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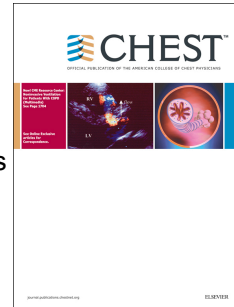
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Translating Basic Research into Clinical Practice: Vitamin D in Asthma – Mechanisms of Action and Considerations for Clinical Trials

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1 **Translating Basic Research into Clinical Practice:**

2 **Vitamin D in Asthma – Mechanisms of Action and Considerations for Clinical Trials**

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29
30
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34
35
36 **Abbreviations:**

37
38 1,25(OH)₂D₃ 1,25 di-hydroxyvitamin D₃
39 25(OH)D 25-hydroxyvitamin D
40 DC dendritic cell
41 IL- interleukin-
42 ILC innate lymphoid cell
43 Th helper CD4+ T lymphocyte
44

45 **Abstract**

46

47 There is increasing interest in the therapeutic utility of vitamin D in asthma, supported by a
48 significant body of evidence on epidemiological associations between vitamin D insufficiency
49 and worse asthma control. In support of a causal relationship, vitamin D beneficially
50 modulates diverse immunological pathways in heterogeneous asthma endotypes, regulating
51 the actions of lymphocytes, mast cells, antigen-presenting cells and structural cells to dampen
52 excessive inflammatory responses.

53

54 Allergic asthma is characterised by a failure of immune tolerance, and development of
55 pathological responses to inhaled aeroallergens, and vitamin D has been extensively shown to
56 support immune regulation. Alarmin cytokines are increasingly implicated in non-allergic
57 eosinophilic inflammation, which vitamin D also regulates. Steroid-resistance and pathological
58 IL-17 responses are features of severe asthma, and vitamin D beneficially enhances the
59 response to steroids in these individuals. Additionally, vitamin D enhances anti-microbial
60 pathways, of relevance to infection-precipitated asthma exacerbations. These mechanisms
61 support a role for vitamin D as secondary prevention to reduce exacerbations and
62 inflammation in asthma. Similar mechanisms, and effects on fetal lung development, likely
63 underlie a primary prevention therapeutic role in pregnancy for vitamin D to reduce the
64 development of asthma in children.

65

66 However, randomised controlled trials of variable design show inconsistent positive outcomes
67 for vitamin D interventions in asthma. Increased understanding of vitamin D biology reveals
68 methodological issues that might explain certain negative outcomes. Importantly, on
69 systematic review of the trials to-date, vitamin D is beneficial in asthma. The evidence

70 discussed in this review supports the importance of optimising vitamin D in holistic asthma
71 care.

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72 **Manuscript**

73

74 The importance of vitamin D in human health is well established, however, for much of the 20th
75 century its actions were only considered influential in calcium and phosphate homeostasis.
76 We have now come to realise that vitamin D has profound paracrine actions throughout the
77 body and in particular to have major effects on the immune system.^{1 2} There is a current
78 epidemic of vitamin D insufficiency³ - in humans the most important source for vitamin D
79 precursors starts with conversion in the skin of 7-dehydrocholesterol to D3 in a reaction
80 requiring solar ultraviolet B radiation, and reduced exposure to sunlight in our modern
81 lifestyle is thought responsible for the high prevalence of vitamin D insufficiency.^{1 3} As part of
82 evolving vitamin D research over the last 15 years there has been intense interest in the role
83 vitamin D may play in airway health and homeostasis, and the effect of vitamin D deficiency on
84 airways pathology. In this review we discuss evidence for vitamin D insufficiency / deficiency
85 contributing to asthma pathology and evidence that vitamin D supplementation should be
86 considered part of holistic treatment of asthma.

87

88 Epidemiological research has revealed strong, significant associations between diverse aspects
89 of asthma pathology and reduced levels of serum 25-hydroxyvitamin (25(OH)D) vitamin D, the
90 major circulating precursor, which is commonly used as a measure of vitamin D status. Cross-
91 sectional studies have consistently found more severe asthma in patients with lower vitamin D
92 levels (Table of studies and discussion in Mann *et al.* 2014⁴). Similar associations seen in other
93 diseases has led to vigorous discussion about whether these associations are due to causation
94 or reverse-causation. Three streams of evidence strongly support causation i.e. that reduced
95 levels of circulating vitamin D can contribute to more severe asthma disease: prospective
96 epidemiological evidence, mechanistic research and vitamin D supplementation studies.

97 Nevertheless reverse-causation (that asthma itself leads to decreased vitamin D levels) is also
98 likely to be true with reduced time spent outside in more severe asthma and the likelihood that
99 asthmatic inflammation is vitamin D consumptive, as has been shown for other types of
100 inflammation.⁵ We propose that there is a complex relationship between vitamin D
101 insufficiency and asthmatic pathology, with a vicious circle of causation and reverse-causation,
102 leading to progressive worsening of asthma, and that this cycle may be broken by including
103 vitamin D supplementation in asthma care. As discussed below this evidence supports a
104 therapeutic role for vitamin D as secondary prevention in asthmatic patients to reduce asthma
105 exacerbations and also airway inflammation with remodelling.

106

107 **Prospective epidemiological evidence**

108 Most vitamin D epidemiological studies have been cross-sectional, comparing vitamin D levels
109 to recent asthma control, and therefore more vulnerable to reverse-causation. Few studies
110 have compared vitamin D levels to future asthma control. However, for example, Brehm *et al.*
111 (2010) have reported an association between baseline serum vitamin D status and risk of a
112 severe exacerbation over the following 4 years in children enrolled into the Childhood Asthma
113 Management Program study.⁶ After adjustment for other variables, including physician judged
114 asthma severity, patients with baseline vitamin D insufficiency (< 30 ng/ml) had higher odds of
115 an asthma exacerbation requiring hospitalisation or Emergency Department attendance over
116 the follow-up period.

117

118 **Mechanistic Research**

119 Translational research over the last 15 years has shown vitamin D to have a major role in
120 regulating immunological responses. Such a role is not surprising given the high frequency of
121 vitamin D response elements in cells of the immune system such as CD4 T lymphocytes.⁷ The

122 immunology of asthma is complex and the disease heterogeneous – we are increasingly aware
123 that multiple independent pathological mechanisms can lead to asthmatic inflammation and
124 that the relative contribution of the pathways will differ from individual to individual patient.
125 These pathways interact and subdivisions described below are to aid discussion. Importantly,
126 vitamin D has beneficial actions at multiple steps in all these proposed pathways.
127 Furthermore, chronic uncontrolled asthmatic inflammation leads to airway remodelling - this
128 is also reduced by vitamin D, likely through its actions on structural cells, as further discussed
129 below.

130

131 **Aero-allergen triggered asthma**

132 Classically asthmatic inflammation was thought primarily due to antigen-dependent immune
133 responses to aeroallergens, with Th2 lymphocytes, IgE-secreting B lymphocytes and mast cells
134 featuring prominently in this patho-mechanism (Figure 1). Naive T lymphocytes become
135 inappropriately primed to respond to otherwise-innocuous inhaled aeroallergens with Th2
136 polarity, and upon re-stimulation by inhaled aeroallergens produce the Th2 cytokines IL-4, IL-
137 5 and IL-13 that promote asthma pathology. IL-4 promotes class-switching of B lymphocytes
138 to IgE production. Aero-allergen specific IgE is produced by these B lymphocytes and coats
139 mast cells. Then on inhalation of aeroallergens the IgE upon mast cells becomes cross-linked
140 leading to rapid release of further pro-inflammatory asthma mediators such as leukotrienes
141 and histamine that cause bronchoconstriction and airway mucus production.

142

143 At the core of this mechanism is a failure of suppression of inappropriate antigen-dependent
144 immune responses to aeroallergens. Adaptive immune responses are regulated by diverse
145 classes of regulatory T lymphocyte (Treg), for example Foxp3 expressing and IL-10 secreting
146 Tregs, and their coordinated action in healthy individuals ensures tolerance to non-harmful

147 antigens. There is an abundance of evidence that vitamin D plays a vital role in Treg responses.
148 ⁸ Our laboratory has previously shown *in vivo* vitamin D status positively correlates with the
149 frequency of Foxp3+ Tregs ⁹ and airway levels of IL-10 ¹⁰ in asthma patients. *In vitro*
150 1,25(OH)₂D₃ promotes distinct IL-10 and Foxp3 expressing CD4+ T cell populations, which is
151 influenced by the local cytokine milieu. ⁹ In addition to Tregs, adaptive immune responses are
152 also regulated through other mechanisms. In particular, the quality of antigen presentation
153 and expression of co-stimulatory signals by dendritic cells (DCs) is important in determining
154 tolerogenic versus inflammatory immune responses. Multiple facets of DC activity are
155 regulated by vitamin D, including antigen presentation and co-stimulatory signals, to promote
156 tolerogenic DCs. ¹¹

157
158 As well as promoting appropriate antigen tolerance, vitamin D also modulates other aspects of
159 allergen-stimulated immune responses. Vitamin D can suppress production of IgE by human B
160 lymphocytes *in vitro* and increase IL-10 production, promoting a regulatory B lymphocyte
161 phenotype. ^{12 13} Notably, in children vitamin D deficiency is associated with increased levels of
162 aeroallergen specific IgE. ^{14 15} Additionally, vitamin D has been shown to have the capacity to
163 suppress mast cell activation, reducing histamine and TNF α release for example, including in
164 the context of IgE-dependent activation. ^{16 17} Vitamin D can also promote mast cell production
165 of anti-inflammatory IL-10. ¹⁸

166
167 In support of these actions of vitamin D being significant, Heine and colleagues have shown in a
168 ovalbumin-sensitisation model that vitamin D deficient mice have higher levels of ovalbumin-
169 specific IgE. ¹⁹ Addition of vitamin D to a desensitisation protocol further reduced airways
170 concentrations of IL-4 and IL-13 post aerosolised ovalbumin challenge, and also further
171 reduced airways hyper-responsiveness. ¹⁹

172

173 **Epithelial damage and Alarmin cytokine elicited asthmatic inflammation**

174 There is increasing interest in the mechanisms underpinning non-allergic asthma. Epithelial
175 damage is now understood to prompt release of cytokines known as Alarmins – e.g. IL-25, IL-
176 33 and TSLP – that directly stimulate multiple cell types including type-2 innate lymphoid cells
177 (ILC2s) and mast cells.²⁰ These stimulated ILC2s then produce Th2-type cytokines including
178 IL-5, which in turn promotes eosinophilic inflammation (Figure 2). Of these Alarmins, IL-33
179 appears particularly important in asthma with both its gene (*IL33*) and the gene for its
180 receptor (*11RL1*) identified in asthma GWAS studies.²¹

181

182 Vitamin D has been shown to modulate the epithelial response to stimulation with a potentially
183 anti-inflammatory role for this action.²² However, of specific relevance to asthma is the
184 capacity of vitamin D to stimulate bronchial epithelial cell production of sST2, a soluble decoy
185 blocker for IL-33, and for this epithelial-produced sST2 to decrease the pro-inflammatory
186 effect of IL-33 on target cells such as mast cells.²³

187

188 There is relatively little evidence as to the effect of vitamin D on ILC2s and eosinophils. Ethier
189 *et al.* have shown vitamin D to be able to enhance eosinophil viability with reduced production
190 of pro-inflammatory necrotic granules.²⁴ Ruiter and colleagues have shown vitamin D to
191 reduce pro-inflammatory cytokine production by stimulated ILC2s.²⁵

192

193 Viral infections in particular trigger epithelial IL-33 release,²⁶ which is important given that
194 Th2-biased asthmatic pathology has been shown to impair appropriate anti-viral immune
195 responses.²⁷ Indeed asthma exacerbations are frequently associated with viral respiratory
196 tract infections. Vitamin D, however, enhances antimicrobial immune responses through many

197 mechanisms. It enhances cellular production of antimicrobial peptides (such as cathelicidin)
198 and autophagy, important in the response to both bacterial and viral infections.^{28 29 30}
199 Consistent with these actions of vitamin D, meta-analysis has shown vitamin D
200 supplementation of appropriate patients to reduce acute respiratory tract infections.³¹

201

202 **Steroid-resistant asthma and IL-17**

203 In a proportion of patients with the most severe asthma the pathology appears to have some
204 distinct features, in particular corticosteroid-resistance and an apparent pathological role for
205 IL-17. Multiple pathways to steroid-resistance in airways disease have been described.³²
206 Airway colonisation with pro-inflammatory bacteria such as Haemophilus influenza, oxidative
207 stress (for example from air pollution) and vitamin D deficiency itself are major contenders for
208 causing acquired steroid-resistance in asthma. Vitamin D enhances anti-microbial pathways
209 and vitamin D also promotes anti-oxidant responses.³³ Additionally vitamin D ameliorates
210 steroid-resistant inflammation through its direct actions on lymphocytes (as discussed below)
211 and monocytes.³⁴ Steroid-insensitive asthmatics have impaired steroid-induced production
212 of anti-inflammatory IL-10. *In vitro* vitamin D enhances steroid-induced lymphocyte
213 production of IL-10 and supplementation of steroid-resistant asthmatic patients with the
214 calcitriol form of vitamin D restores both the clinical and immunological IL-10 response to
215 corticosteroids.^{35 36 37} Furthermore, we have shown that corticosteroids actually promote in
216 steroid-resistant individuals production of IL-17, thought in this context to cause pathological
217 neutrophilic inflammation, with amelioration after vitamin D supplementation.³⁷
218 Subramanian and colleagues have recently reported vitamin D to reduce production of pro-
219 inflammatory cytokines by stimulated neutrophils.³⁸ However vitamin D did enhance the
220 anti-bacterial activity of neutrophils, via enhanced production of antimicrobial peptides rather
221 than increased production of elastase or reactive oxygen species.³⁸ These findings are

222 consistent with earlier findings of vitamin D responsiveness of neutrophils reported by
223 Takahashi and colleagues.³⁹

224

225 **Vitamin D and airway remodelling**

226 The downstream effect of asthmatic immune responses, regardless of the mechanisms
227 precipitating those responses, is airway narrowing. Over the short-term this is due to smooth
228 muscle constriction and mucus secretion, whilst over the longer-term airway remodelling and
229 fibrosis occur. Importantly, vitamin D has actions on airway smooth muscle including
230 inhibiting airway smooth muscle cell proliferation.^{40 41} Consistent with this Gupta and
231 colleagues have previously shown airway smooth muscle volume fraction in endobronchial
232 biopsies to negatively correlate with serum vitamin D concentrations in steroid-refractory
233 severe paediatric patients with asthma.¹⁴

234

235 **Randomised controlled trials (RCTs) of vitamin D to treat asthma**

236 Over the last few years several randomised controlled clinical trials of vitamin D therapies to
237 improve asthma control have completed and published their findings. These studies of vitamin
238 D as secondary prevention to reduce asthma exacerbations have reported a mixture of positive
239 and negative results in their primary outcome measures with, nevertheless, multiple positive
240 secondary outcome measures in some trials with negative primary outcomes. For example, the
241 VIDA trial (Castro *et al.*), despite a negative primary outcome of time to first exacerbation,
242 found in a responder analysis that for each 10ng/ml increase in serum 25(OH)D there was a
243 significant reduction in the rate of treatment failures and of exacerbations.⁴² Furthermore, in
244 a recent Cochrane Review of vitamin D for secondary prevention to reduce exacerbations in
245 asthmatic patients, meta-analysis shows vitamin D supplementation to significantly reduce the
246 rate of severe exacerbations in asthmatic patients.⁴³

247

248 One possible explanation for the divergent results is under-powering in some studies and
249 smaller effect sizes than anticipated. However, we believe that differences and limitations in
250 study design partly explain the differences in study outcome (see Table 1). These
251 methodological issues in vitamin D treatment trials are not specific to asthma and are worthy
252 of detailed discussion.

253

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254 **Table 1: Summary of major vitamin D secondary prevention RCTs in asthma**

255 ACT; Asthma Control Test. ATAQ; Asthma Therapy Assessment Questionnaire. FEV1; Forced

256 Expiratory Volume in 1 second.

257

Trial	
<u>Study Design: Population & Intervention</u>	<u>Primary (1ry) and major Secondary (2ndry) Outcomes</u>
Majak <i>et al.</i> 2011⁴⁴	
Population: 48 children (5-18 years old) with newly diagnosed asthma. Intervention: 500 IU D3 daily (versus placebo). In addition to inhaled steroid.	Outcomes: Significantly lower percentage of participants experiencing an asthma exacerbation in the D3 treated group. No significant difference in improvements in ATAQ score and FEV1 between the two groups.
Lewis <i>et al.</i> 2012⁴⁵	
Population: 30 children (6-17 years age) with asthma; 97% with serum 25(OH)D < 30 ng/ml. 20 completed study (33% drop-out in both groups). Intervention: 1000 IU D3 daily (versus placebo).	Outcomes: No significant effect of D3 therapy on either ACT score or FEV1.
Yadav <i>et al.</i> 2013⁴⁶	
Population: 100 children (5-13 years old) with moderate to severe asthma. Intervention: 60 000 IU D3 per month or placebo.	1ry Outcome: Significantly greater improvement in severity of asthma according to GINA guidelines at 6 months in D3 treated group. 2ndry Outcome: Significantly fewer exacerbations in the D3 treated group.
Castro <i>et al.</i> 2014 (VIDA Study)⁴²	
Population: 408 adult asthmatics all with serum 25(OH)D < 30 ng/ml. Intervention: Placebo versus 100 000 IU D3 bolus dose followed by 4000 IU daily.	1ry Outcome: No significant effect on rate of first asthma treatment failure. 2ndry Outcomes: No significant effect on rate of first asthma exacerbation. Significantly greater taper of inhaled

Tapering inhaled steroids dependent on asthma control in both groups.	steroids in the D3 treated group. In a vitamin D3 responder analysis: Rate of first exacerbation and overall exacerbation rate were both significantly lower in the D3 incremented subgroup.
Martineau <i>et al.</i> 2015 (ViDiAs Study) ⁴⁷	
Population: 250 adult asthmatics; 82% with serum 25(OH)D < 75 nmol/l and 58% with serum 25(OH)D < 50 nmol/l. Intervention: 120 000 IU D3 every two months (versus placebo).	1ry Outcome: No significant effect of D3 treatment on time to first severe asthma exacerbation or time to first upper respiratory infection. No effect modification of baseline vitamin D status on either co-primary outcome.
Tachimoto <i>et al.</i> 2016 ⁴⁸	
Population: 89 schoolchildren (6-15 years age) with asthma. Median average serum 25(OH)D 29 ng/ml. Intervention: 800 IU D3 /day or placebo.	1ry Outcome: Significantly better changes in asthma control levels as defined by GINA in D3 treated arm.

259

260 The first issue is whether vitamin D supplementation is beneficial in all patients or only
261 beneficial where it corrects low vitamin D levels in deficient patients. Unlike in trials of
262 pharmacologic drugs, in trials of vitamins patients at baseline have variable circulating levels
263 of the vitamins and will have variable natural intake over the course of the trial through their
264 normal activities of daily living. Subgroup analyses in two studies of vitamin D as a treatment
265 for COPD have shown significant benefit from vitamin D supplementation in the subgroups of
266 patients with marked vitamin D insufficiency (serum 25(OH)D levels $< 10\text{ng/ml}$ and < 50
267 nmol/l respectively for the two studies).^{49 50} Similarly Martineau and colleagues have
268 reported a significant interaction between baseline vitamin D status and benefit of
269 supplementation in reducing acute respiratory tract infections, with greatest benefit in those
270 with serum 25(OH)D $< 25 \text{ nmol/l}$.³¹ A major limitation of some studies may therefore be that
271 many participants were relatively vitamin D sufficient at baseline and less likely to benefit.

272 Not all supplemented patients in the treatment arms of these trials actually achieved vitamin D
273 sufficiency during the trials. However, that supplementation does achieve vitamin D
274 sufficiency appears an important determinant of benefit. In the VIDA trial approximately one
275 fifth of participants in the vitamin D3 treatment arm did not achieve a serum 25(OH)D3 ≥ 30
276 ng/ml (equivalent to approximately 75 nmol/l). Although the rate of exacerbations was not
277 significantly decreased in the overall treatment group compared to placebo, in an exploratory
278 responder analysis when those not achieving a 25(OH)D3 $\geq 30 \text{ ng/ml}$ in the treatment arm
279 were excluded then there was a significant reduction in exacerbations in the treatment arm
280 compared to placebo.⁴²

281 Furthermore, the optimum vitamin D level in asthma is unclear. A serum 25(OH)D3 target
282 concentration of 75 nmol/l (30ng/ml) is often quoted but this is based on inferences from the
283 actions of vitamin D in calcium-phosphate homeostasis.⁵¹ The responder analysis from the

284 VIDA trial is consistent with this target,⁴² however, that does not mean that this is necessarily
285 the optimum target vitamin D level in asthma or for general health. There are both proponents
286 of lower target vitamin D concentrations and high target serum levels (such as 100nmol/l
287 40ng/ml).^{51 52} In support of the latter are the higher physiological ranges of vitamin D seen in
288 some populations and emerging evidence from vitamin D trials in other medical fields.⁵³
289 However, although in clinical practice we assay serum 25(OH)D concentrations as a measure of
290 patient vitamin D status, whether this is the most appropriate form to measure is uncertain.
291 Vitamin D has a complicated metabolic pathway and it is possible that levels of a precursor or
292 other metabolite are more important.⁵⁴ Additionally different actions of vitamin D may
293 require different threshold concentrations of serum vitamin D. Finally, the bioavailability of
294 circulating vitamin D metabolites is dependent on other factors such as circulating vitamin D
295 binding protein (VDBP), and polymorphisms in VDBP and other vitamin D axis genes may
296 affect the supplementation dose necessary to achieve functional vitamin D sufficiency.⁵⁵
297
298 Secondly, the correct form of vitamin D to give as a supplement and correct dosing strategy is
299 uncertain (reviewed in detail by Hollis and Wagner, 2013⁵⁴). In particular, bolus vitamin D
300 supplementation (as used in some asthma studies) may be immunologically problematic,
301 causing large swings in serum levels and inducible homeostatic enzymes, with dysregulation of
302 other vitamin D regulated pathways.⁵⁶ Indeed a recent meta-analysis of trials of vitamin D to
303 prevent acute respiratory tract infections found regimens with regular vitamin D dosing to be
304 associated with a protective action of vitamin D but regimens with high-dose boluses to not
305 show protective effect.³¹ The commonest forms of vitamin D to give as supplementation are
306 ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3) – although there is little research
307 in to ergocalciferol supplementation in asthma by extrapolating from other diseases
308 cholecalciferol is more likely to be beneficial than ergocalciferol.⁵⁷ Finally, correction of

309 vitamin D insufficiency through increased exposure to ultraviolet radiation may be more
310 physiological and more effective in asthma than oral supplementation.⁵⁸

311

312 Thirdly, vitamin D supplementation may be more beneficial in paediatric asthma^{44 48} than in
313 adult asthma.^{42 47} Notably although Sharief and colleagues found significant associations
314 between serum 25(OH)D and IgE sensitisation to multiple allergens in children and
315 adolescents, significant associations were much fewer in adults.¹⁵ The recent Cochrane
316 Review of vitamin D as secondary prevention in asthma did not directly address this question,
317 however the recent closely-related systematic review of vitamin D to prevent acute respiratory
318 tract infections did not find a significant interaction between age and benefit of vitamin D.³¹ A
319 paediatric versus adult difference in benefit of vitamin D could relate to immunopathological
320 differences in childhood onset, typically atopic asthma, and adult asthma. Different
321 immunological pathways are likely to be of prime importance in childhood-onset asthma
322 compared to adult-onset asthma, and vitamin D may have less effect on the pathways of prime
323 importance in adult-onset asthma. Alternatively, differences in the capacity of vitamin D to
324 regulate immune responses in children and adults may perhaps reflect establishment of
325 epigenetic responses to chronic vitamin D insufficiency in adults after years of vitamin D
326 deficiency, leading to these individuals having less capacity to respond to vitamin D
327 therapies.⁵⁹

328

329 Fourthly whilst the above mechanistic studies show the capacity of vitamin D to beneficially
330 modulate the different pathways thought to underlie different asthma endotypes, it is possible
331 that some of these actions of vitamin D have little clinical efficacy. Therefore vitamin D
332 therapies may be particularly beneficial in discrete endotypes of asthma. The larger RCTs have

333 not selected for specific asthma endotypes, however, we have shown evidence for a particular
334 role for vitamin D therapy in a severe steroid-resistant asthma endotype.^{36 37}

335

336 Further large studies of daily vitamin D supplementation to improve asthma control in vitamin
337 D deficient asthmatic children and adults are needed to better understand these issues.
338 Nevertheless, there is increasing realisation that vitamin D deficiency negatively impacts on
339 general health status and that vitamin D supplementation rarely has side-effects. Therefore,
340 optimising vitamin D status is increasingly becoming part of standard holistic clinical care in
341 asthma (see Box 1 for Clinical Recommendations).

342

343 Box 1: Clinical Recommendations

344

345

CLINICAL RECOMMENDATIONS

346

- Daily supplementation of vitamin D deficient and insufficient patients is safe and has positive benefit to health in asthma and other diseases, but infrequent high-dose bolus supplementation should be avoided.

347

- Supplementation should be given as Vitamin D3 (cholecalciferol).

348

349

- The exact serum 25(OH)D concentration below which supplementation should be started and best dosing regimens remain to be established. We would suggest supplementing those with serum 25(OH)D < 50 nmol/l and starting with a dose of 1000 – 4000 IU/day depending on baseline concentration. Serum vitamin D should be reviewed after a few months of supplementation and if vitamin D sufficiency has not been achieved then the supplementation dose should be increased in order to achieve vitamin D sufficiency.

350

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- Serum vitamin D concentrations should be checked in women planning pregnancy pre-conception and also in all pregnant women, with daily high-dose supplementation given during pregnancy. In those planning pregnancy sufficient vitamin D supplementation should be given to rapidly achieve vitamin D sufficiency.

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364

365 Early life vitamin D and primary prevention of asthma

366 Vitamin D deficiency and insufficiency is extremely common in pregnancy, and is associated
367 with preeclampsia, gestational diabetes mellitus and other co-morbidities of pregnancy.⁶⁰ It
368 has also been associated with increased respiratory infections, and asthma in the newborn and
369 children in many studies.⁶¹ Vitamin D is proposed to beneficially impact fetal and
370 neonatal lung maturation, as well as immunity in a manner likely to promote maternal-fetal
371 tolerance, and to protect mother and fetus from infection.^{61 62} Importantly these associations
372 between maternal vitamin D insufficiency and asthma in the offspring cannot be explained as
373 due to reverse causation although an effect of unmeasured confounding variables in these
374 studies is possible.

375 The mechanisms by which early life vitamin D could help reduce development of asthma
376 include (i) its capacity to support development of tolerogenic immune responses (as discussed
377 above), (ii) facilitating appropriate antiviral and antibacterial immune responses, (iii)
378 enhancing barrier properties of the epidermis and decreasing eczema, and (iv) enhancing
379 appropriate lung development – all factors important in determining whether an individual
380 develops symptomatic chronic airway disease.⁶³ In particular *in utero* vitamin D has been
381 shown to affect expression of genes important in early pulmonary development (for example
382 branching morphogenesis) and to influence multiple aspects of later lung development
383 including alveolar development and surfactant secretion.^{62 64} These vitamin D regulated
384 developmental processes occur at different times in gestation suggesting multiple vital
385 windows for vitamin D actions at different stages of gestation. Furthermore, as vitamin D
386 research continues an increasing number of asthma-relevant development processes are being
387 revealed to be influenced by vitamin D, for example recent evidence for a likely influence of *in*

388 *utero* vitamin D status on the early-life microbiome. ⁶⁵

389 These data, with mechanistic evidence discussed above, has now led to randomised controlled
390 trials of high-dose vitamin D supplementation as primary prevention to avert the development
391 of recurrent wheeze and asthma in offspring.

392 Two large RCT, using doses of vitamin D known to significantly increase vitamin D status of
393 pregnant women, ⁶¹ have recently reported similar and encouraging effects on infant
394 respiratory outcomes. In the COPSAC study, Chawes *et al.*, supplemented pregnant women
395 with 2800 IU/d vitamin D3 in the third trimester of pregnancy, and reported a reduction in
396 episodes of troublesome lung symptoms in the offspring through 3-years though no significant
397 effect on risk of persistent wheeze. ⁶⁶ In the VDAART trial, Litonjua *et al.*, supplemented with
398 4400 IU/d vitamin D3 during the second and third trimester, reporting a significant reduction
399 in allergic sensitizations and a trend for a reduction in recurrent wheeze and asthma in
400 offspring through 3-years. ⁶⁷ Importantly, no increase in adverse effects were reported in
401 these or other comparable pregnancy studies. ^{60 66 67}

402 In an ancillary study of cord blood samples from a subset of babies born to women within the
403 VDAART cohort, Hornsby and colleagues show supplementation to significantly enhance pro-
404 inflammatory cytokine responses to mitogen and TLR-agonist stimulation, increase *TLR2* and
405 *TLR9* gene expression, and IL-17A secretion in response to T cell receptor-ligation. ⁶⁸ Thus,
406 vitamin D supplementation during pregnancy modifies the immune system of the neonate in a
407 manner that is predicted to protect the host against pathogenic infections and reduce
408 development of asthma.

409 However, similar methodological issues exist with primary prevention trials of vitamin D to
410 those discussed above with respect to secondary prevention trials. Stratification of response
411 by baseline vitamin D status is evident - in secondary analyses of the VDAART study infants
412 born to high-dose supplemented mothers who had circulating levels of vitamin D of 30ng/ml

413 or higher at recruitment (sufficiency range) demonstrated a striking and significant reduction
414 in recurrent wheeze and asthma through 3-years. However, infants of high-dose
415 supplemented mothers who were vitamin D deficient at baseline (10-18 weeks gestation) did
416 not have the same significant benefit from randomisation to high-dose vitamin D. This
417 indicates the likelihood of important effects of vitamin D *throughout* neonatal development,
418 likely to include bone, pulmonary and immune effects (as discussed above), and including
419 during the first trimester.⁶⁹ It may also suggest that maternal vitamin D deficiency leads to
420 epigenetic changes in the offspring resulting in relative vitamin D resistance.⁵⁹ Therefore
421 achieving vitamin D sufficiency needs to be a clinical priority pre-conception. High-dose
422 supplementation in both these trials did not include what appears to be a critical window for
423 vitamin D actions in early pregnancy / pre-conception. Notably this influence of baseline
424 vitamin D status on clinical benefit of vitamin D supplementation to prevent development of
425 asthma is opposite to that noted in the secondary prevention RCTs discussed above.

426 Secondly, similar to the secondary prevention trials, not all patients in these primary
427 prevention trials achieved vitamin D sufficiency despite high-dose supplementation (75% in
428 the VDAART trial and 81% in the COPSAC trial).^{67 66} This underscores the likelihood that
429 different individuals require different doses of vitamin D to achieve sufficiency, however, both
430 trials did note a significant proportion of participants not to have been adequately adherent to
431 the vitamin D intervention. Previously infrequent bolus dosing of vitamin D supplements has
432 been suggested as a strategy to improve adherence but as we discuss above such infrequent
433 bolus dosing may be immunologically problematic.

434 It is also too early to determine whether high-dose vitamin D supplementation in these
435 primary prevention studies has successfully reduced the proportion of children going on to
436 develop asthma – reported follow-up to date is for up to age 3 whereas asthma is often
437 diagnosed slightly later in childhood. Long-term follow-up of these patients is eagerly awaited.

438 The longitudinal effects of vitamin D during the first 10 years of life were investigated in a
439 high-risk Australian cohort in order to determine relationships between 25(OH)D levels and
440 susceptibility to allergic sensitization, respiratory tract infections, and asthma.⁷⁰ The study
441 showed that 25(OH)D deficiency in early childhood is associated with increased risk for
442 persistent asthma. The authors proposed that vitamin D may act by influencing susceptibility
443 to early allergic sensitization, and/or upper respiratory tract colonization with bacterial
444 pathogens, or both.

445 Together these very recent studies bring real enthusiasm to investigate strategies that restore
446 vitamin D sufficiency in pregnancy and the first years of life, which offer the potential to reduce
447 both important asthma risk factors, namely allergic sensitizations and respiratory tract
448 infections, as well as asthma itself. A future challenge will be to determine safe and effective
449 regimens of vitamin D3 supplementation in the newborn and infant.

450

451 **Conclusions**

452

453 There is a wealth of epidemiological evidence of a detrimental association between vitamin D
454 insufficiency and asthma, and translational studies have shown vitamin D to have the capacity
455 to beneficially regulate diverse aspects of asthma pathology. Methodological considerations
456 with vitamin D randomised controlled trials to-date may underlie less impressive than hoped
457 clinical effects in the trials. Further clinical trials, with revised protocols learning from the
458 issues discussed above, are needed. Nevertheless, the evidence-base increasingly supports
459 vitamin D supplementation being a safe, practical and beneficial part of the comprehensive
460 management of asthma.

461

462 **Figure Legends**

463

464 **Figure 1: Illustrative schematic of the mechanistic pathway of aero-allergen stimulated**
465 **asthmatic inflammation, and steps at which vitamin D acts.**

466 Vitamin D can act beneficially at multiple steps along the pathway to prevent / reduce
467 asthmatic inflammation.

468

469 **Figure 2: Schematic for non-allergic eosinophilic asthmatic inflammation.**

470 Epithelial injury, for instance from proteases or viral infection, stimulates epithelial release of
471 Alarmin cytokines such as IL-25 and IL-33. These cytokines act on immune cells such as ILC2s
472 and mast cells to elicit production of Th2-type cytokines that stimulate eosinophilic
473 inflammation. Vitamin D can inhibit this pathway at multiple steps.

474

475 **Figure 3: Diverse stimuli can elicit steroid-resistant asthmatic inflammation**

476 Th17-mediated neutrophilic airways inflammation can follow diverse precipitants such as
477 oxidative stress that are sensitive to modulation by vitamin D. Furthermore, Th17
478 lymphocytes and neutrophils themselves are also beneficially sensitive to vitamin D.

479

480

481

482 **References**

483

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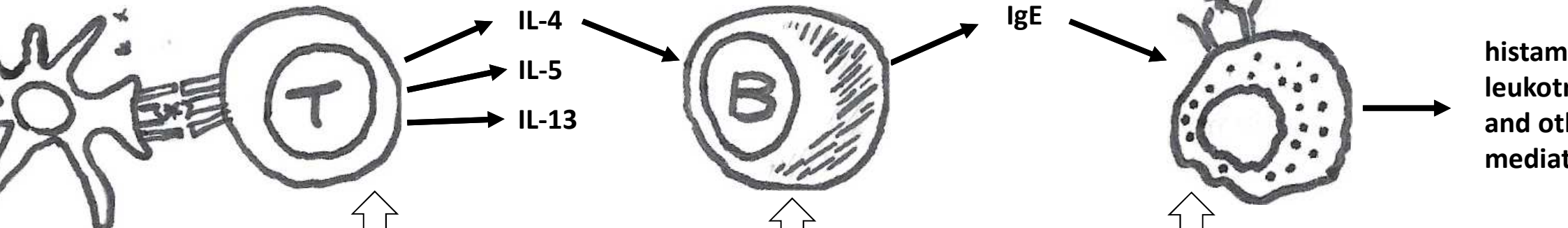
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- 683

allergen stimulated Th2 cells

IgE secreting B cells

activated mast cells



promotion of tolerogenic dendritic cells (DCs)

enhanced Treg action to suppress inappropriate Th2 responses

reduced class-switching to IgE synthesis, increased IL-10 secretion

decreased mast cell activation

- Szeles *et al.* 2009

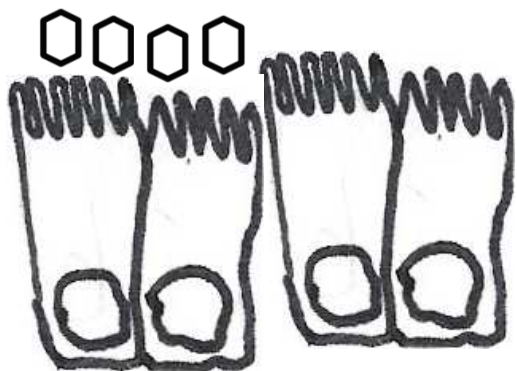
- Urry *et al.* 2009
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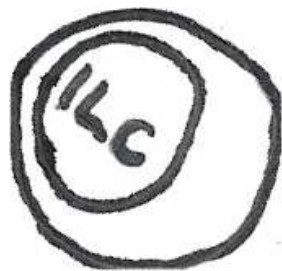
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- Liu *et al.* 2016

Figure 1

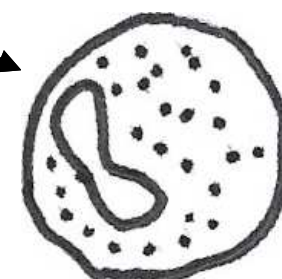
viral stimulated epithelial cells



stimulated ILC2s



activated eosinophils



eosino
cationi
protein
and ot
mediat

IL-25
IL-33

IL-5

anti-
microbial
actions

modulated
epithelial
stimulation
(anti-inflammatory)

stimulated sST2
production
inhibits IL-33
actions

decreased ILC2
stimulation

actions on
eosinophils

- Liu *et al.* 2006
- Handsottir *et al.* 2010
- Beard *et al.* 2011
- Fabri *et al.* 2011

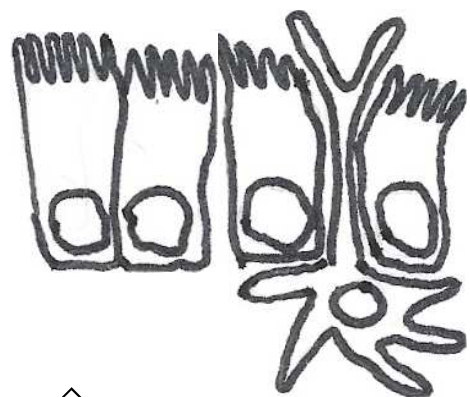
- Pfeffer *et al.* 2015

- Ruitter *et al.* 2015

- Ethier *et al.* 2016

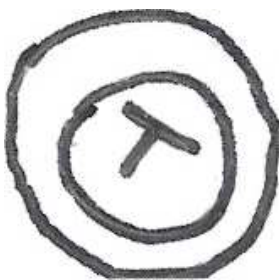
Figure 2

stimulation e.g. bacteria, pollution



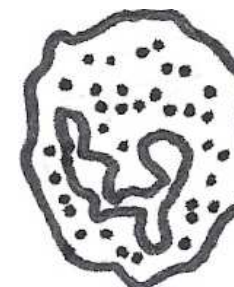
IL-6
IL-23
IL-1 β

stimulated Th17 cells



IL-17

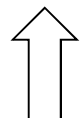
activated neutrophils



neutro
elastas
and oth
mediat



anti-oxidant and
anti-microbial actions of
vitamin D



suppression of Th17
responses and
enhancement of steroid-
induced IL-10 secretion



reduced pro-inflammatory
cytokine production by
neutrophils but with enhanced
anti-bacterial activity

- Liu *et al.* 2006
- Fabri *et al.* 2011
- Lan *et al.* 2014

- Xystrakis *et al.* 2006
- Nanzer *et al.* 2014
- Chambers *et al.* 2015

- Takahashi *et al.* 2002
- Subramanian *et al.* 2017

Figure 3