHO) recommends that infants should be exclusively breastfed for 6 months. Human milk contains very little iron so exclusively breastfed infants are forced to utilise their birth endowment of liver ferritin and fetal haemoglobin to meet the needs of growth and tissue development. In low-income countries many infants are born prematurely, at low birthweight or to iron deficient mothers. These infants start life with low iron reserves and hence frequently become very iron deficient by 6 months of age. In many high-income countries it is recommended that such infants should receive iron supplements from soon after birth. Although not specifically precluded by WHO's recommendation on exclusive breastfeeding, the provision of supplements is widely viewed as being unnecessary and as undermining the ethos of the recommendation.

Added value of this study

In this proof-of-principle trial we demonstrated that providing 7.5mg iron per day to exclusively breastfed Gambian infants from 6 weeks of age substantially improved all markers of iron status at 6 months of age. There were no signals of adverse effects on growth or on infections.

Implications of all the available evidence

Serious iron deficiency in many exclusively breastfed infants in low-income countries impairs iron supply to rapidly developing tissues including immune and neural cells and the expanding erythroid pool. Early introduction of iron supplements can reverse this deficiency and warrants further testing in large-scale trials with additional measurements of functional outcomes and safety.

SUMMARY

Background: We have previously shown that breastfed Gambian children have depleted their neonatal iron endowment before 6 months. We measured the effect of daily iron supplementation for 14 weeks on serum iron concentration and other iron markers among breastfed Gambian infants.

Method: In a double-blind trial, healthy exclusively breastfed rural Gambian infants aged 6 to 10 weeks were identified from vaccination clinics and local communities. Eligible children (n=101) were individually randomised to 14 weeks of daily supplementation with either iron (7.5mg as ferrous sulphate in sorbitol solution) or placebo (sorbitol solution). The primary outcome was serum iron

concentration after 99 days of supplementation (98 days intervention plus 1-day washout). We used intention-to-treat analysis with multiple imputation to replace missing values. This trial was registered with clinicaltrials.gov (NCT04751994).

Findings: Iron administration increased serum iron concentration (crude difference in means: 2.5 $\mu\text{mol/L}$; 95%CI: 0.6 to 4.3 $\mu\text{mol/L}$, $p=0.0091$) and meaningfully improved additional markers of iron and haematological status. There were 10 serious adverse events (5 iron/5 placebo) and 106 nonserious adverse events (54 iron/52 placebo) with no deaths. There were no marked group differences in maternally-reported episodes of diarrhoea, fever, cough, skin infection, eye infection and nasal discharge.

Interpretation: In exclusively breastfed infants, early introduction of iron supplements can enhance iron supply to rapidly developing tissues in early infancy and warrants further investigation in large-scale trials with additional measurements of functional outcomes and safety. Funding UK Department for International Development, Medical Research Council UK, National Institute for Health Research and Care Research, Wellcome Trust.

64 Key words: Iron deficiency; exclusive breastfeeding; iron supplements; Gambia; infants; iron

65 status; safety

INTRODUCTION

There are increasing doubts whether the iron supplied by exclusive breastfeeding is adequate to meet infant requirements in the first 6 months of life. The iron content of human milk is low (at ~0.35 mg/L).¹ So a young infant typically receiving 750-1,000 mL breastmilk per day will receive around 0.25-0.35 mg iron daily. In calculating iron requirements, the US Institute of Medicine (IOM) assumed that breastmilk iron is adequate and hence set the requirement at 0.27 mg/d for 06 months.² At 6 months, based upon factorial calculations of iron losses and the needs for growth, the IOM recommendation jumps 40-fold to 11 mg/d.² This non-physiological disjuncture is an artefact, and back-extrapolation of factorial calculations to ages below 6 months, when infants are growing faster, would indicate a true need of at least 10 mg/d if supplied by diet. Breastmilk iron is largely bound to lactoferrin and benefits from efficient receptor-mediated absorption compared to non-milk iron but, even allowing for this, breastmilk provides an order of magnitude less iron than an infant requires.¹ Hence, exclusively breastfed infants must draw on their endowed iron reserves (liver ferritin and recycled iron from fetal haemoglobin) to support their daily needs and for growth (especially of their expanding red cell mass).³

The fetus accrues most of its iron during the final trimester of pregnancy and is dependent upon an adequate maternal iron status to supply these needs.⁴ Infants born prematurely, at low birthweight, or to iron deficient mothers, and whose cord is clamped rapidly after birth, start life with smaller iron stores and hence a lower capacity to subsidise the iron requirements of growth and metabolism whilst being breastfed. Thus, in many high-income countries, it is recommended that they should receive supplemental iron at rates of 2-4 mg/kg when given orally, starting soon after birth.¹

In establishing the recommendation that infants should be exclusively breastfed until 6 months the World Health Organisation's expert panel acknowledged evidence that many children would become iron deficient if not given supplemental iron but offered no solution.⁵

In low-income settings infants are much more likely to be born with low iron reserves and are also more likely to be exclusively breastfed in early infancy.⁶ In two independent community cohorts we have previously shown that breastfed Gambian children have utilised their birth endowment of iron before 6 months, and that functional indicators of iron deficiency (concentrations of transferrin and soluble transferrin receptor) start to rise at 2 months of age.³ Haemoglobin falls and the proportion of anaemic children rises.³ Most remarkably, concentrations of serum iron were within the standard range at birth (~15 µmol/L) but fell rapidly to levels below 8 µmol/L at 2 months and around 2-4

μmol/L by 6 months; these are well below the reference levels for well-nourished children (10-20 μmol/L).³

All human cells require iron and many tissues undergoing critical phases of development in a young infant (eg, neurones and lineages involved in adaptive immunity) have high iron needs.⁷ These cells can only access iron from circulating plasma and hence the very low levels of serum iron in breastfed infants likely constitute a substantial limitation on development.³

In this proof-of-principle, placebo-controlled, randomised trial we measured the effect of supplying 7.5 mg/d iron as ferrous sulphate for 14 weeks to exclusively breastfed Gambian infants aged 6-10 weeks, with a view to improve their iron status. The primary outcome was serum iron, and safety was assessed by daily review of possible adverse events. Secondary outcomes included a range of additional markers of iron and haematological status.

SUBJECTS AND METHODS

Trial design: This was a double-blind, individually randomised placebo-controlled trial conducted in Jarra West in the Lower River Region of The Gambia. Malaria used to be endemic but is now virtually non-existent in this area, as indicated by the low prevalence of Plasmodium infection.

(0.4%) among under-five children according to the 2019 Demographic and Health Survey (DHS).⁸ Full details of study design are available in the published protocol.⁹

The study was approved by the Scientific Coordinating Committee of the Medical Research Council (MRC) Unit The Gambia and the Joint Gambia Government MRC Ethics Committee and the Ethics Committee at the London School of Hygiene and Tropical Medicine (SCC19092). The trial was conducted according to the principles of Good Clinical Practice, had oversight from a Data Safety and Monitoring Board and a Trial Steering Committee and was monitored by the Clinical Trials Office at the MRCG@LSHTM. No interim analysis for efficacy was done. This trial was registered with clinicaltrials.gov (NCT04751994).

Recruitment: Participants were identified from vaccination clinics and in the communities by field workers. Following sensitisation those willing to participate were followed up in their homes for consenting. After obtaining written informed consent, participants were invited to the Jarra Soma Health Centre and screened for eligibility before randomisation (Day 0). All trial participants were generally healthy and were exclusively breastfed as reported by their mothers. Eligibility criteria included willingness to stay in the participating communities and to adhere to the trial protocol.

Formula-fed infants and those with fever, haemoglobin <7.0 g/dL, or acute or chronic illnesses were excluded.

On Day 0 (baseline), weight (Seca 336 infant scale, Hamburg, Germany; to the nearest 10 g) and length (Seca 417 lengths board; to the nearest 1 mm) were measured. Eligibility screening and blood sample collection was done by the research nurses. Participants that met all the inclusion criteria were enrolled and a venous blood sample was collected and stored on ice until analysis. Full blood count and reticulocyte parameters were analysed in EDTA stabilised blood within 4 hours after collection using an automated haematology analyser (Sysmex XN-Series 1500, Sysmex Corporation, Japan), and a serum sample was stored at -80°C for subsequent measurement of iron and inflammation markers. Children were randomised either on Day 0 or 1 after haemoglobin concentration was measured and eligibility to randomisation had been fully established.

Randomisation and blinding: Permuted block randomisation (with a fixed block size of six) with an allocation ratio of 1:1 was used. The randomisation sequence was produced using RStudio by the trial statistician and uploaded as an electronic case report form (eCRF) into the RedCap database by the data manager. The iron and placebo groups were sub-divided into 3 groups each (total 6 groups) to further conceal the treatment allocation. The eCRF was a random number generator used to assign each enrolled participant into one of the six intervention codes by the data manager. The interpretation of these codes was only known by the independent pharmacist. The randomisation code was broken after data were cleaned and locked.

The ferrous sulphate supplement (containing 7.5 mg/day iron in sorbitol solution) and placebo (sorbitol solution USP 70%) were supplied in liquid form by Pharmacy Innovation (NC, USA). To ensure masking, both supplements were repackaged in identical bottles by a pharmacist independent from the study.

Follow up: Enrolled participants were provided with infant mosquito nets to decrease the risk of subsequent malaria infection. Participants were supplemented with 0.5 mL supplemental solution (7.5 mg/day iron or placebo) by fieldworkers from Day 1 to Day 98 (14 weeks) with the support of parents/guardians. The investigational products were returned daily to the field office and the storage temperature was monitored. Daily iron supplementation, daily health and weekly feeding questionnaires were completed for 98 days.

An endline sample of venous blood (3.0 mL) was collected on Day 99 to allow one day of wash-out. The full blood count and reticulocyte parameters were assessed, and a serum sample was collected

as described for baseline. Post-supplementation follow-up to assess health status was continued daily up to Day 112 (16 weeks).

Laboratory measurements: Baseline and endline serum samples were used to measure concentrations of iron, ferritin, soluble transferrin receptor (sTfR), unsaturated iron binding capacity (UIBC), transferrin saturation, C-reactive protein (CRP) and α_1 -acid glycoprotein (AGP), using an automated biochemistry analyser (Cobas Integra 400 plus; Roche Diagnostics, Rotkreuz, Switzerland). Serum hepcidin (DRG instruments, manufacturer code HS EIA-5782, Marburg, Germany), erythropoietin (Human Erythropoietin SimpleStep Kit, ABCAM, ab274397, Cambridge, UK) and erythroferrone (IE, Intrinsic Life Science, SKU# ERF-001 California, USA) concentrations were all analysed by ELISA.

Outcomes: The primary outcome was serum iron concentration at Day 99. Secondary outcomes included anaemia, iron deficiency and iron deficiency anaemia, erythroferrone, erythropoietin, hepcidin with other iron and haematological markers (see full list of variables in Tables 1 and 2) at Day 99. Safety was monitored by daily assessment of morbidity and adverse events during the intervention period. Adverse events were defined as any untoward or unfavourable medical occurrence whether considered related to the child's participation in the study or not. Serious adverse events were defined as adverse events that were life-threatening, resulted in death, required hospitalization or prolongation of hospitalisation, or resulted in a persistent and significant disability or incapacity, and were always investigated by the trial clinician. In addition, we daily asked mothers about the occurrence of specific events of interest (diarrhoea, fever, cough, vomiting, skin infection, eye infection, nasal discharge).

Statistical issues

Sample size calculations: Sample size was calculated assuming a geometric SD for serum iron concentration of 2.39 for both groups, based on results from a previous study.³ The geometric SD is a dimensionless factor that indicates variation that is equivalent to subtraction or addition of 1 SD on a log-transformed scale.³ Assuming <10% drop-out, recruitment targeted 100 infants, providing 80% probability that an increase in geometric mean serum iron concentration by at least 70% in the intervention group compared to the placebo would exclude no effect (ie, ratio of geometric means of 1) from the 95% CI. In absolute terms, assuming a geometric mean serum iron concentration in the placebo group of 4.3 $\mu\text{mol/L}$,³ an increase by 70% would correspond to +3.0 $\mu\text{mol/L}$.

Definitions: Transferrin saturation was calculated as serum iron concentration ($\mu\text{mol/L}$)/total ironbinding capacity ($\mu\text{mol/L}$) $\times$ 100. Anthropometric z-scores for were derived using the WHO international growth reference.¹⁰ Anaemia was defined as haemoglobin concentration <11.0 g/dL even though there are no agreed definitions for infants aged below 6 months.¹¹ Adherence was defined as the percentage of scheduled days (98) that the supplement/placebo was actually ingested.

Group descriptions: Analyses were conducted using Stata version 17 (Stata Corp, College Station, TX) and R version 4.2.1 (<https://www.R-project.org/>). We visually inspected histograms of variables and residuals by intervention group to assess the shape of their distribution and to identify possible outliers. Skewed variables were normalised by log transformation as appropriate. Groups were described using mean (SD) for normally distributed variables, geometric mean (geometric SD, GSD) for log-transformed data and proportion/percentage for categorical variables.

GSD was calculated as the exponentiated SD of the log-transformed variable.

Endpoints: The primary endpoint was serum iron concentration at Day 99 in the modified intention-to-treat population (i.e., all participants that were randomised and had received at least one supplemental dose). Secondary efficacy endpoints included other markers of iron status, haemoglobin, and reticulocyte haemoglobin, hepcidin, erythroferrone, and erythropoietin.

Intervention effects: We estimated crude intervention effects on continuous outcomes by simple linear regression. To assess the potential role of confounding due to imbalances in baseline variables, we also used multiple linear regression to estimate intervention effects adjusted for the outcome variable measured at baseline, sex (binary variable), age (continuous variable), and haemoglobin concentration and serum concentrations of ferritin and soluble transferrin receptor at baseline (all continuous variables); except for anthropometric outcomes that were adjusted for the outcome variable measured at baseline, sex (binary variable) and age (continuous variable). In these analyses, continuous variables were entered as fractional polynomials to allow for possible non-linear relationships with outcome.¹² In all models, we accounted for heteroscedasticity using robust variance estimators. Intervention effects are reported as absolute differences in means for normally distributed outcomes, or as relative differences in geometric means for log-transformed outcomes. When log-transformation failed to normalise the distribution of residuals within groups, we assessed group differences by two-sample Wilcoxon rank-sum (Mann–Whitney) tests. In per protocol analysis,

we used logistic regression models with the adjrr command in Stata package st0306.pkg to estimate prevalence differences adjusted for baseline characteristics.

As planned, the primary analysis was by modified intention to treat analysis, i.e., excluding children who were randomised but withdrawn before they received the first supplemental dose of iron or placebo. In practice, such withdrawals did not occur, so the primary analysis was de facto by intention-to-treat. To account for missing values, we used multiple imputation (see details of the imputation model in the Annex). As supportive analyses, we also report per protocol analyses with pairwise deletion of missing values.

Adverse events: We used per protocol analyses of adverse events and daily maternal reports of adverse symptoms of interest. Adverse events were reported as the number of events within each treatment group. No formal statistical analysis was done due to the small number of adverse events.

In the analysis of daily maternal reports of adverse symptoms of interest (diarrhoea, fever, cough, vomiting, skin infections, eye infections, nasal discharge), we defined a new episode as one or more successive days that such symptoms were reported, separated from a previous episode by successive days without such symptoms (diarrhoea, 5 days; all other symptoms, 3 days). Thus, new episodes could be recurrent within individual children. Details of case definitions and analysis are provided in the Annex.

RESULTS

Between 3 August 2021 and 9 March 2022, 103 children were invited, and 101 children were enrolled. Of these, 50 were randomised to iron (26 females) and 51 to placebo (29 females). Two participants were excluded from the study before randomisation (one failed the eligibility criteria and the second was excluded due to technical error) (Figure 1). Three participants were lost to follow up, one had a clotted sample (haematology analysis could not done) and one refused to give a sample at endline blood in the per-protocol population. At the start of the study, reticulocyte number, reticulocyte percentage, immature reticulocyte fraction and reticulocyte haemoglobin content were not measured for 17 children (9 intervention; 8 control) due to delays in equipment installation and calibration.

Intervention groups were similar regarding baseline characteristics (Table 1). Adherence to directly observed daily supplementation was 91% and was similar between groups (iron group: 92.3%; placebo group: 89.1%). Participants travelling outside the study area were responsible for the majority of non-adherence events (Figure S1).

Results of intention to treat analyses are shown in Table 2 (for results of per protocol analyses: see Annex, Tables S1 and S2). After 98 days of supplementation, the primary outcome, serum iron was higher in the iron group compared to placebo (crude difference in means: 2.5 $\mu\text{mol/L}$; 95%CI: 0.6 to 4.3 $\mu\text{mol/L}$). All other markers of iron status improved in the treatment group including decreased soluble transferrin receptor (crude difference in geometric means: -7.2%; 95%CI: -3.7 to -10.5%), decreased UIBC (crude difference in means: -8.7 $\mu\text{mol/L}$; 95%CI: -13.1 to -4.3 $\mu\text{mol/L}$), increased transferrin saturation (crude difference in geometric means: 45.4%; 95%CI: 18.8 to 78.0%), increased ferritin (crude difference in geometric means: 111.2%; 95%CI: 49.4% to 198.6%) and increased hepcidin (crude difference in geometric means: 79.0%; 95%CI: 20.4% to 165.9%) (Table 2). There was strong evidence of higher erythroferrone concentrations in the placebo group compared to the ferrous sulphate group ($p=0.0052$).

Haemoglobin concentration (crude difference in means: 0.6 g/dL; 95%CI: 0.2 to 1.0 g/dL) and other haematological markers including MCV, MCH, MCHC, reticulocytes haemoglobin and reticulocyte number also increased in the iron supplemented group compared to placebo (Table 2). There was good evidence of decreased prevalence of anaemia (crude prevalence difference: -24.2% (-43.7% to -4.7%). Figure 2 summarises the benefits for all the iron and haematological markers expressed as standardised differences of means (i.e. expressed as standard deviations for each variable) or as the ratio of geometric means for skewed variables.

There were no marked differences between groups for immature reticulocyte fractions, erythropoietin, α_1 -acid glycoprotein, C-reactive protein, height-for-age z-score, weight-for-age zscore or weight-for-height z-score.

There were no deaths during the intervention period. Adverse events, serious adverse events and self-reported illnesses are reported in Tables 3, 4 and S4 and Figure S3. A total of 10 serious adverse events (5 intervention/5 control) and 106 non-serious adverse events (54 intervention/52 control) were reported and were similar between groups. There was no evidence of differences between groups in the incidence of maternal-reported episodes of diarrhoea, fever, cough, skin infection, eye infection and nasal discharge. However, there was a decreased incidence of vomiting in the iron compared to the placebo group (incidence difference: 0.37 episodes per 100 child-days of observation; 95%CI: 0.08 to 0.67) (Table 4).

DISCUSSION

This proof-of-principle trial of early iron supplementation in fully breastfed Gambian children achieved substantial benefit across all markers of iron and haematological status. Although a full assessment of the safety of the intervention will require a much larger trial, it was reassuring that we detected no adverse effects on infections or growth. The target population was typical of many similar rural African settings where there is a high prevalence of moderate iron deficiency amongst pregnant mothers, and high proportions of infants born small and/or premature, all of which reduce an infant's iron endowment at birth.

Iron is the nutrient most lacking in human breastmilk.⁷ and exclusively breastfed infants born with low iron reserves rapidly become very iron deficient.⁶ This might appear contrary to evolutionary theory which posits that breastfeeding has evolved as the optimal means for nourishing a young infant. However, breastfeeding evolved under very different dietary conditions to today's predominantly cereal-based diets associated with the agrarian revolution which started only ~12,000 years ago. For the vast majority of human evolution our hunter-gatherer predecessors consumed a mixed omnivorous diet with a high proportion of their food needs derived from animal sources and with high intakes of vitamin C (a promoter of iron absorption).¹⁴ Although direct evidence is lacking, it is assumed that these populations would have been replete in iron and that it was the ecological transition to cereal-based diets with low amounts of animal foods that caused a rise in deficiency diseases such as anaemia.¹⁴ Thus, when the composition of human milk was evolving, infants would likely have been born with a high iron endowment that would cover their needs for the first post-partum months and permit a very low milk iron content selected on the basis that it promotes a more favourable (lactobacillus and bifidobacterial dominant) gut microbiome.¹⁴ In contrast modern breastfed infants become iron-deficient unless supplemented, and they will become progressively more iron deficient with increasing duration of exclusive breastfeeding.⁶

In high-income countries it is standard practice to recommend iron supplements soon after birth in infants at risk of iron deficiency,⁷ but in low-income settings, where the risk of deficiency is much higher, supplementation is viewed as potentially undermining the message that exclusive breastfeeding is the best way to feed an infant, and hence early iron supplementation has rarely been tested.¹⁵

To our knowledge, there are no previous trials of early iron supplementation for breastfed infants that have assessed the impact on serum iron and other markers of cellular iron supply. Most prior trials have started supplementation later (between 3 to 4 months of age) and have usually assessed haemoglobin and/or ferritin.¹⁶ Other trials have recruited children between 1-36 months^{17 18} and 2-

36 months but have not analysed outcomes separately for the youngest children. A trial in Northeast India started iron supplementation very early post-natally.^{19,20} Supplementation with iron at 2 mg/kg (as ferrous ascorbate) from 36h after birth improved haemoglobin at 6 months from 97.0 to 103.7 g/L (P <0.0001) and ferritin from 78 to 134 ng/ml (P<0.001).¹⁹ In China, a 4-arm trial (total n=1,276) with iron offered in pregnancy and/or infancy (with ~1mg/kg iron as iron protein succinylate oral solution from 6 weeks to 9 months) measured haemoglobin, ferritin, zinc protoporphyrin and sTfR at 9 months.²¹ Iron supplementation reduced zinc protoporphyrin but was not found to improve other outcomes.²¹ In addition, in a trial in Honduras, infants who had been exclusively breastfed for 4 months were randomised to either continue being exclusively breastfed until 6 months or to receive iron-fortified foods in addition to breast milk from 4 to 6 months. The introduction of complementary foods in addition to breastfeeding resulted in an increase in iron intake, and in increased haemoglobin concentrations, haematocrit, and ferritin concentrations at 6 months of age.²² In a similarly designed randomised trial in Iceland, the introduction of complementary foods in addition to breastfeeding resulted in an increase in serum ferritin concentrations, but there was no evidence that it improved haemoglobin concentration or other iron markers.²³

Ideally, we would have assessed the change in our primary outcome, serum iron, over a 24h period, but this is not feasible in young infants. In light of this limitation the observed improvement in serum iron was particularly impressive given that it was assessed 24h after the last iron dose. The absorption of a bolus dose of ferrous sulphate occurs rapidly with a peak, in adults, at 3-4h post dose and with a subsequent decline towards baseline after 8h.²⁴ In pilot work for a previous study we estimated that the peak serum iron level in young Gambian children was also at 3-4h post-dose.²⁵ We also demonstrated that at 6 m of age serum iron increased 5-fold (from 5.8 to 26.5 µmol/L) 4h after a 2 mg/kg dose of ferrous sulphate. Hence, in the current study, the aggregate benefit in supplying iron to iron-demanding tissues is likely to be much larger (estimated to be 3-4 fold higher) than indicated by the 24 h post-dose serum iron level. This is confirmed by Figure 2 which summarises all measures of iron status expressed as an effect size relative to the population standard deviation. The effects on the other longer-term metrics of iron status (of which reticulocyte haemoglobin (0.88 SD) and transferrin (-0.70 SD)) are particularly important) were larger than the 24-h post-dose serum iron (+0.53 SD).

A strength of our trial was the daily visits by fieldworkers who directly supervised consumption of the iron or placebo (and hence maintained a high compliance). The fieldworkers also asked mothers if their infant was sick and referred any worrying conditions to study nurses for assessment of adverse

events, and onwards to a physician for any serious adverse events. There were no deaths, there were ten illnesses categorised as serious adverse events (5 intervention/5 control), all of which resolved, and there were 106 adverse events (54 intervention/52 control) all of which resolved. None of these conditions were deemed to be related to the intervention. In line with the low prevalence of Plasmodium infection in our study area,⁸ there were no incident cases of malaria in our study. Surprisingly we found weak evidence of a reduction in episodes of vomiting in the infants receiving iron.

Two prior trials in Honduras and Sweden reported that iron supplementation was associated with impaired growth.²⁶ We failed to find evidence of any such effect, and no other trials have replicated this finding.

The main limitation of our trial is that it is a proof-of-principle trial with a small sample size and will require replication in a much larger sample.

The benefits of early iron supplementation shown here are similar to those reported in a meta analysis of 12 studies of delayed cord-clamping in low- and middle-income countries with ferritin, haemoglobin and MCV assessed between 2-12 months,²⁷ and in the 3 studies among these that made assessments at 6 months.²⁸ Although widely adopted as best practice it has proven difficult to implement delayed cord clamping in many settings and especially those, as in our study, with high proportions of home deliveries. It would be useful to test the combined effects of delayed clamping and early iron supplementation.

An improved haemoglobin at 6 months of age should enhance infants' ability to learn through exploring their environment and improving attention, although these benefits have been surprisingly hard to demonstrate in studies of older children.²⁹ The health and developmental benefits of an enhanced systemic iron supply, as achieved here, might be greater for both cognitive and immune function because the critical neural and lymphoid tissues cannot access iron from liver ferritin or haemoglobin (the major body iron stores) and must utilise transferrin-bound iron. Based on our proof-of-principle success in the current trial further trials are warranted with adequate power to properly assess safety and to assess functional outcomes.

ROLE OF THE FUNDING SOURCE

The funder had no role in the design or implementation of the study or the analysis and publication of results. All authors had full access to the data and approved the manuscript prior to publication.

CONTRIBUTORS

MB contributed to the study design, led the study as part of his PhD, oversaw all aspects of study implementation including data collection, data analysis and interpretation and writing of the original report and reviewing and editing. IS contributed to the study protocol design, project administration, reviewing and editing of the report and was supervised by SEM. CC conceived the study idea and acquisition of funding, design, and implementation, was overall principal investigator, contributed to the writing of the original report, reviewing, and editing and supervised MB. HV contributed to the study design, data analysis and interpretation, writing of the original report, reviewing, and editing and co-supervised MB. AMP contributed to the study idea and acquisition of funding, design, data visualisation, interpretation, writing of the original report, reviewing, and editing. BS oversaw clinical care of the participants, contributed to data collection and reviewing and editing.

DECLARATION OF INTERESTS

We declare no competing interests.

ACKNOWLEDGMENTS

This study was jointly funded by the UK Department for International Development (DFID), the Medical Research Council UK (MRC), the National Institute for Health Research and Care Research the Wellcome Trust and was awarded to the Medical Research Council Unit The Gambia at London School of Hygiene & Tropical Medicine (Grant Ref: MR/T003960/1). CC, BS and AMP were funded through MR CG@LSHTM. MB was funded through MR CG@LSHTM and Wageningen University and Research. HV was funded through Wageningen University and Research. IS and SEM are funded through a Wellcome Trust Senior Research Fellowship to SEM [220225]. MR CG@LSHTM is corefunded by MCA760-5QX00 from the MRC and the UK Department for International Development (DFID) under the MRC/DFID Concordat agreement. We sincerely thank Iron Babies Study Team at MR CG@LSHTM for their dedicated support. We also thank the participants and communities of Jarra Soma.

ANNEXES

Supplementary Materials (including trial protocol) (Bah_etal_Supplementary Annex_FINAL)

Supplementary Figure S4 (Bah_etal_Supplementary_Fig S4)

<https://www.medrxiv.org/content/10.1101/2023.01.12.22284059v1.supplementary-material>

REFERENCES

- 1 Baker RD, Greer FR, Bhatia JJS, et al. Diagnosis and prevention of iron deficiency and iron deficiency anemia in infants and young children (0–3 years of age). *Pediatrics* 2010; 126: 1040–50.
- 2 IOM. Panel on micronutrients: dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington (DC): National Academies Press (US); 2001.
- 3 Armitage AE, Agbla SC, Betts M, et al. Rapid growth is a dominant predictor of hepcidin suppression and declining ferritin in Gambian infants. *Haematologica* 2019; 104: 1542.
- 4 Mwangi MN, Mzembe G, Moya E, Verhoef H. Iron deficiency anaemia in sub-Saharan Africa: a review of current evidence and primary care recommendations for high-risk groups. *Lancet Haematol* 2021; 8: e732–43.
- 5 WHO. The optimal duration of exclusive breastfeeding report of an expert consultation: report of an expert consultation. Geneva, 2001 <http://www.who.int/child-adolescenthealth> (accessed Dec 21, 2022).
- 6 Marques RFSV, Taddei JAAC, Lopez FA, Braga JAP. Breastfeeding exclusively and iron deficiency anemia during the first 6 months of age. *Rev Assoc Med Bras (1992)* 2014; 60: 18–22.
- 7 Friel J, Qasem W, Cai C. Iron and the Breastfed Infant. *Antioxidants* 2018; 7.
- 8 GBoS, ICF. The Gambia demographic and health survey 2019-20. Banjul, The Gambia, 2021 <https://dhsprogram.com/publications/publication-FR369-DHS-Final-Reports.cfm> (accessed Dec 16, 2022).
- 9 Stelle I, Bah M, Silverio SA, et al. Iron supplementation of breastfed Gambian infants from 6 weeks to 6 months of age: protocol for a randomised controlled trial. *Wellcome Open Res* 2022; 7: 16.
- 10 Mei Z, Laurence &, Grummer-Strawn M. Bulletin of the World Health Organization. *Bull World Health Organ* 2007; 85: 441–8.
- 11 WHO. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Geneva, 2011 <http://www.who.int/vmnis/indicators/haemoglobin> (accessed Nov 28, 2022).
- 12 Royston P, Sauerbrei W. Multivariable Model - Building: A Pragmatic Approach to Regression Analysis based on Fractional Polynomials for Modelling Continuous Variables. Chichester, England: Wiley, 2008 <https://mfp.imbi.uni-freiburg.de/book> (accessed Nov 28, 2022).
13. Gustin, J. et al. Characterizing exclusively breastfed infant stool via a novel infant stool scale. *J Par Ent Nutr* 2018 42, S5–S11.
14. Alt KW, Al-Ahmad A, Woelber JP. Nutrition and Health in Human Evolution—Past to Present.

Nutrients 2022; 14.

15. UNICEF. From the first hour of life: making the case for improved infant and young child feeding everywhere. New York, 2016 moz-extension://13c12fee-5b00-433a-90aad1268ee244f9/enhancedreader.html?openApp&pdf=https%3A%2F%2Fwww.unicef.org%2Fmedia%2F49801%2Ffile%2FFrom-the-first-hour-of-life-ENG.pdf (accessed Nov 28, 2022).
16. Stelle I, Venkatesan S, Edmond K, Moore SE. Acknowledging the gap: a systematic review of micronutrient supplementation in infants under six months of age. Wellcome Open Res 2020; 5: 238.
17. Sazawal S, Black RE, Ramsan M, et al. Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial. Lancet 2006; 367: 133–43.
18. Verhoef H, West CE, Nzyuko SM, et al. Intermittent administration of iron and sulfadoxinepyrimethamine to control anaemia in Kenyan children: a randomised controlled trial. Lancet 2002; 360: 908–14.
19. Bora R, Ramasamy S, Brown B, Wolfson J, Rao R. Effect of iron supplementation from neonatal period on the iron status of 6-month-old infants at-risk for early iron deficiency: a randomized interventional trial. J Matl-Fetal Neonat Med 2021; 34: 1421–9.
20. Tielsch JM, Khatry SK, Stoltzfus RJ, et al. Effect of routine prophylactic supplementation with iron and folic acid on preschool child mortality in southern Nepal: community-based, cluster-randomised, placebo-controlled trial. Lancet 2006; 367: 144–52.
21. Lozoff B, Jiang Y, Li X, et al. Low-Dose Iron Supplementation in Infancy Modestly Increases Infant Iron Status at 9 Mo without Decreasing Growth or Increasing Illness in a Randomized Clinical Trial in Rural China. J Nutr 2016; 146: 612.
22. Dewey KG, Cohen RJ, Rivera LL, Brown KH. Effects of age of introduction of complementary foods on iron status of breast-fed infants in Honduras. Am J Clin Nutr 1998; 67: 878–84.
23. Jonsdottir OH, Thorsdottir I, Hibberd PL, et al. Timing of the introduction of complementary foods in infancy: a randomized controlled trial. Pediatrics 2012; 130: 1038–45.
24. Brittenham GM, Andersson M, Egli I, et al. Circulating non-transferrin-bound iron after oral administration of supplemental and fortification doses of iron to healthy women: a randomized study. Am J Clin Nutr 2014; 100: 813.
25. Cerami C, Ledger E, Jallow A, et al. Clinical and sub-clinical infections driving hepcidin-mediated inhibition of iron absorption in African children: the IDeA Studies. In preparation.
26. Domellöf M, Cohen RJ, Dewey KG, Hernell O, Rivera LL, Lönnerdal B. Iron supplementation of breast-fed Honduran and Swedish infants from 4 to 9 months of age. J Pediatr 2001; 138: 679–87.

- 476 27. Fu X, Dang D, Li S, Xu Z, Wu H. Effect of delayed versus early cord clamping on improving anemia
477 interm infants aged two months or older - a meta-analysis. Indian Pediatr 2020; 57:
478 815–9.
- 479 28. Chaparro CM, Neufeld LM, Tena Alavez G, Eguia-Líz Cedillo R, Dewey KG. Effect of timing of
480 umbilical cord clamping on iron status in Mexican infants: a randomised controlled trial.
481 Lancet 2006; 367: 1997–2004.
- 482 29. Pasricha S-R, Hasan MI, Braat S, et al. Benefits and Risks of Iron Interventions in Infants in Rural
483 Bangladesh. N Engl J Med 2021; 385: 982–95.

554 TABLE 1. Characteristics of the study population at baseline (intention-to-treat population)

Baseline variable	Iron	Placebo
n, as randomised	50	51
Age, weeks		
6-6.99 weeks	29 (58.0%)	25 (49.0%)
7-7.99 weeks	12 (24.0%)	12 (23.5%)
8-8.99 weeks	3 (6.0%)	8 (15.7%)
9+ weeks	6 (12.0%)	6 (11.8%)
Female	26 (52.0%)	29 (56.9%)
Number of children born to the mother	3.9 (2.2)	3.9 (2.1)
Serum iron, $\mu\text{mol/L}$	12.6 (4.5)	12.5 (6.9)
Serum ferritin, $\mu\text{g/L}$ †	283.2 (1.81)	262.6 (1.76)
Serum soluble transferrin receptor, mg/L †	2.81 (1.28)	2.76 (1.30)
Serum sTfR/ $\log_{10}(\text{ferritin})$ index †	1.15 (1.34)	1.15 (1.31)
Serum transferrin, g/L	2.2 (0.4)	2.1 (0.4)
UIBC, $\mu\text{mol/L}$	37.0 (11.0)	35.2 (11.0)
TSAT, % †	24.0 (1.65)	22.6 (2.00)
Haemoglobin, g/dL †	10.9 (1.6)	11.0 (1.2)
MCV, fL	89.6 (5.3)	89.2 (5.4)
MCH, pg	29.4 (1.7)	28.9 (2.1)
MCHC, g/dL	32.9 (1.4)	32.6 (1.3)
Reticulocyte count, $\times 10^9/\text{L}$ †	0.07 (1.44)	0.07 (1.49)
Reticulocyte count, % †	1.83 (1.57)	1.91 (1.54)
Immature reticulocyte fraction, % †	17.2 (1.50)	16.4 (1.52)
Reticulocyte haemoglobin, pg	31.8 (1.8)	31.3 (2.4)
Serum hepcidin, $\mu\text{g/L}$ †	20.3 (2.21)	19.6 (2.36)

Serum erythropoietin, IU/L †	12.7 (2.05)	10.8 (1.85)
Serum α_1 -acid glycoprotein, g/L †	0.66 (1.48)	0.61 (1.80)
Serum erythroferrone, $\mu\text{g/L}$ * §		
Below or equal to LOQ ($\leq 0.16 \mu\text{g/L}$)	66.0% (33)	74.5% (38)
0.16-0.499	22.0% (11)	3.9% (2)
0.5-0.99	10.0% (5)	5.9% (3)
1.0+	2.0% (1)	15.7% (8)
Serum C-reactive protein *		
Below or equal to LOQ ($\leq 0.60 \text{ mg/L}$)	34 (68.0%)	33 (64.7%)
0.60-4.99 mg/L	12 (24.0%)	12 (23.5%)
5-14.99 mg/L	2 (4.0%)	1 (2.0%)
15+ mg/L	2 (4.0%)	5 (9.8%)
Height-for-age z-score, SD	-0.59 (1.18)	-0.68 (1.59)
Weight-for-age z-score, SD	-0.71 (1.19)	-0.70 (1.25)
Weight-for-height z-score, SD	-0.22 (1.53)	-0.11 (1.11)

0

1 LOQ: limit of quantification; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular
2 haemoglobin concentration; MCV: mean corpuscular volume; sTfR: soluble transferrin receptor; TSAT:
3 transferrin saturation; UIBC: unsaturated iron-binding capacity. Values indicate n, mean (SD); n, geometric
4 mean (geometric SD); † or prevalence n (%). * median (25-, and 75%-percentiles); The geometric SD is a
5 dimensionless, multiplicative factor such that dividing or multiplication of the geometric mean by this
6 ratio indicates a variation that is equivalent to subtraction or addition of one standard deviation on a
7 logtransformed scale. ‡ Excluding one observation with an outlying value (86.8).

TABLE 2. Effect of iron supplementation on primary and secondary outcomes (intention-to-treat population)

Outcome variable	Iron	Placebo	Group difference, unadjusted (95% CI)	p-value	Group difference, * adjusted (95% CI)	p-value
Serum iron, $\mu\text{mol/L}$	11.8 (4.9)	9.4 (4.4)	2.5 (0.6 to 4.3)	0.0091	2.3 (0.5 to 4.1)	0.013
Serum ferritin, $\mu\text{g/L}$ †	94.0 (1.98)	44.5 (2.83)	111.2% (49.4% to 198.6%)	<0.0005	115.8% (60.9% to 189.4%)	<0.0005
Serum soluble transferrin receptor, mg/L †	2.75 (1.11)	2.96 (1.09)	-7.2% (-3.7% to -10.5%)	<0.0005	-7.7% (-4.6% to -12.1%)	<0.0005
Serum transferrin, g/L	2.4 (0.4)	2.7 (0.5)	-0.33 (-0.51 to -0.15)	0.00052	0.37 (0.21 to 0.53)	<0.0005
UIBC, $\mu\text{mol/L}$	47.1 (10.4)	55.8 (12.0)	-8.7 (-13.0 to -4.3)	<0.0005	-9.5 (-13.5 to -5.5)	<0.0005
TSAT †	18.6 (1.59)	12.8 (1.74)	45.4% (18.8% to 78.0%)	<0.0005	5.72 (2.84 to 8.59)	0.00020
Serum sTfR/log ₁₀ (ferritin) index †	1.41 (1.21)	1.79 (1.27)	-21.3% (-27.7% to -14.3%)	<0.0005	-22.7% (-28.6% to -16.4%)	<0.0005
Haemoglobin, g/dL	11.2 (1.2)	10.6 (0.9)	0.6 (0.2 to 1.0)	0.0072	0.6 (0.2 to 1.0)	0.0082
Anaemia (haemoglobin <11.0 g/dL)	42.7%	66.9%	-24.2% (-43.7% to -4.7%)	0.018	.. ‡ ..	..
MCV, fL	77.7 (4.0)	74.9 (4.4)	2.8 (1.1 to 4.5)	0.0012	2.7 (1.4 to 4.1)	<0.0005
MCH, pg	24.5 (1.4)	23.3 (1.5)	1.2 (0.6 to 1.8)	<0.0005	1.2 (0.6 to 1.7)	<0.0005
MCHC, g/dL	31.6 (0.8)	31.2 (0.9)	0.4 (0.1 to 0.8)	0.016	0.34 (0.03 to 0.64)	0.031
Reticulocyte count, $\times 10^9/\text{L}$ †	0.06 (1.46)	0.05 (1.43)	19.8% (3.4% to 38.8%)	0.017	23.5% (6.4% to 43.3%)	0.0061
Reticulocyte count, % †	1.36 (1.48)	1.14 (1.45)	19.4% (2.7% to 38.9%)	0.021	23.2% (5.5% to 43.8%)	0.009
Immature reticulocyte fraction, % †	9.6 (1.68)	10.4 (1.48)	-7.9% (-23.3% to 10.5%)	0.37	-8.5% (-24.1% to 10.3)	0.35
Reticulocyte haemoglobin, pg	30.1 (2.7)	27.4 (3.1)	2.8 (1.6 to 3.9)	<0.0005	2.72 (1.55 to 3.90)	<0.0005
Serum hepcidin, $\mu\text{g/L}$ †	13.54 (2.21)	7.56 (3.26)	79.0% (20.4% to 165.9%)	0.0044	72.4% (14.5% to 159.6%)	0.010
Serum erythropoietin, IU/L †	8.66 (2.17)	10.59 (1.73)	-18.2% (-37.3% to 6.4%)	0.14	-18.7% (-37.3% to 5.3%)	0.12
Serum α_1 -acid glycoprotein, g/L †	0.92 (1.52)	1.01 (1.52)	-9.8% (-23.5% to 6.4%)	0.22	-8.8% (-22.7% to 7.5%)	0.27
Serum erythroferrone, $\mu\text{g/L}$ † §	0.16 (0.16, 0.58)	0.42 (0.16, 1.26)		0.005		..
Serum C-reactive protein, mg/L † §	1.3 (0.7, 4.2)	1.4 (0.6, 4.6)		0.93		..
Height-for-age z-score, SD	-0.81 (1.24)	-0.68 (1.18)	-0.14 (-0.62 to 0.35)	0.58	-0.21 (-0.16 to 0.57)	0.27
Weight-for-age z-score, SD	-0.64 (1.27)	-0.70 (1.13)	0.05 (-0.42 to 0.53)	0.82	0.02 (-0.37 to 0.41)	0.92
Height-for-weight z-score, SD	-0.07 (1.11)	-0.24 (1.10)	0.18 (-0.26 to 0.61)	0.43	0.19 (-0.23 to 0.60)	0.38

MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; MCV: mean corpuscular volume; sTfR: soluble transferrin receptor; TSAT: transferrin saturation; UIBC: unsaturated iron-binding capacity. Values for group descriptors indicate n, mean (SD); n, geometric mean (geometric SD); † prevalence % (SD) or median (25-, 75-percentiles). Analysis was based on the sample as randomised (iron: n=50, placebo: n=51) with multiple imputation of missing values. The geometric SD is a dimensionless, multiplicative factor such that dividing or multiplication of the geometric mean by this ratio indicates a variation that is equivalent to subtraction or addition of one standard deviation on a log-transformed scale. Intervention effects are reported as absolute differences, relative differences† or prevalence ratios. * Effect estimates adjusted for the outcome variable measured at baseline, sex (binary variable), age (continuous), haemoglobin concentration and serum concentrations of ferritin and soluble transferrin receptor at baseline (all continuous); except for anthropometric outcomes that were adjusted for the outcome variable measured at baseline, sex (binary variable) and age (continuous variable). Continuous covariates were modelled as fractional polynomials (see text). ‡ Estimates not reported because, to our knowledge, no method is available to compute differences in proportions, adjusted for covariates, with multiply imputed data. § Variables were left-censored at the limit of quantification (erythroferrone: 0.16 µg/L; C-reactive protein: 0.60 µg/L). In the iron group, most erythroferrone values (26/47) were left-censored, so that the median equals the 25-percentile. Because logtransformation failed to normalise the distribution of residuals within groups, we neither conducted parametric analyses nor imputed missing values; p-values reported were obtained by two-sample Wilcoxon rank-sum (Mann–Whitney) tests.

TABLE 3. Effect of iron supplementation on adverse events

	Iron	Placebo
Serious adverse events		
Bronchiolitis	1	1
Constipation	0	1
Lower respiratory tract infection	1	0
Pneumonia	1	0
Sepsis	1	0
Severe malnutrition	0	2
Upper respiratory tract infection	1	1
Adverse events		
Conjunctivitis	1/1	4/4
Fever due to immunisation	18/16	25/17

Multiple abscesses	1/1	0
Skin rashes	6/6	5/5
Upper respiratory tract infection	27/24	16/15
Hospitalisation for somatic illness	1/1	2/2
Category of adverse events		
Grade 1	44	47
Grade 2	7	3
Grade 3	3	2

Values indicate the number of events or the number of events/number of children with at least one adverse event. Grade 1: Awareness of sign or symptom, but easily tolerated. Grade 2: Enough discomfort to cause interference with usual activity. Grade 3: Incapacitating with inability to do usual activity.

TABLE 4. Group comparisons of maternally reported adverse events

Adverse event/ group	Analysis of first episode				Analysis of recurrent new episodes			
	Risk, * % (n)	Risk difference	(95% CI)	p-value	Observed incidence †	Predicted incidence † (95% CI)	Incidence (95% CI) difference ‡	p-value
Diarrhoea §								
Iron	54.0% (27)	18.7% (−0.3% to 37.8%)		0.06	1.21 (51/4,227)	1.26 (0.81 to 1.70)	0.20 (−0.49 to 0.88)	0.57
Placebo	35.3% (18)	Reference (..)		..	0.93 (39/4,194)	1.06 (0.54 to 1.58)	Reference (..)	..
Fever "								
Iron	90.0% (45)	1.8% (−10.4% to 13.9)		0.78	2.80 (116/4,146)	2.80 (2.24 to 3.35)	0.16 (−0.64 to 0.95)	0.70
Placebo	88.2% (45)	Reference (..)		..	2.64 (108/4,088)	2.64 (2.07 to 3.22)	Reference (..)	..
Cough "								
Iron	80.0% (40)	−4.3% (−19.2% to 10.6%)		0.57	2.94 (120/4,083)	3.03 (2.31 to 3.75)	0.37 (−0.60 to 1.33)	0.45
Placebo	84.3% (43)	Reference (..)		..	2.65 (108/4,083)	2.65 (2.01 to 3.31)	Reference (..)	..
Vomiting "								
Iron	32.0% (16)	−22.9% (−41.7% to −4.1%)		0.02	0.43 (19/4,470)	0.43 (0.24 to 0.61)	−0.37 (−0.67 to −0.08)	0.01
Placebo	54.9% (28)	Reference (..)		..	0.80 (35/4,363)	0.80 (0.57 to 1.03)	Reference (..)	..
Skin infections "								
Iron	34.0% (17)	10.5% (−7.1% to 28.0%)		0.25	0.65 (29/4,430)	0.67 (0.30 to 1.03)	0.32 (−0.08 to 0.73)	0.12

Placebo	23.5% (12)	Reference (..)	..	0.34 (15/4,425)	0.34 (0.16 to 0.52)	Reference (..)	..
Eye infections "							
Iron	18.0% (9)	-5.5% (-21.3% to 10.2%)	0.49	0.27 (12/4,484)	0.27 (0.09 to 0.45)	-0.00 (-0.02 to 0.02)	0.98
Placebo	23.5% (12)	Reference (..)	..	0.27 (12/4,438)	0.27 (0.14 to 0.40)	Reference (..)	..
Nasal discharge "							
Iron	72.0% (36)	-2.5% (-19.7% to 14.8%)	0.78	1.94 (82/4,233)	1.96 (1.45 to 2.47)	-0.15 (-0.89 to 0.59)	0.69
Placebo	74.5% (38)	Reference (..)	..	2.10 (88/4,194)	2.11 (1.57 to 2.65)	Reference (..)	..

9

.. : not applicable

* Computed as the percent of children who were randomised (iron: n=50; placebo: n=51) and who experienced at least one new episode during the follow-up period.

† Number of recurrent new episodes per 100 child-days, calculated as marginal effects from count models with zero-inflation as appropriate.

‡ Expressed per 100 child-days. All count models used Poisson regression except for skin infections, which were modelled using negative binomial (NB2) regression. For diarrhoea, cough and nasal discharge, we accounted for excess zero values by using zero-inflated regression models (see text and Table S4).

§ A new episode was defined as a maternal report of the child having one or more successive days with 3 or more liquid or fluid stools (Gustin et al. ¹³: Figure 6, type 4, 5A or 5B), separated by at least 5 successive days without diarrhoea (Gustin et al. ¹³: Figure 6, types ≤3).

" For other symptoms, episodes were defined as a maternal report of the child having one or more successive days with that symptom, separated by at least 3 successive days without the symptom.

FIGURE LEGENDS

Figure 1. CONSORT diagram

Figure 2. Aggregate effects of iron supplementation on selected outcomes, intention-to-treat analysis

MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; MCV: mean corpuscular volume; sTfR: soluble transferrin receptor; UIBC: unsaturated iron-binding capacity. For outcomes with normally distributed residuals, effect sizes are standardised, i.e., they are computed as the difference in group means divided by the SD of all imputed values (upper panel). For outcomes with log-normally distributed residuals, effect sizes are computed as the ratio of geometric means (lower panel). Note that reductions in transferrin, UIBC and sTfR indicate improved iron status.

Figure 1

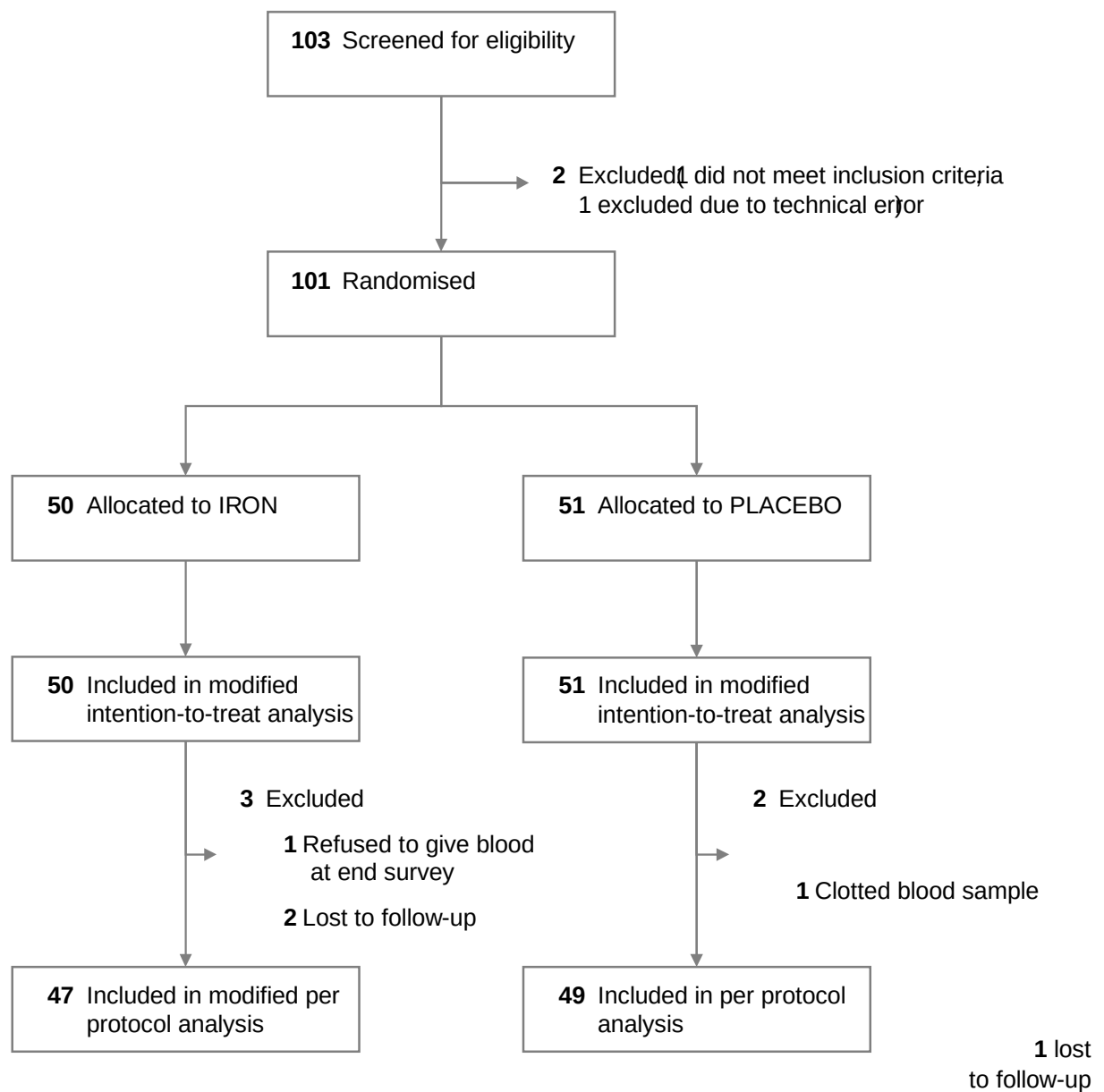
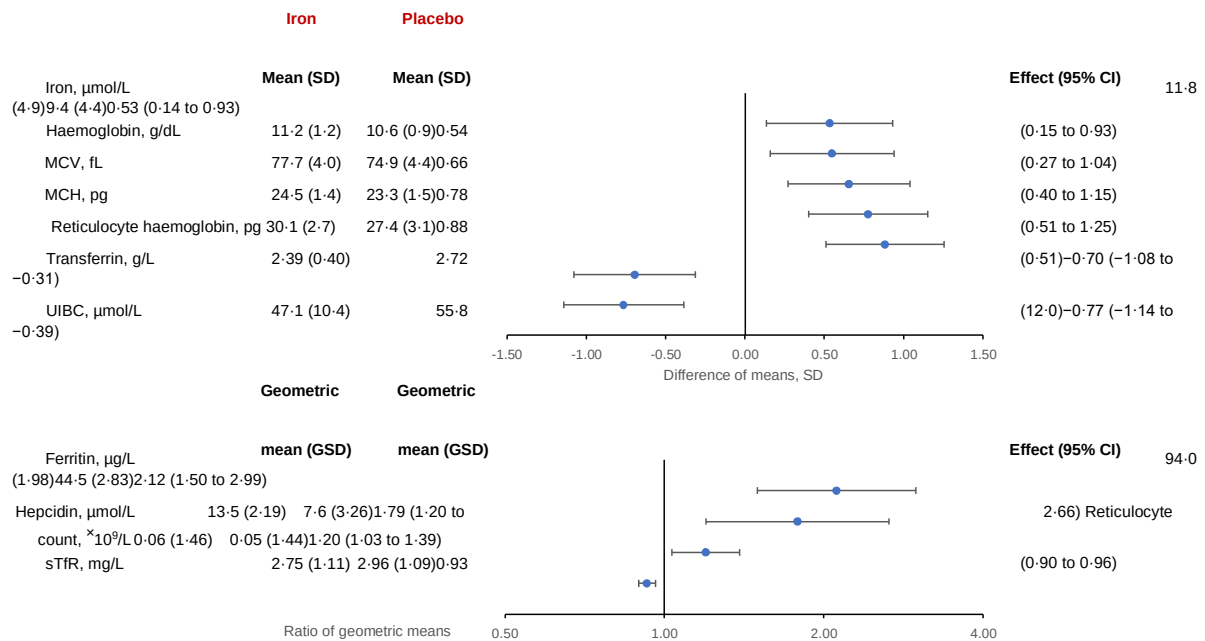


Figure 2



REFERENCES

1. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet*. 2017;390(10100):1211-59.
2. Miller JL. Iron deficiency anemia: a common and curable disease. *Cold Spring Harb Perspect Med*. 2013;3(7).
3. Global Nutrition Targets 2025: anaemia policy brief. Geneva, Switzerland World Health Organization; 2014.
4. Armitage AE, Moretti D. The Importance of Iron Status for Young Children in Low- and Middle-Income Countries: A Narrative Review. *Pharmaceuticals (Basel)*. 2019;12(2).
5. Butte N, López-Alarcón M, Garza C. Nutrient Adequacy of Exclusive Breastfeeding for the Term Infant During the First Six Months of Life. 2002:1-49.
6. Armitage AE, Agbla SC, Betts M, Sise EA, Jallow MW, Sambou E, et al. Rapid growth is a dominant predictor of hepcidin suppression and declining ferritin in Gambian infants. *Haematologica*. 2019;104(8):1542-53.
7. Eriksen KG, Johnson W, Sonko B, Prentice AM, Darboe MK, Moore SE. Following the World Health Organization's Recommendation of Exclusive Breastfeeding to 6 Months of Age Does Not Impact the Growth of Rural Gambian Infants. *J Nutr*. 2017;147(2):248-55.
8. WHO. Nutritional anaemias: tools for effective prevention and control: World Health Organization; 2017 [Available from: <https://www.who.int/publications-detail/9789241513067>].
9. Johns Hopkins Hospital KK, Lauren McDaniel, Matthew Molloy. The Harriet Lane Handbook. 22nd ed: Elsevier; 2020 April 22, 2020. 1216 p.
10. Bah A, Muhammad AK, Wegmuller R, Verhoef H, Goheen MM, Sanyang S, et al. Hepcidin-guided screen-and-treat interventions against iron-deficiency anaemia in pregnancy: a randomised controlled trial in The Gambia. *Lancet Glob Health*. 2019;7(11):e1564-e74.
11. Prentice S, Jallow AT, Sinjanka E, Jallow MW, Sise EA, Kessler NJ, et al. Hepcidin mediates hypoferremia and reduces the growth potential of bacteria in the immediate post-natal period in human neonates. *Scientific Reports*. 2019;9(1):16596.
12. Darboe MK, Thurnham DI, Morgan G, Adegbola RA, Secka O, Solon JA, et al. Effectiveness of an early supplementation scheme of high-dose vitamin A versus standard WHO protocol in Gambian mothers and infants: a randomised controlled trial. *Lancet*. 2007;369(9579):2088-96.
13. Jallow MW, Campino S, Saidykhan A, Prentice AM, Cerami C. Common Variants in the TMPRSS6 Gene Alter Hepcidin but not Plasma Iron in Response to Oral Iron in Healthy Gambian Adults: A Recall-by-Genotype Study. *Curr Dev Nutr*. 2021;5(3):nzab014.
14. Stelle I, Bah M, Silverio S, Verhoef H, Comma E, Prentice A, et al. Iron supplementation of breastfed Gambian infants from 6 weeks to 6 months of age: protocol for a randomised controlled trial [version 1; peer review: awaiting peer review]. *Wellcome Open Research*. 2022;7(16).
15. Muckenthaler MU, Rivella S, Hentze MW, Galy B. A Red Carpet for Iron Metabolism. *Cell*. 2017;168(3):344-61.
16. Pena-Rosas JP, De-Regil LM, Garcia-Casal MN, Dowswell T. Daily oral iron supplementation during pregnancy. *Cochrane Database Syst Rev*. 2015(7):CD004736.
17. Grantham-McGregor S, Ani C. A review of studies on the effect of iron deficiency on cognitive development in children. *J Nutr*. 2001;131(2S-2):649S-66S; discussion 66S-68S.
18. Lozoff B. Iron deficiency and child development. *Food Nutr Bull*. 2007;28(4 Suppl):S560-71.
19. Georgieff MK. Long-term brain and behavioral consequences of early iron deficiency. *Nutr Rev*. 2011;69 Suppl 1(Suppl 1):S43-8.
20. Domellöf M, Braegger C, Campoy C, Colomb V, Decsi T, Fewtrell M, et al. Iron requirements of infants and toddlers. *J Pediatr Gastroenterol Nutr*. 2014;58(1):119-29.

21. Prentice AM, Mendoza YA, Pereira D, Cerami C, Wegmuller R, Constable A, et al. Dietary strategies for improving iron status: balancing safety and efficacy. *Nutr Rev.* 2017;75(1):49-60.
22. Horton S, Ross J. The economics of iron deficiency. *Food Policy.* 2003;28(1):51-75.
23. WHO. WHO recommendations on antenatal care for a positive pregnancy experience: World Health Organization 2016 [Available from: http://www.who.int/reproductivehealth/publications/maternal_perinatal_health/anc-positive-pregnancy-experience/en/].
24. WHO. Guideline: Daily iron supplementation in infants and children. . Geneva, Switzerland: World Health Organization 2016.
25. Widdowson EM. Trace elements in foetal and early postnatal development. *Proc Nutr Soc.* 1974;33(3):275-84.
26. Pasricha SR, Drakesmith H, Black J, Hipgrave D, Biggs BA. Control of iron deficiency anemia in low- and middle-income countries. *Blood.* 2013;121(14):2607-17.
27. Conclusions and recommendations of the WHO Consultation on prevention and control of iron deficiency in infants and young children in malaria-endemic areas. *Food Nutr Bull.* 2007;28(4 Suppl):S621-7.
28. Anaemia: overview, symptoms, and treatment: World Health Organization [Available from: https://www.who.int/health-topics/anaemia#tab=tab_1].
29. WHO UNICEF UNU. Iron Deficiency Anaemia Assessment, Prevention and Control: A Guide for Programme Managers. 1st Edition ed. Geneva: World Health Organisation 2011.
30. Qasem WA, Friel JK. An Overview of Iron in Term Breast-Fed Infants. *Clin Med Insights Pediatr.* 2015;9:79-84.
31. WHO. Worldwide prevalence of anaemia 1993-2005 : WHO global database on anaemia. / Edited by Bruno de Benoist, Erin McLean, Ines Egli and Mary Cogswell. Geneva: World Health Organization; 2008.
32. Pasricha SR, Armitage AE, Prentice AM, Drakesmith H. Reducing anaemia in low income countries: control of infection is essential. *Bmj.* 2018;362:k3165.
33. WHO. The Global Prevalence of Anaemia in 2011 Geneva, Switzerland: World Health Organisation; 2015 [Available from: https://apps.who.int/iris/bitstream/handle/10665/177094/9789241564960_eng.pdf].
34. Stevens GA, Finucane MM, De-Regil LM, Paciorek CJ, Flaxman SR, Branca F, et al. Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for 1995-2011: a systematic analysis of population-representative data. *Lancet Glob Health.* 2013;1(1):e16-25.
35. Helman SL, Anderson GJ, Frazer DM. Dietary iron absorption during early postnatal life. *Biometals.* 2019;32(3):385-93.
36. Domellöf M, Lönnerdal B, Abrams SA, Hernell O. Iron absorption in breast-fed infants: effects of age, iron status, iron supplements, and complementary foods. *Am J Clin Nutr.* 2002;76(1):198-204.
37. Anderson GJ, Frazer DM, McLaren GD. Iron absorption and metabolism. *Curr Opin Gastroenterol.* 2009;25(2):129-35.
38. Ganz T. Systemic iron homeostasis. *Physiol Rev.* 2013;93(4):1721-41.
39. Drakesmith H, Prentice A. Viral infection and iron metabolism. *Nat Rev Microbiol.* 2008;6(7):541-52.
40. Rivera S, Nemeth E, Gabayan V, Lopez MA, Farshidi D, Ganz T. Synthetic hepcidin causes rapid dose-dependent hypoferremia and is concentrated in ferroportin-containing organs. *Blood.* 2005;106(6):2196-9.
41. Abera Teka T. Factors Affecting Iron Absorption and Mitigation Mechanisms: A review. *International Journal of Agricultural Science and Food Technology.* 2019.
42. Kautz L, Jung G, Valore EV, Rivella S, Nemeth E, Ganz T. Identification of erythroferrone as an erythroid regulator of iron metabolism. *Nat Genet.* 2014;46(7):678-84.

43. Mirciov CSG, Wilkins SJ, Hung GCC, Helman SL, Anderson GJ, Frazer DM. Circulating iron levels influence the regulation of hepcidin following stimulated erythropoiesis. *Haematologica*. 2018;103(10):1616-26.
44. Schwartz AJ, Das NK, Ramakrishnan SK, Jain C, Jurkovic MT, Wu J, et al. Hepatic hepcidin/intestinal HIF-2 α axis maintains iron absorption during iron deficiency and overload. *J Clin Invest*. 2019;129(1):336-48.
45. Nemeth E, Ganz T. Anemia of inflammation. *Hematol Oncol Clin North Am*. 2014;28(4):671-81, vi.
46. Armitage AE, Eddowes LA, Gileadi U, Cole S, Spottiswoode N, Selvakumar TA, et al. Hepcidin regulation by innate immune and infectious stimuli. *Blood*. 2011;118(15):4129-39.
47. Nemeth E, Rivera S, Gabayan V, Keller C, Taudorf S, Pedersen BK, et al. IL-6 mediates hypoferraemia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. *J Clin Invest*. 2004;113(9):1271-6.
48. Verga Falzacappa MV, Vujic Spasic M, Kessler R, Stolte J, Hentze MW, Muckenthaler MU. STAT3 mediates hepatic hepcidin expression and its inflammatory stimulation. *Blood*. 2007;109(1):353-8.
49. Wrighting DM, Andrews NC. Interleukin-6 induces hepcidin expression through STAT3. *Blood*. 2006;108(9):3204-9.
50. Armitage AE, Lim PJ, Frost JN, Pasricha SR, Soilleux EJ, Evans E, et al. Induced Disruption of the Iron-Regulatory Hormone Hepcidin Inhibits Acute Inflammatory Hypoferraemia. *J Innate Immun*. 2016;8(5):517-28.
51. Arezes J, Jung G, Gabayan V, Valore E, Ruchala P, Gulig PA, et al. Hepcidin-induced hypoferraemia is a critical host defense mechanism against the siderophilic bacterium *Vibrio vulnificus*. *Cell Host Microbe*. 2015;17(1):47-57.
52. Stefanova D, Raychev A, Arezes J, Ruchala P, Gabayan V, Skurnik M, et al. Endogenous hepcidin and its agonist mediate resistance to selected infections by clearing non-transferrin-bound iron. *Blood*. 2017;130(3):245-57.
53. Guida C, Altamura S, Klein FA, Galy B, Boutros M, Ulmer AJ, et al. A novel inflammatory pathway mediating rapid hepcidin-independent hypoferraemia. *Blood*. 2015;125(14):2265-75.
54. Weiss G, Ganz T, Goodnough LT. Anemia of inflammation. *Blood*. 2019;133(1):40-50.
55. Zimmermann MB, Fucharoen S, Winichagoon P, Sirankapracha P, Zeder C, Gowachirapant S, et al. Iron metabolism in heterozygotes for hemoglobin E (HbE), alpha-thalassemia 1, or beta-thalassemia and in compound heterozygotes for HbE/beta-thalassemia. *Am J Clin Nutr*. 2008;88(4):1026-31.
56. Weatherall DJ. The inherited diseases of hemoglobin are an emerging global health burden. *Blood*. 2010;115(22):4331-6.
57. Sanchaisuriya K, Fucharoen S, Ratanasiri T, Sanchaisuriya P, Fucharoen G, Dietz E, et al. Effect of the maternal betaE-globin gene on hematologic responses to iron supplementation during pregnancy. *Am J Clin Nutr*. 2007;85(2):474-9.
58. Joint World Health Organization/Centers for Disease C, Prevention Technical Consultation on the Assessment of Iron Status at the Population L. Assessing the iron status of populations: including literature reviews: report of a Joint World Health Organization/Centers for Disease Control and Prevention Technical Consultation on the Assessment of Iron Status at the Population Level, Geneva, Switzerland, 6-8 April 2004. 2nd ed ed. Geneva: World Health Organization; 2007 2007.
59. Pfeiffer CM, Looker AC. Laboratory methodologies for indicators of iron status: strengths, limitations, and analytical challenges. *The American Journal of Clinical Nutrition*. 2017;106:1606S-14S.
60. WHO. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity: The World Health Organisation; 2011 [Available from: https://apps.who.int/iris/bitstream/handle/10665/85839/WHO_NMH_NHD_MNM_11.1_eng.pdf?ua=1].

61. Cusick SE, Georgieff MK, Rao R. Approaches for Reducing the Risk of Early-Life Iron Deficiency-Induced Brain Dysfunction in Children. *Nutrients*. 2018;10(2).
62. Lynch S, Pfeiffer CM, Georgieff MK, Brittenham G, Fairweather-Tait S, Hurrell RF, et al. Biomarkers of Nutrition for Development (BOND)-Iron Review. *J Nutr*. 2018;148(suppl_1):1001s-67s.
63. Brugnara C, Zurakowski D, DiCanzio J, Boyd T, Platt O. Reticulocyte hemoglobin content to diagnose iron deficiency in children. *Jama*. 1999;281(23):2225-30.
64. Gelaw Y, Woldu B, Melku M. The Role of Reticulocyte Hemoglobin Content for Diagnosis of Iron Deficiency and Iron Deficiency Anemia, and Monitoring of Iron Therapy: a Literature Review. *Clin Lab*. 2019;65(12).
65. Namaste SM, Rohner F, Huang J, Bhushan NL, Flores-Ayala R, Kupka R, et al. Adjusting ferritin concentrations for inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. *Am J Clin Nutr*. 2017;106(Suppl 1):359s-71s.
66. Mei Z, Flores-Ayala RC, Grummer-Strawn LM, Brittenham GM. Is Erythrocyte Protoporphyrin a Better Single Screening Test for Iron Deficiency Compared to Hemoglobin or Mean Cell Volume in Children and Women? *Nutrients*. 2017;9(6).
67. Mwangi MN, Maskey S, Andang o PE, Shinali NK, Roth JM, Trijsburg L, et al. Diagnostic utility of zinc protoporphyrin to detect iron deficiency in Kenyan pregnant women. *BMC Med*. 2014;12:229.
68. Leventi E, Aksan A, Nebe CT, Stein J, Farrag K. Zinc Protoporphyrin Is a Reliable Marker of Functional Iron Deficiency in Patients with Inflammatory Bowel Disease. *Diagnostics (Basel)*. 2021;11(2).
69. Dallman PR, Reeves JD, Driggers DA, Edward Lo YT. Diagnosis of iron deficiency: The limitations of laboratory tests in predicting response to iron treatment in 1-year-old infants. *The Journal of Pediatrics*. 1981;99(3):376-81.
70. Pasricha SR, Atkinson SH, Armitage AE, Khandwala S, Veenemans J, Cox SE, et al. Expression of the iron hormone hepcidin distinguishes different types of anemia in African children. *Sci Transl Med*. 2014;6(235):235re3.
71. Moretti D, Goede JS, Zeder C, Jiskra M, Chatzinakou V, Tjalsma H, et al. Oral iron supplements increase hepcidin and decrease iron absorption from daily or twice-daily doses in iron-depleted young women. *Blood*. 2015;126(17):1981-9.
72. Prentice AM, Doherty CP, Abrams SA, Cox SE, Atkinson SH, Verhoef H, et al. Hepcidin is the major predictor of erythrocyte iron incorporation in anemic African children. *Blood*. 2012;119(8):1922-8.
73. Srinivasan B, O'Dell D, Finkelstein JL, Lee S, Erickson D, Mehta S. ironPhone: Mobile device-coupled point-of-care diagnostics for assessment of iron status by quantification of serum ferritin. *Biosens Bioelectron*. 2018;99:115-21.
74. Srinivasan B, Finkelstein JL, O'Dell D, Erickson D, Mehta S. Rapid diagnostics for point-of-care quantification of soluble transferrin receptor. *EBioMedicine*. 2019;42:504-10.
75. Boucher AA, Pfeiffer A, Bedel A, Young J, McGann PT. Utilization trends and safety of intravenous iron replacement in pediatric specialty care: A large retrospective cohort study. *Pediatric blood & cancer*. 2018;65(6):e26995.
76. Cook JD. Diagnosis and management of iron-deficiency anaemia. *Best Pract Res Clin Haematol*. 2005;18(2):319-32.
77. Daru J, Colman K, Stanworth SJ, De La Salle B, Wood EM, Pasricha SR. Serum ferritin as an indicator of iron status: what do we need to know? *Am J Clin Nutr*. 2017;106(Suppl 6):1634S-9S.
78. Abdullah K, Birken CS, Maguire JL, Fehlings D, Hanley AJ, Thorpe KE, et al. Re-Evaluation of Serum Ferritin Cut-Off Values for the Diagnosis of Iron Deficiency in Children Aged 12-36 Months. *J Pediatr*. 2017;188:287-90.
79. Powers JM, Buchanan GR. Potential for Improved Screening, Diagnosis, and Treatment for Iron Deficiency and Iron Deficiency Anemia in Young Children. *J Pediatr*. 2017;188:8-10.
80. Domellof M, Cohen RJ, Dewey KG, Hernell O, Rivera LL, Lonnerdal B. Iron supplementation of breast-fed Honduran and Swedish infants from 4 to 9 months of age. *J Pediatr*. 2001;138(5):679-87.

81. Ganz T, Nemeth E. Iron homeostasis in host defence and inflammation. *Nat Rev Immunol*. 2015;15(8):500-10.
82. Kang JO. Chronic iron overload and toxicity: clinical chemistry perspective. *Clin Lab Sci*. 2001;14(3):209-19; quiz 22.
83. Kling PJ, Schmidt RL, Roberts RA, Widness JA. Serum erythropoietin levels during infancy: associations with erythropoiesis. *J Pediatr*. 1996;128(6):791-6.
84. Linderkamp O, Zilow EP, Zilow G. [The critical hemoglobin value in newborn infants, infants and children]. *Beitr Infusionsther*. 1992;30:235-46; discussion 47-64.
85. Cusick SE, Georgieff MK. The Role of Nutrition in Brain Development: The Golden Opportunity of the "First 1000 Days". *The Journal of pediatrics*. 2016;175:16-21.
86. Fretham SJ, Carlson ES, Wobken J, Tran PV, Petryk A, Georgieff MK. Temporal manipulation of transferrin-receptor-1-dependent iron uptake identifies a sensitive period in mouse hippocampal neurodevelopment. *Hippocampus*. 2012;22(8):1691-702.
87. Siddappa AJ, Rao RB, Wobken JD, Leibold EA, Connor JR, Georgieff MK. Developmental changes in the expression of iron regulatory proteins and iron transport proteins in the perinatal rat brain. *J Neurosci Res*. 2002;68(6):761-75.
88. Georgieff MK, Brunette KE, Tran PV. Early life nutrition and neural plasticity. *Dev Psychopathol*. 2015;27(2):411-23.
89. Algarín C, Nelson CA, Peirano P, Westerlund A, Reyes S, Lozoff B. Iron-deficiency anemia in infancy and poorer cognitive inhibitory control at age 10 years. *Dev Med Child Neurol*. 2013;55(5):453-8.
90. Guiang SF, 3rd, Georgieff MK, Lambert DJ, Schmidt RL, Widness JA. Intravenous iron supplementation effect on tissue iron and hemoproteins in chronically phlebotomized lambs. *Am J Physiol*. 1997;273(6):R2124-31.
91. Petry CD, Eaton MA, Wobken JD, Mills MM, Johnson DE, Georgieff MK. Iron deficiency of liver, heart, and brain in newborn infants of diabetic mothers. *J Pediatr*. 1992;121(1):109-14.
92. Zhang DL, Senecal T, Ghosh MC, Ollivierre-Wilson H, Tu T, Rouault TA. Hcpidin regulates ferroportin expression and intracellular iron homeostasis of erythroblasts. *Blood*. 2011;118(10):2868-77.
93. Zhang D-L, Ghosh MC, Ollivierre H, Li Y, Rouault TA. Ferroportin deficiency in erythroid cells causes serum iron deficiency and promotes hemolysis due to oxidative stress. *Blood*. 2018;132(19):2078-87.
94. Georgieff MK, Schmidt RL, Mills MM, Radmer WJ, Widness JA. Fetal iron and cytochrome c status after intrauterine hypoxemia and erythropoietin administration. *Am J Physiol*. 1992;262(3 Pt 2):R485-91.
95. Dallman PR, Siimes MA, Manies EC. Brain iron: persistent deficiency following short-term iron deprivation in the young rat. *Br J Haematol*. 1975;31(2):209-15.
96. Rao R, Tkac I, Townsend EL, Ennis K, Gruetter R, Georgieff MK. Perinatal iron deficiency predisposes the developing rat hippocampus to greater injury from mild to moderate hypoxia-ischemia. *J Cereb Blood Flow Metab*. 2007;27(4):729-40.
97. Lozoff B, Georgieff MK. Iron Deficiency and Brain Development. *Seminars in Pediatric Neurology*. 2006;13(3):158-65.
98. Pasricha SR, Hayes E, Kalumba K, Biggs BA. Effect of daily iron supplementation on health in children aged 4-23 months: a systematic review and meta-analysis of randomised controlled trials. *Lancet Glob Health*. 2013;1(2):e77-e86.
99. Bryan J, Osendarp S, Hughes D, Calvaresi E, Baghurst K, van Klinken JW. Nutrients for cognitive development in school-aged children. *Nutr Rev*. 2004;62(8):295-306.
100. Stoltzfus RJ, Kvalsvig JD, Chwaya HM, Montresor A, Albonico M, Tielsch JM, et al. Effects of iron supplementation and anthelmintic treatment on motor and language development of preschool children in Zanzibar: double blind, placebo controlled study. *Bmj*. 2001;323(7326):1389-93.

101. McCann S, Perapoch Amadó M, Moore SE. The Role of Iron in Brain Development: A Systematic Review. *Nutrients*. 2020;12(7).
102. Yang Z, Lönnerdal B, Adu-Afarwuah S, Brown KH, Chaparro CM, Cohen RJ, et al. Prevalence and predictors of iron deficiency in fully breastfed infants at 6 mo of age: comparison of data from 6 studies. *Am J Clin Nutr*. 2009;89(5):1433-40.
103. Michaelsen KF, Milman N, Samuelson G. A longitudinal study of iron status in healthy Danish infants: effects of early iron status, growth velocity and dietary factors. *Acta Paediatr*. 1995;84(9):1035-44.
104. Thorsdottir I, Gunnarsson BS, Atladottir H, Michaelsen KF, Palsson G. Iron status at 12 months of age — effects of body size, growth and diet in a population with high birth weight. *European Journal of Clinical Nutrition*. 2003;57(4):505-13.
105. Stelle I, Venkatesan S, Edmond K, Moore SE. Acknowledging the gap: a systematic review of micronutrient supplementation in infants under six months of age. *Wellcome Open Res*. 2020;5:238.
106. Angeles IT, Schultink WJ, Matulessi P, Gross R, Sastroamidjojo S. Decreased rate of stunting among anemic Indonesian preschool children through iron supplementation. *Am J Clin Nutr*. 1993;58(3):339-42.
107. Majumdar I, Paul P, Talib VH, Ranga S. The effect of iron therapy on the growth of iron-replete and iron-deplete children. *J Trop Pediatr*. 2003;49(2):84-8.
108. Samuel A, Brouwer ID, Feskens EJM, Adish A, Kebede A, De-Regil LM, et al. Effectiveness of a Program Intervention with Reduced-Iron Multiple Micronutrient Powders on Iron Status, Morbidity and Growth in Young Children in Ethiopia. *Nutrients*. 2018;10(10).
109. Iannotti LL, Tielsch JM, Black MM, Black RE. Iron supplementation in early childhood: health benefits and risks. *Am J Clin Nutr*. 2006;84(6):1261-76.
110. Dewey KG, Domellöf M, Cohen RJ, Landa Rivera L, Hernell O, Lönnerdal B. Iron Supplementation Affects Growth and Morbidity of Breast-Fed Infants: Results of a Randomized Trial in Sweden and Honduras. *The Journal of Nutrition*. 2002;132(11):3249-55.
111. Idjradinata P, Watkins WE, Pollitt E. Adverse effect of iron supplementation on weight gain of iron-replete young children. *Lancet*. 1994;343(8908):1252-4.
112. Sachdev H, Gera T, Nestel P. Effect of iron supplementation on physical growth in children: systematic review of randomised controlled trials. *Public Health Nutr*. 2006;9(7):904-20.
113. Ramakrishnan U, Nguyen P, Martorell R. Effects of micronutrients on growth of children under 5 y of age: meta-analyses of single and multiple nutrient interventions. *Am J Clin Nutr*. 2009;89(1):191-203.
114. Lönnerdal B. Development of iron homeostasis in infants and young children. *Am J Clin Nutr*. 2017;106(Suppl 6):1575S-80S.
115. Jaeggi T, Kortman GA, Moretti D, Chassard C, Holding P, Dostal A, et al. Iron fortification adversely affects the gut microbiome, increases pathogen abundance and induces intestinal inflammation in Kenyan infants. *Gut*. 2015;64(5):731-42.
116. Brittenham GM, Andersson M, Egli I, Foman JT, Zeder C, Westerman ME, et al. Circulating non-transferrin-bound iron after oral administration of supplemental and fortification doses of iron to healthy women: a randomized study. *Am J Clin Nutr*. 2014;100(3):813-20.
117. Paganini D, Uyoga MA, Kortman GAM, Cercamondi CI, Moretti D, Barth-Jaeggi T, et al. Prebiotic galacto-oligosaccharides mitigate the adverse effects of iron fortification on the gut microbiome: a randomised controlled study in Kenyan infants. *Gut*. 2017;66(11):1956-67.
118. Arrieta M-C, Stiemsma LT, Amenyogbe N, Brown EM, Finlay B. The Intestinal Microbiome in Early Life: Health and Disease. *Frontiers in Immunology*. 2014;5.
119. Zimmermann MB, Chassard C, Rohner F, N'Goran E K, Nindjin C, Dostal A, et al. The effects of iron fortification on the gut microbiota in African children: a randomized controlled trial in Cote d'Ivoire. *Am J Clin Nutr*. 2010;92(6):1406-15.

120. Paganini D, Zimmermann MB. The effects of iron fortification and supplementation on the gut microbiome and diarrhea in infants and children: a review. *Am J Clin Nutr*. 2017;106(Suppl 6):1688S-93S.
121. Cross JH, Bradbury RS, Fulford AJ, Jallow AT, Wegmuller R, Prentice AM, et al. Oral iron acutely elevates bacterial growth in human serum. *Sci Rep*. 2015;5:16670.
122. Mullen A, Gosset L, Larke N, Manno D, Chisenga M, Kasonka L, et al. The effects of micronutrient-fortified complementary/replacement food on intestinal permeability and systemic markers of inflammation among maternally HIV-exposed and unexposed Zambian infants. *Br J Nutr*. 2012;107(6):893-902.
123. Tibble J, Sigthorsson G, Foster R, Sherwood R, Fagerhol M, Bjarnason I. Faecal calprotectin and faecal occult blood tests in the diagnosis of colorectal carcinoma and adenoma. *Gut*. 2001;49(3):402-8.
124. Prentice AM. Iron metabolism, malaria, and other infections: what is all the fuss about? *J Nutr*. 2008;138(12):2537-41.
125. Zlotkin S, Newton S, Aimone AM, Azindow I, Amenga-Etego S, Tchum K, et al. Effect of iron fortification on malaria incidence in infants and young children in Ghana: a randomized trial. *JAMA*. 2013;310(9):938-47.
126. Jaeggi T, Moretti D, Kvalsvig J, Holding PA, Tjalsma H, Kortman GA, et al. Iron status and systemic inflammation, but not gut inflammation, strongly predict gender-specific concentrations of serum hepcidin in infants in rural Kenya. *PLoS One*. 2013;8(2):e57513.
127. Mayo-Wilson E, Imdad A, Junior J, Dean S, Bhutta ZA. Preventive zinc supplementation for children, and the effect of additional iron: a systematic review and meta-analysis. *BMJ Open*. 2014;4(6):e004647.
128. Simonyté Sjödin K, Domellöf M, Lagerqvist C, Hernell O, Lönnerdal B, Szymlek-Gay EA, et al. Administration of ferrous sulfate drops has significant effects on the gut microbiota of iron-sufficient infants: a randomised controlled study. *Gut*. 2019;68(11):2095-7.
129. De-Regil LM, Jefferds ME, Sylvetsky AC, Dowswell T. Intermittent iron supplementation for improving nutrition and development in children under 12 years of age. *Cochrane Database Syst Rev*. 2011(12):CD009085.
130. Dostal A, Baumgartner J, Riesen N, Chassard C, Smuts CM, Zimmermann MB, et al. Effects of iron supplementation on dominant bacterial groups in the gut, faecal SCFA and gut inflammation: a randomised, placebo-controlled intervention trial in South African children. *Br J Nutr*. 2014;112(4):547-56.
131. Pena-Rosas JP, De-Regil LM, Gomez Malave H, Flores-Urrutia MC, Dowswell T. Intermittent oral iron supplementation during pregnancy. *Cochrane Database Syst Rev*. 2015(10):CD009997.
132. Stelle I, Kinshella M-LW, Moore SE. Caregiver perceptions of nutrition interventions in infants and children under 24 months of age: a systematic review. *Public Health Nutrition*. 2023;1-24.
133. Chang S, El Arifeen S, Bari S, Wahed MA, Rahman KM, Rahman MT, et al. Supplementing iron and zinc: double blind, randomized evaluation of separate or combined delivery. *Eur J Clin Nutr*. 2010;64(2):153-60.
134. Richard SA, Zavaleta N, Caulfield LE, Black RE, Witzig RS, Shankar AH. Zinc and iron supplementation and malaria, diarrhea, and respiratory infections in children in the Peruvian Amazon. *Am J Trop Med Hyg*. 2006;75(1):126-32.
135. Sharrow D, Hug L, You D, Alkema L, Black R, Cousens S, et al. Global, regional, and national trends in under-5 mortality between 1990 and 2019 with scenario-based projections until 2030: a systematic analysis by the UN Inter-agency Group for Child Mortality Estimation. *The Lancet Global Health*. 2022;10(2):e195-e206.
136. Dowling DJ, Levy O. Ontogeny of early life immunity. *Trends Immunol*. 2014;35(7):299-310.

137. Kollmann TR, Kampmann B, Mazmanian SK, Marchant A, Levy O. Protecting the Newborn and Young Infant from Infectious Diseases: Lessons from Immune Ontogeny. *Immunity*. 2017;46(3):350-63.
138. Wang H. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet*. 2016;388(10053):1459-544.
139. Grassly NC, Kang G, Kampmann B. Biological challenges to effective vaccines in the developing world. *Philosophical transactions of the Royal Society of London Series B, Biological sciences*. 2015;370(1671):20140138.
140. Kollmann T. Variation between Populations in the Innate Immune Response to Vaccine Adjuvants. *Frontiers in Immunology*. 2013;4.
141. Olin A, Henckel E, Chen Y, Lakshmikanth T, Pou C, Mikes J, et al. Stereotypic Immune System Development in Newborn Children. *Cell*. 2018;174(5):1277-92.e14.
142. Brodin P, Jojic V, Gao T, Bhattacharya S, Angel CJ, Furman D, et al. Variation in the human immune system is largely driven by non-heritable influences. *Cell*. 2015;160(1-2):37-47.
143. Carr EJ, Dooley J, Garcia-Perez JE, Lagou V, Lee JC, Wouters C, et al. The cellular composition of the human immune system is shaped by age and cohabitation. *Nat Immunol*. 2016;17(4):461-8.
144. Ned RM, Swat W, Andrews NC. Transferrin receptor 1 is differentially required in lymphocyte development. *Blood*. 2003;102(10):3711-8.
145. Belkaid Y, Harrison OJ. Homeostatic Immunity and the Microbiota. *Immunity*. 2017;46(4):562-76.
146. Ganz T. Iron in innate immunity: starve the invaders. *Curr Opin Immunol*. 2009;21(1):63-7.
147. Núñez G, Sakamoto K, Soares MP. Innate Nutritional Immunity. *The Journal of Immunology*. 2018;201(1):11-8.
148. Cassat JE, Skaar EP. Iron in infection and immunity. *Cell Host Microbe*. 2013;13(5):509-19.
149. Martins AC, Almeida JI, Lima IS, Kapitião AS, Gozzelino R. Iron Metabolism and the Inflammatory Response. *IUBMB Life*. 2017;69(6):442-50.
150. Soares MP, Weiss G. The Iron age of host-microbe interactions. *EMBO Rep*. 2015;16(11):1482-500.
151. Nairz M, Haschka D, Demetz E, Weiss G. Iron at the interface of immunity and infection. *Front Pharmacol*. 2014;5:152.
152. Pearce EL, Poffenberger MC, Chang CH, Jones RG. Fueling immunity: insights into metabolism and lymphocyte function. *Science*. 2013;342(6155):1242-454.
153. Sinclair LV, Rolf J, Emslie E, Shi YB, Taylor PM, Cantrell DA. Control of amino-acid transport by antigen receptors coordinates the metabolic reprogramming essential for T cell differentiation. *Nat Immunol*. 2013;14(5):500-8.
154. Jabara HH, Boyden SE, Chou J, Ramesh N, Massaad MJ, Benson H, et al. A missense mutation in TFRC, encoding transferrin receptor 1, causes combined immunodeficiency. *Nat Genet*. 2016;48(1):74-8.
155. Savy M, Edmond K, Fine PE, Hall A, Hennig BJ, Moore SE, et al. Landscape analysis of interactions between nutrition and vaccine responses in children. *J Nutr*. 2009;139(11):2154s-218s.
156. Seligman PA, Kovar J, Gelfand EW. Lymphocyte proliferation is controlled by both iron availability and regulation of iron uptake pathways. *Pathobiology*. 1992;60(1):19-26.
157. Neckers LM, Cossman J. Transferrin receptor induction in mitogen-stimulated human T lymphocytes is required for DNA synthesis and cell division and is regulated by interleukin 2. *Proc Natl Acad Sci U S A*. 1983;80(11):3494-8.
158. Ahluwalia N, Sun J, Krause D, Mastro A, Handte G. Immune function is impaired in iron-deficient, homebound, older women. *Am J Clin Nutr*. 2004;79(3):516-21.
159. Thibault H, Galan P, Selz F, Preziosi P, Olivier C, Badoual J, et al. The immune response in iron-deficient young children: effect of iron supplementation on cell-mediated immunity. *Eur J Pediatr*. 1993;152(2):120-4.

160. Galan P, Thibault H, Preziosi P, Hercberg S. Interleukin 2 production in iron-deficient children. *Biological Trace Element Research*. 1992;32(1):421-6.
161. Spottiswoode N, Armitage AE, Williams AR, Fyfe AJ, Biswas S, Hodgson SH, et al. Role of Activins in Hepcidin Regulation during Malaria. *Infect Immun*. 2017;85(12):e00191-17.
162. de Mast Q, Nadjm B, Reyburn H, Kemna EH, Amos B, Laarakkers CM, et al. Assessment of urinary concentrations of hepcidin provides novel insight into disturbances in iron homeostasis during malarial infection. *J Infect Dis*. 2009;199(2):253-62.
163. Howard CT, McKakpo US, Quakyi IA, Bosompem KM, Addison EA, Sun K, et al. Relationship of hepcidin with parasitemia and anemia among patients with uncomplicated *Plasmodium falciparum* malaria in Ghana. *Am J Trop Med Hyg*. 2007;77(4):623-6.
164. Armitage AE, Stacey AR, Giannoulitou E, Marshall E, Sturges P, Chatha K, et al. Distinct patterns of hepcidin and iron regulation during HIV-1, HBV, and HCV infections. *Proc Natl Acad Sci U S A*. 2014;111(33):12187-92.
165. Minchella PA, Donkor S, Owolabi O, Sutherland JS, McDermid JM. Complex anemia in tuberculosis: the need to consider causes and timing when designing interventions. *Clin Infect Dis*. 2015;60(5):764-72.
166. Kerkhoff AD, Meintjes G, Burton R, Vogt M, Wood R, Lawn SD. Relationship Between Blood Concentrations of Hepcidin and Anemia Severity, Mycobacterial Burden, and Mortality Among Patients With HIV-Associated Tuberculosis. *J Infect Dis*. 2016;213(1):61-70.
167. Darton TC, Blohmke CJ, Giannoulitou E, Waddington CS, Jones C, Sturges P, et al. Rapidly Escalating Hepcidin and Associated Serum Iron Starvation Are Features of the Acute Response to Typhoid Infection in Humans. *PLoS neglected tropical diseases*. 2015;9(9):e0004029-e.
168. Prentice AM, Bah A, Jallow MW, Jallow AT, Sanyang S, Sise EA, et al. Respiratory infections drive hepcidin-mediated blockade of iron absorption leading to iron deficiency anemia in African children. *Sci Adv*. 2019;5(3):eaav9020.
169. Szabó M, Vászárhelyi B, Balla G, Szabó T, Machay T, Tulassay T. Acute postnatal increase of extracellular antioxidant defence of neonates: the role of iron metabolism. *Acta Paediatrica*. 2001;90(10):1167-70.
170. Cross JH, Jarjou O, Mohammed NI, Rayment Gomez S, Touray BJB, Prentice AM, et al. Early postnatal hypoferremia in low birthweight and preterm babies: A prospective cohort study in hospital-delivered Gambian neonates. *eBioMedicine*. 2020;52.
171. Drakesmith H, Prentice AM. Hepcidin and the iron-infection axis. *Science*. 2012;338(6108):768-72.
172. Glinz D, Kamiyango M, Phiri KS, Munthali F, Zeder C, Zimmermann MB, et al. The effect of timing of iron supplementation on iron absorption and haemoglobin in post-malaria anaemia: a longitudinal stable isotope study in Malawian toddlers. *Malaria Journal*. 2014;13(1):397.
173. Cercamondi CI, Egli IM, Ahouandjinou E, Dossa R, Zeder C, Salami L, et al. Afebrile *Plasmodium falciparum* parasitemia decreases absorption of fortification iron but does not affect systemic iron utilization: a double stable-isotope study in young Beninese women. *The American journal of clinical nutrition*. 2010;92(6):1385-92.
174. McCance RA, Widdowson EM. ABSORPTION AND EXCRETION OF IRON. *The Lancet*. 1937;230(5951):680-4.
175. Hentze MW, Muckenthaler MU, Galy B, Camaschella C. Two to tango: regulation of Mammalian iron metabolism. *Cell*. 2010;142(1):24-38.
176. Stoltzfus RJ, Albonico M, Chwaya HM, Savioli L, Tielsch J, Schulze K, et al. Hemoquant determination of hookworm-related blood loss and its role in iron deficiency in African children. *Am J Trop Med Hyg*. 1996;55(4):399-404.
177. Loukas A, Hotez PJ, Diemert D, Yazdanbakhsh M, McCarthy JS, Correa-Oliveira R, et al. Hookworm infection. *Nat Rev Dis Primers*. 2016;2:16088.

178. Lauer JM, Ghosh S, Ausman LM, Webb P, Bashaasha B, Agaba E, et al. Markers of Environmental Enteric Dysfunction Are Associated with Poor Growth and Iron Status in Rural Ugandan Infants. *The Journal of nutrition*. 2020;150(8):2175-82.
179. Humphrey JH. Child undernutrition, tropical enteropathy, toilets, and handwashing. *Lancet*. 2009;374(9694):1032-5.
180. Kosek M, Haque R, Lima A, Babji S, Shrestha S, Qureshi S, et al. Fecal markers of intestinal inflammation and permeability associated with the subsequent acquisition of linear growth deficits in infants. *The American journal of tropical medicine and hygiene*. 2013;88(2):390-6.
181. Fahim SM, Das S, Sanin KI, Gazi MA, Mahfuz M, Islam MM, et al. Association of Fecal Markers of Environmental Enteric Dysfunction with Zinc and Iron Status among Children at First Two Years of Life in Bangladesh. *Am J Trop Med Hyg*. 2018;99(2):489-94.
182. Muriuki JM, Mentzer AJ, Kimita W, Ndungu FM, Macharia AW, Webb EL, et al. Iron Status and Associated Malaria Risk Among African Children. *Clin Infect Dis*. 2019;68(11):1807-14.
183. Gwamaka M, Kurtis JD, Sorensen BE, Holte S, Morrison R, Mutabingwa TK, et al. Iron deficiency protects against severe *Plasmodium falciparum* malaria and death in young children. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2012;54(8):1137-44.
184. Jonker FAM, Calis JCJ, van Hensbroek MB, Phiri K, Geskus RB, Brabin BJ, et al. Iron status predicts malaria risk in Malawian preschool children. *PloS one*. 2012;7(8):e42670-e.
185. Nyakeriga AM, Troye-Blomberg M, Dorfman JR, Alexander ND, Bäck R, Kortok M, et al. Iron deficiency and malaria among children living on the coast of Kenya. *J Infect Dis*. 2004;190(3):439-47.
186. Sazawal S, Black RE, Ramsan M, Chwaya HM, Stoltzfus RJ, Dutta A, et al. Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial. *Lancet*. 2006;367(9505):133-43.
187. Esan MO, van Hensbroek MB, Nkhoma E, Musicha C, White SA, Ter Kuile FO, et al. Iron supplementation in HIV-infected Malawian children with anemia: a double-blind, randomized, controlled trial. *Clin Infect Dis*. 2013;57(11):1626-34.
188. Veenemans J, Milligan P, Prentice AM, Schouten LR, Inja N, van der Heijden AC, et al. Effect of supplementation with zinc and other micronutrients on malaria in Tanzanian children: a randomised trial. *PLoS Med*. 2011;8(11):e1001125.
189. Krebs NF, Domellöf M, Ziegler EE. Balancing Benefits and Risks of Iron Fortification in Resource-Rich Countries. *The Journal of pediatrics*. 2015;167 4 Suppl:S20-5.
190. Bressler JP, Olivi L, Cheong JH, Kim Y, Bannona D. Divalent metal transporter 1 in lead and cadmium transport. *Ann N Y Acad Sci*. 2004;1012:142-52.
191. Collins JF, Prohaska JR, Knutson MD. Metabolic crossroads of iron and copper. *Nutrition reviews*. 2010;68(3):133-47.
192. Sharp P. The molecular basis of copper and iron interactions. *Proc Nutr Soc*. 2004;63(4):563-9.
193. Gutowska K, Formanowicz D, Formanowicz P. Interrelations between Iron and Vitamin A- Studied Using Systems Approach. *International journal of molecular sciences*. 2022;23(3):1189.
194. NIH Consensus conference. Optimal calcium intake. NIH Consensus Development Panel on Optimal Calcium Intake. *Jama*. 1994;272(24):1942-8.
195. Hallberg L, Hultén L, Gramatkovski E. Iron absorption from the whole diet in men: how effective is the regulation of iron absorption? *Am J Clin Nutr*. 1997;66(2):347-56.
196. Sotelo A, González-Osnaya L, Sánchez-Chinchillas A, Trejo A. Role of oxate, phytate, tannins and cooking on iron bioavailability from foods commonly consumed in Mexico. *Int J Food Sci Nutr*. 2010;61(1):29-39.
197. Schlemmer U, Frølich W, Prieto RM, Grases F. Phytate in foods and significance for humans: food sources, intake, processing, bioavailability, protective role and analysis. *Mol Nutr Food Res*. 2009;53 Suppl 2:S330-75.

198. Mao X, Yao G. Effect of vitamin C supplementations on iron deficiency anemia in Chinese children. *Biomed Environ Sci.* 1992;5(2):125-9.
199. Sharma DC, Mathur R. Correction of anemia and iron deficiency in vegetarians by administration of ascorbic acid. *Indian J Physiol Pharmacol.* 1995;39(4):403-6.
200. Low MS, Speedy J, Styles CE, De-Regil LM, Pasricha SR. Daily iron supplementation for improving anaemia, iron status and health in menstruating women. *Cochrane Database Syst Rev.* 2016;4:CD009747.
201. Siimes AS, Siimes MA. Changes in the concentration of ferritin in the serum during fetal life in singletons and twins. *Early Hum Dev.* 1986;13(1):47-52.
202. Harthoorn-Lasthuizen EJ, Lindemans J, Langenhuijsen MM. Does iron-deficient erythropoiesis in pregnancy influence fetal iron supply? *Acta Obstet Gynecol Scand.* 2001;80(5):392-6.
203. Singla PN, Tyagi M, Shankar R, Dash D, Kumar A. Fetal iron status in maternal anemia. *Acta Paediatr.* 1996;85(11):1327-30.
204. Rasmussen K. Is There a Causal Relationship between Iron Deficiency or Iron-Deficiency Anemia and Weight at Birth, Length of Gestation and Perinatal Mortality? *J Nutr.* 2001;131(2s-2):590S-601S; discussion S-3S.
205. Ghosh S, Sinha S, Thomas T, Sachdev HS, Kurpad AV. Revisiting Dietary Iron Requirement and Deficiency in Indian Women: Implications for Food Iron Fortification and Supplementation. *J Nutr.* 2019;149(3):366-71.
206. Institute of Medicine (US) Panel on Micronutrients. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Washington (DC): National Academies Press (US); 2001.
207. Iron: vitamins and minerals: NHS; 2020 [Available from: <https://www.nhs.uk/conditions/vitamins-and-minerals/iron/#:~:text=The%20amount%20of%20iron%20you,day%20for%20women%20over%2050>].
208. NIH. Iron: Fact Sheet for Health Professionals. National Institutes of Health (NIH) Office of Dietary Supplements (ODS); 2021.
209. Aggett PJ, Agostoni C, Axelsson I, Bresson JL, Goulet O, Hernell O, et al. Iron metabolism and requirements in early childhood: do we know enough?: a commentary by the ESPGHAN Committee on Nutrition. *J Pediatr Gastroenterol Nutr.* 2002;34(4):337-45.
210. Baker RD, Greer FR. Diagnosis and prevention of iron deficiency and iron-deficiency anemia in infants and young children (0-3 years of age). *Pediatrics.* 2010;126(5):1040-50.
211. Dewey KG, Chaparro CM. Session 4: Mineral metabolism and body composition iron status of breast-fed infants. *Proc Nutr Soc.* 2007;66(3):412-22.
212. Friel JK. There Is No Iron in Human Milk. *J Pediatr Gastroenterol Nutr.* 2017;64(3):339-40.
213. Griffin I, Cooke RJ. Iron retention in preterm infants fed low iron intakes: a metabolic balance study. *Early Hum Dev.* 2010;86 Suppl 1:49-53.
214. Agostoni C, Buonocore G, Carnielli VP, De Curtis M, Darmaun D, Decsi T, et al. Enteral nutrient supply for preterm infants: commentary from the European Society of Paediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition. *J Pediatr Gastroenterol Nutr.* 2010;50(1):85-91.
215. Doyle JJ ZA. Neonatal blood disorders. New York: Oxford University Press; 1992.
216. WHO. Guideline: Daily iron supplementation in adult women and adolescent girls. . Geneva, Switzerland World Health Organization 2016.
217. WHO. Optimal feeding of low-birth-weight infants : technical review. / Karen Edmond, Rajiv Bahl. Geneva: World Health Organization; 2006.
218. WHO. WHO Guideline: use of multiple micronutrient powders for point-of-use fortification of foods consumed by infants and young children aged 6–23 months and children aged 2–12 years :

World Health Organisation 2016 [Available from:

<https://www.who.int/publications/i/item/9789241549943>.

219. Marriott LD, Foote KD. Advances in the nutrition of preterm infants. *Journal of the Royal Society for the Promotion of Health*. 2003;123(3):159-64.
220. Infant Feeding Recommendation. In: Health Do, editor. Online2003.
221. Recommendations to Prevent and Control Iron Deficiency in the United States. Centers for Diseases Control and Prevention; 1998.
222. NATIONAL MATERNAL&NEONATAL CARE GUIDELINES AND SERVICE DELIVERY STANDARDS. In: UNIT RCHP, editor. Banjul, The Gambia: MINISTRY OF HEALTH & SOCIAL WELFARE; 2017.
223. Guideliens for Control of Iron Deficiency Anaemia. In: Division A, editor.: Ministry of Health and Family Welfare Government of India; 2013.
224. WHO. Multiple micronutrient supplementation during pregnancy: World Health Organisation; 2020 [Available from: [https://www.who.int/elena/titles/micronutrients_pregnancy/en/#:~:text=The%20most%20current%20evidence%20shows,SGA\)%20is%20difficult%20to%20interpret](https://www.who.int/elena/titles/micronutrients_pregnancy/en/#:~:text=The%20most%20current%20evidence%20shows,SGA)%20is%20difficult%20to%20interpret).
225. Vitamins, supplements and nutrition in pregnancy: Iron in pregnancy United Kingdom: National Health Service (NHS); [Available from: <https://www.nhs.uk/pregnancy/keeping-well/vitamins-supplements-and-nutrition/>].
226. Mwangi MN, Roth JM, Smit MR, Trijsburg L, Mwangi AM, Demir AY, et al. Effect of Daily Antenatal Iron Supplementation on Plasmodium Infection in Kenyan Women: A Randomized Clinical Trial. *JAMA*. 2015;314(10):1009-20.
227. Zeng L, Dibley MJ, Cheng Y, Dang S, Chang S, Kong L, et al. Impact of micronutrient supplementation during pregnancy on birth weight, duration of gestation, and perinatal mortality in rural western China: double blind cluster randomised controlled trial. *Bmj*. 2008;337:a2001.
228. Andersson O, Hellstrom-Westas L, Andersson D, Domellof M. Effect of delayed versus early umbilical cord clamping on neonatal outcomes and iron status at 4 months: a randomised controlled trial. *BMJ*. 2011;343:d7157.
229. McDonald SJ, Middleton P, Dowswell T, Morris PS. Effect of timing of umbilical cord clamping of term infants on maternal and neonatal outcomes. *Cochrane Database Syst Rev*. 2013;2013(7):Cd004074.
230. Baker RD, Greer FR, Committee on Nutrition American Academy of P. Diagnosis and prevention of iron deficiency and iron-deficiency anemia in infants and young children (0-3 years of age). *Pediatrics*. 2010;126(5):1040-50.
231. Bradley CK, Hillman L, Sherman AR, Leedy D, Cordano A. Evaluation of two iron-fortified, milk-based formulas during infancy. *Pediatrics*. 1993;91(5):908-14.
232. Tuthill DP, Cosgrove M, Dunstan F, Stuart ML, Wells JC, Davies DP. Randomized double-blind controlled trial on the effects on iron status in the first year between a no added iron and standard infant formula received for three months. *Acta Paediatr*. 2002;91(2):119-24.
233. Lönnnerdal B, Hernell O. Iron, zinc, copper and selenium status of breast-fed infants and infants fed trace element fortified milk-based infant formula. *Acta Paediatr*. 1994;83(4):367-73.
234. Hernell O, Lönnnerdal B. Iron status of infants fed low-iron formula: no effect of added bovine lactoferrin or nucleotides. *Am J Clin Nutr*. 2002;76(4):858-64.
235. Moffatt ME, Longstaffe S, Besant J, Dureski C. Prevention of iron deficiency and psychomotor decline in high-risk infants through use of iron-fortified infant formula: a randomized clinical trial. *J Pediatr*. 1994;125(4):527-34.
236. Thompson J, Biggs BA, Pasricha SR. Effects of daily iron supplementation in 2- to 5-year-old children: systematic review and meta-analysis. *Pediatrics*. 2013;131(4):739-53.
237. Zlotkin S, Arthur P, Schauer C, Antwi KY, Yeung G, Piekarz A. Home-fortification with iron and zinc sprinkles or iron sprinkles alone successfully treats anemia in infants and young children. *J Nutr*. 2003;133(4):1075-80.

238. Zlotkin SH, Schauer C, Christofides A, Sharieff W, Tondeur MC, Hyder SMZ. Micronutrient Sprinkles to Control Childhood Anaemia. *PLOS Medicine*. 2005;2(1):e1.
239. De-Regil LM, Suchdev PS, Vist GE, Walleser S, Peña-Rosas JP. Home fortification of foods with multiple micronutrient powders for health and nutrition in children under two years of age. *Cochrane Database Syst Rev*. 2011(9):Cd008959.
240. Stoffel NU, Zeder C, Brittenham GM, Moretti D, Zimmermann MB. Iron absorption from supplements is greater with alternate day than with consecutive day dosing in iron-deficient anemic women. *Haematologica*. 2019.
241. Camaschella C. Iron-deficiency anemia. *N Engl J Med*. 2015;372(19):1832-43.
242. Fernandez-Gaxiola AC, De-Regil LM. Intermittent iron supplementation for reducing anaemia and its associated impairments in adolescent and adult menstruating women. *Cochrane Database Syst Rev*. 2019;1:CD009218.
243. Hackl LS, Abizari AR, Speich C, Zungbey-Garti H, Cercamondi CI, Zeder C, et al. Micronutrient-fortified rice can be a significant source of dietary bioavailable iron in schoolchildren from rural Ghana. *Sci Adv*. 2019;5(3):eaau0790.
244. Troesch B, Egli I, Zeder C, Hurrell RF, de Pee S, Zimmermann MB. Optimization of a phytase-containing micronutrient powder with low amounts of highly bioavailable iron for in-home fortification of complementary foods. *The American Journal of Clinical Nutrition*. 2009;89(2):539-44.
245. WHO. Guidelines on food fortification with micronutrients World Health Organisation; 2006 [Available from: <https://www.who.int/publications/i/item/9241594012>].
246. Diego Quintaes K, Barberá R, Cilla A. Iron bioavailability in iron-fortified cereal foods: The contribution of in vitro studies. *Critical Reviews in Food Science and Nutrition*. 2017;57(10):2028-41.
247. Lee K, Clydesdale FM, Tannenbaum SR. Iron sources used in food fortification and their changes due to food processing. *C R C Critical Reviews in Food Science and Nutrition*. 1979;11(2):117-53.
248. Blanco-Rojo R, Pérez-Granados AM, Toxqui L, González-Vizcayno C, Delgado MA, Vaquero MP. Efficacy of a microencapsulated iron pyrophosphate-fortified fruit juice: a randomised, double-blind, placebo-controlled study in Spanish iron-deficient women. *Br J Nutr*. 2011;105(11):1652-9.
249. Toxqui L, Pérez-Granados AM, Blanco-Rojo R, Wright I, González-Vizcayno C, Vaquero MP. Effects of an iron or iron and vitamin D-fortified flavored skim milk on iron metabolism: a randomized controlled double-blind trial in iron-deficient women. *J Am Coll Nutr*. 2013;32(5):312-20.
250. Navas-Carretero S, Pérez-Granados AM, Sarriá B, Vaquero MP. Iron absorption from meat pate fortified with ferric pyrophosphate in iron-deficient women. *Nutrition*. 2009;25(1):20-4.
251. Connorton JM, Balk J. Iron Biofortification of Staple Crops: Lessons and Challenges in Plant Genetics. *Plant Cell Physiol*. 2019;60(7):1447-56.
252. de Valença AW, Bake A, Brouwer ID, Giller KE. Agronomic biofortification of crops to fight hidden hunger in sub-Saharan Africa. *Global Food Security*. 2017;12:8-14.
253. Finkelstein JL, Haas JD, Mehta S. Iron-biofortified staple food crops for improving iron status: a review of the current evidence. *Curr Opin Biotechnol*. 2017;44:138-45.
254. CADTH Rapid Response Reports. Oral Iron for Anemia: A Review of the Clinical Effectiveness, Cost-effectiveness and Guidelines. Ottawa (ON): Canadian Agency for Drugs and Technologies in Health Copyright © 2016 Canadian Agency for Drugs and Technologies in Health.; 2016.
255. Sangkhav V, Nemeth E. Regulation of the Iron Homeostatic Hormone Heparin. *Adv Nutr*. 2017;8(1):126-36.
256. Gordeuk VR, Brittenham GM, Hughes M, Keating LJ, Oppl J. High-dose carbonyl iron for iron deficiency anemia: a randomized double-blind trial. *Am J Clin Nutr*. 1987;46(6):1029-34.
257. Stoffel NU, von Siebenthal HK, Moretti D, Zimmermann MB. Oral iron supplementation in iron-deficient women: How much and how often? *Molecular Aspects of Medicine*. 2020;75:100865.
258. Cancelo-Hidalgo MJ, Castelo-Branco C, Palacios S, Haya-Palazuelos J, Ciria-Recasens M, Manasanch J, et al. Tolerability of different oral iron supplements: a systematic review. *Curr Med Res Opin*. 2013;29(4):291-303.

259. Paganini D, Uyoga MA, Cercamondi CI, Moretti D, Mwasi E, Schwab C, et al. Consumption of galacto-oligosaccharides increases iron absorption from a micronutrient powder containing ferrous fumarate and sodium iron EDTA: a stable-isotope study in Kenyan infants. *Am J Clin Nutr*. 2017;106(4):1020-31.
260. Hilty FM, Arnold M, Hilbe M, Teleki A, Knijnenburg JT, Ehrensperger F, et al. Iron from nanocompounds containing iron and zinc is highly bioavailable in rats without tissue accumulation. *Nat Nanotechnol*. 2010;5(5):374-80.
261. von Moos LM, Schneider M, Hilty FM, Hilbe M, Arnold M, Ziegler N, et al. Iron phosphate nanoparticles for food fortification: Biological effects in rats and human cell lines. *Nanotoxicology*. 2017;11(4):496-506.
262. Powell JJ, Bruggraber SF, Faria N, Poots LK, Hondow N, Pennycook TJ, et al. A nano-disperse ferritin-core mimetic that efficiently corrects anemia without luminal iron redox activity. *Nanomedicine*. 2014;10(7):1529-38.
263. Pisani A, Riccio E, Sabbatini M, Andreucci M, Del Rio A, Visciano B. Effect of oral liposomal iron versus intravenous iron for treatment of iron deficiency anaemia in CKD patients: a randomized trial. *Nephrol Dial Transplant*. 2015;30(4):645-52.
264. Pereira DIA, Mohammed NI, Ofordile O, Camara F, Baldeh B, Mendy T, et al. A novel nano-iron supplement to safely combat iron deficiency and anaemia in young children: The IHAT-GUT double-blind, randomised, placebo-controlled trial protocol. *Gates Open Res*. 2018;2:48.
265. Mohammed NI, Wason J, Mendy T, Nass SA, Ofordile O, Camara F, et al. A novel nano-iron supplement versus standard treatment for iron deficiency anaemia in children 6 months (IHAT-GUT trial): a double-blind, randomised, placebo-controlled non-inferiority phase II trial in The Gambia. *eClinicalMedicine*. 2023;56.
266. Shah AA, Donovan K, Seeley C, Dickson EA, Palmer AJR, Doree C, et al. Risk of Infection Associated With Administration of Intravenous Iron: A Systematic Review and Meta-analysis. *JAMA Network Open*. 2021;4(11):e2133935-e.
267. Pasricha SR, Gilbertson M, Indran T, Bennett A, van Dam M, Coughlin E, et al. Safety of rapid injection of undiluted ferric carboxymaltose to patients with iron-deficiency anaemia: a Phase II single-arm study. *Intern Med J*. 2021;51(8):1304-11.
268. Lewkowicz AK, Gupta A, Simon L, Sabol BA, Stoll C, Cooke E, et al. Intravenous compared with oral iron for the treatment of iron-deficiency anemia in pregnancy: a systematic review and meta-analysis. *J Perinatol*. 2019;39(4):519-32.
269. Bonovas S, Fiorino G, Allocca M, Lytras T, Tsantes A, Peyrin-Biroulet L, et al. Intravenous Versus Oral Iron for the Treatment of Anemia in Inflammatory Bowel Disease: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Medicine (Baltimore)*. 2016;95(2):e2308.
270. Radhika AG, Sharma AK, Perumal V, Sinha A, Sriganesh V, Kulshreshtha V, et al. Parenteral Versus Oral Iron for Treatment of Iron Deficiency Anaemia During Pregnancy and post-partum: A Systematic Review. *Journal of obstetrics and gynaecology of India*. 2019;69(1):13-24.
271. Pasricha SR, Flecknoe-Brown SC, Allen KJ, Gibson PR, McMahon LP, Olynyk JK, et al. Diagnosis and management of iron deficiency anaemia: a clinical update. *Med J Aust*. 2010;193(9):525-32.
272. Pasricha S-R, Mwangi MN, Moya E, Ataide R, Mzembe G, Harding R, et al. Ferric carboxymaltose versus standard-of-care oral iron to treat second-trimester anaemia in Malawian pregnant women: a randomised controlled trial. *The Lancet*. 2023;401(10388):1595-609.
273. Powers JM, Shamoun M, McCavit TL, Adix L, Buchanan GR. Intravenous Ferric Carboxymaltose in Children with Iron Deficiency Anemia Who Respond Poorly to Oral Iron. *The Journal of pediatrics*. 2017;180:212-6.
274. Cerami C. Iron Nutrition of the Fetus, Neonate, Infant, and Child. *Annals of nutrition & metabolism*. 2017;71 Suppl 3(Suppl 3):8-14.
275. Badruddin SH. Nutrition at a Glance. *Maternal & Child Nutrition*. 2008;4(4):311-2.

276. Hurrell R, Egli I. Iron bioavailability and dietary reference values. *Am J Clin Nutr*. 2010;91(5):1461s-7s.
277. Mills RJ, Davies MW. Enteral iron supplementation in preterm and low birth weight infants. *Cochrane Database Syst Rev*. 2012(3):CD005095.
278. Covidence systematic review software [Internet]. Veritas Health Innovation. Available from: www.covidence.org.
279. The World Bank 2019 [cited 2020 March 30]. Available from: <https://www.worldbank.org/en/country/gambia/overview>.
280. Soma [Available from: <https://zhujiworld.com/gm/1343829-soma/>].
281. Population and Housing Census; Economic Characteristics The Republic of The Gambia: Gambia Bureau of Statistics; 2013.
282. Population and Housing Census Report. The Republic of The Gambia: The Gambia Bureau of Statistics; 2013.
283. Clark A, Forde, E.R.A., and Gailey, H.A. The Gambia 2021 [Available from: <https://www.britannica.com/place/The-Gambia>].
284. Population and Housing Census Report, Education Characteristics The Republic of The Gambia: The Gambia Bureau of Statistics 2013.
285. World Climate Guide - The Gambia: Climates to Travel; [06 January 2022]. Available from: <https://www.climatestotravel.com/climate/gambia>.
286. Hennig BJ, Unger SA, Dondeh BL, Hassan J, Hawkesworth S, Jarjou L, et al. Cohort Profile: The Kiang West Longitudinal Population Study (KWLPs)-a platform for integrated research and health care provision in rural Gambia. *Int J Epidemiol*. 2017;46(2):e13.
287. Agriculture and Farming in Gambia The Gambia: Access Gambia; 2022 [Available from: <https://www.accessgambia.com/information/farming-agriculture.html>].
288. Ceesay SM, Prentice AM, Cole TJ, Foord F, Weaver LT, Poskitt EM, et al. Effects on birth weight and perinatal mortality of maternal dietary supplements in rural Gambia: 5 year randomised controlled trial. *Bmj*. 1997;315(7111):786-90.
289. Prentice AM, Whitehead RG, Roberts SB, Paul AA. Long-term energy balance in child-bearing Gambian women. *Am J Clin Nutr*. 1981;34(12):2790-9.
290. Johnson W, Elmrayed SA, Sosseh F, Prentice AM, Moore SE. Preconceptional and gestational weight trajectories and risk of delivering a small-for-gestational-age baby in rural Gambia. *Am J Clin Nutr*. 2017;105(6):1474-82.
291. Rayco-Solon P, Fulford AJ, Prentice AM. Differential effects of seasonality on preterm birth and intrauterine growth restriction in rural Africans. *Am J Clin Nutr*. 2005;81(1):134-9.
292. Moore SE, Cole TJ, Collinson AC, Poskitt EM, McGregor IA, Prentice AM. Prenatal or early postnatal events predict infectious deaths in young adulthood in rural Africa. *Int J Epidemiol*. 1999;28(6):1088-95.
293. Gambia Bureau of Statistics G, Icf. The Gambia Demographic and Health Survey 2019-20. Banjul, The Gambia: GBoS/ICF; 2021.
294. Gambia: Healthy Newborn Network; 2017 [06 January 2021]. Available from: <https://www.healthynewbornnetwork.org/country/gambia/>.
295. Population and Housing Census, The Children Report. The Republic of The Gambia: Gambia Bureau of Statistics 2013.
296. Ali Z, Scheelbeek PFD, Dalzell S, Hadida G, Segnon AC, M'Boob S, et al. Socio-economic and food system drivers of nutrition and health transitions in The Gambia from 1990 to 2017. *Global Food Security*. 2023;37:100695.
297. The Global Impact of Respiratory Disease Sheffield, European Respiratory Society; 2017.
298. Bryce J, Boschi-Pinto C, Shibuya K, Black RE. WHO estimates of the causes of death in children. *The Lancet*. 2005;365(9465):1147-52.
299. Boschi-Pinto C, Velebit L, Shibuya K. Estimating child mortality due to diarrhoea in developing countries. *Bull World Health Organ*. 2008;86(9):710-7.

300. Levine MM, Nasrin D, Acácio S, Bassat Q, Powell H, Tennant SM, et al. Diarrhoeal disease and subsequent risk of death in infants and children residing in low-income and middle-income countries: analysis of the GEMS case-control study and 12-month GEMS-1A follow-on study. *The Lancet Global Health*. 2020;8(2):e204-e14.
301. Bah M, Stelle I, Verhoef H, Saidykhan A, Moore SE, Susso B, et al. Early iron supplementation of exclusively breastfed African infants: a proof-of-principle, placebo-controlled, randomised, double-blinded efficacy trial. *medRxiv*. 2023:2023.01.12.22284059.
302. Levels and trends in child malnutrition. United Nations Children's Fund, World Health Organization, World Bank Group; 2021.
303. de Onis M, Garza C, Onyango AW, Rolland-Cachera MF. [WHO growth standards for infants and young children]. *Archives de pediatrie : organe officiel de la Societe francaise de pediatrie*. 2009;16(1):47-53.
304. WHO Anthro. World Health Organization.
305. Rowland MG, Barrell RA, Whitehead RG. Bacterial contamination in traditional Gambian weaning foods. *Lancet*. 1978;1(8056):136-8.
306. Bernstein RM, O'Connor GK, Vance EA, Affara N, Drammeh S, Dunger DB, et al. Timing of the Infancy-Childhood Growth Transition in Rural Gambia. *Front Endocrinol (Lausanne)*. 2020;11:142.
307. Stelle I, Bah M, Saidykhan M, Verhoef H, Prentice AM, Cerami C, et al. Maternal acceptability of iron supplementation in young breastfed rural Gambian infants. *Reproductive, Female and Child Health*. 2023;n/a(n/a).
308. Sekhon M, Cartwright M, Francis JJ. Acceptability of healthcare interventions: an overview of reviews and development of a theoretical framework. *BMC Health Serv Res*. 2017;17(1):88.
309. Becker CB, Darius E, Schaumberg K. An analog study of patient preferences for exposure versus alternative treatments for posttraumatic stress disorder. *Behav Res Ther*. 2007;45(12):2861-73.
310. Sidani S, Epstein DR, Bootzin RR, Moritz P, Miranda J. Assessment of preferences for treatment: validation of a measure. *Res Nurs Health*. 2009;32(4):419-31.
311. Tarrier N, Liversidge T, Gregg L. The acceptability and preference for the psychological treatment of PTSD. *Behav Res Ther*. 2006;44(11):1643-56.
312. Dillip A, Alba S, Mshana C, Hetzel MW, Lengeler C, Mayumana I, et al. Acceptability – a neglected dimension of access to health care: findings from a study on childhood convulsions in rural Tanzania. *BMC Health Services Research*. 2012;12(1):113.
313. Staniszevska S, Crowe S, Badenoch D, Edwards C, Savage J, Norman W. The PRIME project: developing a patient evidence-base. *Health Expect*. 2010;13(3):312-22.
314. Diepeveen S, Ling T, Suhrcke M, Roland M, Marteau TM. Public acceptability of government intervention to change health-related behaviours: a systematic review and narrative synthesis. *BMC Public Health*. 2013;13(1):756.
315. Stok FM, de Ridder DTD, de Vet E, Nureeva L, Luszczynska A, Wardle J, et al. Hungry for an intervention? Adolescents' ratings of acceptability of eating-related intervention strategies. *BMC Public Health*. 2016;16(1):5.
316. Fisher P, McCarney R, Hasford C, Vickers A. Evaluation of specific and non-specific effects in homeopathy: feasibility study for a randomised trial. *Homeopathy*. 2006;95(4):215-22.
317. Hommel KA, Hente E, Herzer M, Ingerski LM, Denson LA. Telehealth behavioral treatment for medication nonadherence: a pilot and feasibility study. *Eur J Gastroenterol Hepatol*. 2013;25(4):469-73.
318. Borrelli B, Sepinwall D, Ernst D, Bellg AJ, Czajkowski S, Breger R, et al. A new tool to assess treatment fidelity and evaluation of treatment fidelity across 10 years of health behavior research. *J Consult Clin Psychol*. 2005;73(5):852-60.
319. Proctor E, Silmere H, Raghavan R, Hovmand P, Aarons G, Bunger A, et al. Outcomes for implementation research: conceptual distinctions, measurement challenges, and research agenda. *Adm Policy Ment Health*. 2011;38(2):65-76.

320. Brooks J, McCluskey S, Turley E, King N. The Utility of Template Analysis in Qualitative Psychology Research. *Qualitative Research in Psychology*. 2015;12(2):202-22.
321. Swanson CA. Iron intake and regulation: implications for iron deficiency and iron overload. *Alcohol*. 2003;30(2):99-102.
322. World Health Organization. Guideline: Iron supplementation in postpartum women. Geneva: World Health Organization; 2016 2016.
323. Ziegler EE, Fomon SJ, Nelson SE, Jeter JM, Theuer RC. Dry cereals fortified with electrolytic iron or ferrous fumarate are equally effective in breast-fed infants. *J Nutr*. 2011;141(2):243-8.
324. Friel JK, Aziz K, Andrews WL, Harding SV, Courage ML, Adams RJ. A double-masked, randomized control trial of iron supplementation in early infancy in healthy term breast-fed infants. *The Journal of pediatrics*. 2003;143(5):582-6.
325. Pasricha S-R, Hayes E, Kalumba K, Biggs B-A. Effect of daily iron supplementation on health in children aged 4-23 months: a systematic review and meta-analysis of randomised controlled trials. *The Lancet Global health*. 2013;1(2):e77-e86.
326. Hussain I, Bhoyroo J, Butcher A, Koch TA, He A, Bregman DB. Direct Comparison of the Safety and Efficacy of Ferric Carboxymaltose versus Iron Dextran in Patients with Iron Deficiency Anemia. *Anemia*. 2013;2013:169107.
327. Domellöf M. Iron requirements, absorption and metabolism in infancy and childhood. *Current Opinion in Clinical Nutrition & Metabolic Care*. 2007;10(3).
328. Liyanage G, Anupama K, Sudarshini MLP. Acceptability of home fortification with multiple micro-nutrients among Sri Lankan children. *PLoS One*. 2021;16(12):e0261516.
329. Bhutta ZA, Das JK, Rizvi A, Gaffey MF, Walker N, Horton S, et al. Evidence-based interventions for improvement of maternal and child nutrition: what can be done and at what cost? *Lancet*. 2013;382(9890):452-77.
330. WHO. Accelerated implementation of maternal and newborn continuum of care as part of improving reproductive health: mapping of in-country activities: joint country support. Geneva, Switzerland 2010.
331. Humphrey JH, Mbuya MNN, Ntozini R, Moulton LH, Stoltzfus RJ, Tavengwa NV, et al. Independent and combined effects of improved water, sanitation, and hygiene, and improved complementary feeding, on child stunting and anaemia in rural Zimbabwe: a cluster-randomised trial. *The Lancet Global health*. 2019;7(1):e132-e47.
332. Tofail F, Fernald LC, Das KK, Rahman M, Ahmed T, Jannat KK, et al. Effect of water quality, sanitation, hand washing, and nutritional interventions on child development in rural Bangladesh (WASH Benefits Bangladesh): a cluster-randomised controlled trial. *The Lancet Child and Adolescent Health*. 2018;2(4):255-68.
333. Null C, Stewart CP, Pickering AJ, Dentz HN, Arnold BF, Arnold CD, et al. Effects of water quality, sanitation, handwashing, and nutritional interventions on diarrhoea and child growth in rural Kenya: a cluster-randomised controlled trial. *Lancet Glob Health*. 2018;6(3):e316-e29.
334. Shaw JG, Friedman JF. Iron deficiency anemia: focus on infectious diseases in lesser developed countries. *Anemia*. 2011;2011:260380.
335. Prentice AM, Moore SE, Fulford AJ. Growth faltering in low-income countries. *World Rev Nutr Diet*. 2013;106:90-9.
336. Nabwera HM, Fulford AJ, Moore SE, Prentice AM. Growth faltering in rural Gambian children after four decades of interventions: a retrospective cohort study. *Lancet Glob Health*. 2017;5(2):e208-e16.
337. Crane RJ, Berkley JA. Progress on growth faltering. *Lancet Glob Health*. 2017;5(2):e125-e6.
338. Black AP, Vally H, Morris PS, Daniel M, Esterman AJ, Smith FE, et al. Health outcomes of a subsidised fruit and vegetable program for Aboriginal children in northern New South Wales. *The Medical journal of Australia*. 2013;199(1):46-50.
339. Allen LH. Current Information Gaps in Micronutrient Research, Programs and Policy: How Can We Fill Them? *World review of nutrition and dietetics*. 2016;115:109-17.

340. Pongcharoen T, DiGirolamo AM, Ramakrishnan U, Winichagoon P, Flores R, Martorell R. Long-term effects of iron and zinc supplementation during infancy on cognitive function at 9 y of age in northeast Thai children: a follow-up study. *Am J Clin Nutr*. 2011;93(3):636-43.
341. Sudfeld CR, Manji KP, Darling AM, Kisenge R, Kvestad I, Hysing M, et al. Effect of antenatal and infant micronutrient supplementation on middle childhood and early adolescent development outcomes in Tanzania. *Eur J Clin Nutr*. 2019;73(9):1283-90.
342. Allen LH, Hampel D, Shahab-Ferdows S, Andersson M, Barros E, Doel AM, et al. The Mothers, Infants, and Lactation Quality (MILQ) Study: A Multi-Center Collaboration. *Curr Dev Nutr*. 2021;5(10):nzab116.
343. Sandström B, Davidsson L, Cederblad A, Lönnerdal B. Oral iron, dietary ligands and zinc absorption. *J Nutr*. 1985;115(3):411-4.
344. Haschke F, Vanura H, Male C, Owen G, Pietschnig B, Schuster E, et al. Iron nutrition and growth of breast- and formula-fed infants during the first 9 months of life. *Journal of pediatric gastroenterology and nutrition*. 1993;16(2):151-6.
345. Faber J, Fonseca LM. How sample size influences research outcomes. *Dental Press J Orthod*. 2014;19(4):27-9.
346. Evans SR. Fundamentals of clinical trial design. *J Exp Stroke Transl Med*. 2010;3(1):19-27.
347. Butte NF, Lopez-Alarcon MG, Garza C, Expert Consultation on the Optimal Duration of Exclusive B. Nutrient adequacy of exclusive breastfeeding for the term infant during the first six months of life / Nancy F. Butte, Mardia G. Lopez-Alarcon, Cutberto Garza. Geneva: World Health Organization; 2002.
348. Jáuregui-Lobera I. Iron deficiency and cognitive functions. *Neuropsychiatr Dis Treat*. 2014;10:2087-95.
349. Allen L, Black RE, Brandes N, Brittenham G, Chazot G, Chunming C, et al. [Conclusions and recommendations of a WHO expert consultation meeting on iron supplementation for infants and young children in malaria endemic areas]. *Med Trop (Mars)*. 2008;68(2):182-8.
350. Finkelstein JL, Herman HS, Guetterman HM, Peña-Rosas JP, Mehta S. Daily iron supplementation for prevention or treatment of iron deficiency anaemia in infants, children, and adolescents. *Cochrane Database of Systematic Reviews*. 2018(12).
351. Chmielewska A, Chmielewski G, Domellof M, Lewandowski Z, Szajewska H. Effect of iron supplementation on psychomotor development of non-anaemic, exclusively or predominantly breastfed infants: a randomised, controlled trial. *BMJ Open*. 2015;5(11):e009441.
352. Intermittent malaria treatment and iron supplementation for the control of malaria and anaemia in infants in the Forest Belt of rural Ghana: a double-blind randomised controlled trial who.int/ictpr: World Health Organisation; [January 2021]. Available from: <http://apps.who.int/trialsearch/Trial2.aspx?TrialID=ISRCTN85737357>.
353. Intervention study to reduce the level of anaemia among infants who.int/ictpr: World Health Organisation; [January 2021]. Available from: <http://apps.who.int/trialsearch/Trial2.aspx?TrialID=ISRCTN63098210>.
354. Effectiveness of daily and weekly iron supplementation who.int/ictpr: World Health Organisation; [January 2021]. Available from: <http://apps.who.int/trialsearch/Trial2.aspx?TrialID=TCTR20150824001>.
355. Sutharson R. A clinical trial to evaluate the effects of iron therapy in term newborns who.int/ictpr: World Health Organisation; 2016 [cited 2020 January 2021]. Available from: <http://apps.who.int/trialsearch/Trial2.aspx?TrialID=CTRI/2016/09/007242>.
356. Bhattacharya D. to Assess the Effect of Early vs Routine Iron Supplementation on Iron Store and Growth in Term Infants In: ClinicalTrials.gov [cited 2020 January 21]. Available from: <https://clinicaltrials.gov/ct2/show/NCT03171324>.
357. Iron supplementation in early infancy who.int/ictpr: World Health Organisation; [January 2021]. Available from: <http://apps.who.int/trialsearch/Trial2.aspx?TrialID=CTRI/2019/04/018460>.

358. Aydin A, Gur E, Erener-Ercan T, Can G, Arvas A. Comparison of Different Iron Preparations in the Prophylaxis of Iron-deficiency Anemia. *J Pediatr Hematol Oncol*. 2017;39(7):495-9.
359. Berger J, Ninh NX, Khan NC, Nhien NV, Lien DK, Trung NQ, et al. Efficacy of combined iron and zinc supplementation on micronutrient status and growth in Vietnamese infants. *Eur J Clin Nutr*. 2006;60(4):443-54.
360. Bhandari N, Taneja S, Mazumder S, Bahl R, Fontaine O, Bhan MK, et al. Adding zinc to supplemental iron and folic acid does not affect mortality and severe morbidity in young children. *J Nutr*. 2007;137(1):112-7.
361. Bora R, Ramasamy S, Brown B, Wolfson J, Rao R. Effect of iron supplementation from neonatal period on the iron status of 6-month-old infants at-risk for early iron deficiency: a randomized interventional trial. *J Matern Fetal Neonatal Med*. 2019:1-9.
362. Carter RC, Kupka R, Manji K, McDonald CM, Aboud S, Erhardt JG, et al. Zinc and multivitamin supplementation have contrasting effects on infant iron status: a randomized, double-blind, placebo-controlled clinical trial. *Eur J Clin Nutr*. 2018;72(1):130-5.
363. McDonald CM, Manji KP, Kisenge R, Aboud S, Spiegelman D, Fawzi WW, et al. Daily Zinc but Not Multivitamin Supplementation Reduces Diarrhea and Upper Respiratory Infections in Tanzanian Infants: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial. *J Nutr*. 2015;145(9):2153-60.
364. Desai MR, Mei JV, Kariuki SK, Wannemuehler KA, Phillips-Howard PA, Nahlen BL, et al. Randomized, controlled trial of daily iron supplementation and intermittent sulfadoxine-pyrimethamine for the treatment of mild childhood anemia in western Kenya. *J Infect Dis*. 2003;187(4):658-66.
365. Dijkhuizen MA, Wieringa FT, West CE, Martuti S, Muhilal. Effects of iron and zinc supplementation in Indonesian infants on micronutrient status and growth. *J Nutr*. 2001;131(11):2860-5.
366. Ermis B, Demirel F, Demircan N, Gurel A. Effects of three different iron supplementations in term healthy infants after 5 months of life. *J Trop Pediatr*. 2002;48(5):280-4.
367. Fahmida U, Rumawas JS, Utomo B, Patmonodewo S, Schultink W. Zinc-iron, but not zinc-alone supplementation, increased linear growth of stunted infants with low haemoglobin. *Asia Pac J Clin Nutr*. 2007;16(2):301-9.
368. Friel JK, Aziz K, Andrews WL, Harding SV, Courage ML, Adams RJ. A double-masked, randomized control trial of iron supplementation in early infancy in healthy term breast-fed infants. *J Pediatr*. 2003;143(5):582-6.
369. Liu E, Duggan C, Manji KP, Kupka R, Aboud S, Bosch RJ, et al. Multivitamin supplementation improves haematologic status in children born to HIV-positive women in Tanzania. *J Int AIDS Soc*. 2013;16:18022.
370. Duggan C, Manji KP, Kupka R, Bosch RJ, Aboud S, Kisenge R, et al. Multiple micronutrient supplementation in Tanzanian infants born to HIV-infected mothers: a randomized, double-blind, placebo-controlled clinical trial. *Am J Clin Nutr*. 2012;96(6):1437-46.
371. Lozoff B, Jiang Y, Li X, Zhou M, Richards B, Xu G, et al. Low-Dose Iron Supplementation in Infancy Modestly Increases Infant Iron Status at 9 Mo without Decreasing Growth or Increasing Illness in a Randomized Clinical Trial in Rural China. *J Nutr*. 2016;146(3):612-21.
372. Angulo-Barroso RM, Li M, Santos DC, Bian Y, Sturza J, Jiang Y, et al. Iron Supplementation in Pregnancy or Infancy and Motor Development: A Randomized Controlled Trial. *Pediatrics*. 2016;137(4).
373. Massaga JJ, Kitua AY, Lemnge MM, Akida JA, Malle LN, Ronn AM, et al. Effect of intermittent treatment with amodiaquine on anaemia and malarial fevers in infants in Tanzania: a randomised placebo-controlled trial. *Lancet*. 2003;361(9372):1853-60.
374. Menendez C, Kahigwa E, Hirt R, Vounatsou P, Aponte JJ, Font F, et al. Randomised placebo-controlled trial of iron supplementation and malaria chemoprophylaxis for prevention of severe anaemia and malaria in Tanzanian infants. *Lancet*. 1997;350(9081):844-50.

375. Menendez C, Schellenberg D, Quinto L, Kahigwa E, Alvarez L, Aponte JJ, et al. The effects of short-term iron supplementation on iron status in infants in malaria-endemic areas. *Am J Trop Med Hyg.* 2004;71(4):434-40.
376. Nagpal J, Sachdev HP, Singh T, Mallika V. A randomized placebo-controlled trial of iron supplementation in breastfed young infants initiated on complementary feeding: effect on haematological status. *J Health Popul Nutr.* 2004;22(2):203-11.
377. Rahimy MC, Fanou L, Somasse YE, Gangbo A, Ahouignan G, Alihonou E. When to start supplementary iron to prevent iron deficiency in early childhood in sub-Saharan Africa setting. *Pediatr Blood Cancer.* 2007;48(5):544-9.
378. Schellenberg D, Kahigwa E, Sanz S, Aponte JJ, Mshinda H, Alonso P, et al. A randomized comparison of two anemia treatment regimens in Tanzanian children. *Am J Trop Med Hyg.* 2004;71(4):428-33.
379. Tielsch JM, Khatri SK, Stoltzfus RJ, Katz J, LeClerq SC, Adhikari R, et al. Effect of routine prophylactic supplementation with iron and folic acid on preschool child mortality in southern Nepal: community-based, cluster-randomised, placebo-controlled trial. *Lancet.* 2006;367(9505):144-52.
380. Wasantwisut E, Winichagoon P, Chitchumroonchokchai C, Yamborisut U, Boonpradern A, Pongcharoen T, et al. Iron and zinc supplementation improved iron and zinc status, but not physical growth, of apparently healthy, breast-fed infants in rural communities of northeast Thailand. *J Nutr.* 2006;136(9):2405-11.
381. Wieringa FT, Dijkhuizen MA, West CE, Thurnham DI, Muhilal, Van der Meer JW. Redistribution of vitamin A after iron supplementation in Indonesian infants. *Am J Clin Nutr.* 2003;77(3):651-7.
382. Yurdakok K, Temiz F, Yalcin SS, Gumruk F. Efficacy of daily and weekly iron supplementation on iron status in exclusively breast-fed infants. *J Pediatr Hematol Oncol.* 2004;26(5):284-8.
383. Ziegler EE, Nelson SE, Jeter JM. Iron supplementation of breastfed infants from an early age. *Am J Clin Nutr.* 2009;89(2):525-32.
384. Lauer JM, McDonald CM, Kisenge R, Aboud S, Fawzi WW, Liu E, et al. Markers of Systemic Inflammation and Environmental Enteric Dysfunction Are Not Reduced by Zinc or Multivitamins in Tanzanian Infants: A Randomized, Placebo-Controlled Trial. *J Pediatr.* 2019;210:34-40 e1.
385. Locks LM, Manji KP, McDonald CM, Kupka R, Kisenge R, Aboud S, et al. Effect of zinc and multivitamin supplementation on the growth of Tanzanian children aged 6-84 wk: a randomized, placebo-controlled, double-blind trial. *Am J Clin Nutr.* 2016;103(3):910-8.
386. Manji KP, McDonald CM, Kupka R, Bosch RJ, Kisenge R, Aboud S, et al. Effect of multivitamin supplementation on the neurodevelopment of HIV-exposed Tanzanian infants: a randomized, double-blind, placebo-controlled clinical trial. *J Trop Pediatr.* 2014;60(4):279-86.

