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Ultrasound classification and grading of lipohypertrophy and its impact on glucose variability in Type 1 Diabetes (the TITANIC Study)
an exploratory study

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Ultrasound Classification and Grading of Lipohypertrophy and Its Impact on Glucose Variability in Type 1 Diabetes (the TITANIC Study)- An Exploratory Study

by

Rabab Hashem

A Thesis Presented for the Degree of Doctor of Philosophy

King's College London

Florence Nightingale Faculty of Nursing, Midwifery and Palliative Care

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Abstract

Background

Lipohypertrophy (LH) is a common insulin injection site problem that occurs with repeated exposure to insulin injections in the subcutaneous tissue. Subcutaneous insulin exposure can increase tissue density with hypertrophy and hyperplasia of adipocytes. The effect of insulin when injected into an LH area can be attenuated, potentially leading to glucose variability. Thereby, potentially increasing the risks of diabetes complications, hypoglycaemia and individual distress. The aim of this study was to explore the association between ultrasonographically characterised LH lesions with time in range (TIR 4 to 10 mmol/L) and glucose variability (GV) in adults with Type 1 diabetes (T1DM). The study also aimed to characterise the LH observed in participants.

Methods

The study involved two integrated arms, the glucose variability arm (the GV study) and LH characterisation arm (the LH characterisation study). All participants were recruited from clinics at Guys and St Thomas' Hospitals. The main screening criteria were: people with T1DM using insulin; with evident GV based on the standard deviation (SD) of the mean glucose from their self-monitored blood glucose downloads (SD≥ 4.0mmol/L). In the GV study participants TIR and GV (SD of glucose, coefficient of variation (CV), mean amplitude of glucose excursions (MAGE), continuous overlapping net glycaemic action (CONGA), means of the daily differences (MODD), and mean absolute glucose (MAG)) were assessed in two conditions using blind continuous glucose monitoring (CGM): Condition 1, usual insulin injecting behaviour; and Condition 2, injecting in areas assessed to be free of LH- participants insulin doses were reduced at this stage, to reduce the risk of hypoglycaemia. Additional outcomes included: glycaemic control; insulin satisfaction; diabetes distress; and quality of life. Data were also collected on participant injecting behaviours; and at the end of the

study interviews were conducted to explore participants' views of the study. In the LH characterisation study, the participants injection areas were screened using ultrasound (US) with a blind comparison to physical clinical assessment (digital palpation) following a standard protocol for the assessment and recording the LH. These participants were shown were their LH lesions were and given advise on insulin site management (avoiding LH affected tissue) and insulin doses were reviewed to reduce the risk of potential hypoglycaemia.

Findings

A total of 27 participants were enrolled into the GV study, of which 15 completed the study and were included in the analysis. The median age of the completing participants was 32 (IQR, 25-60) years (range 20-71 years), with a median duration of T1DM of 14 (IQR, 10-23) years. In terms of the impact of LH on TIR and GV, one third of those participants demonstrated improvement in TIR (4-10mmol/L) $\geq$10%, the remainder showed limited improvement and one participant showed a reduction in TIR >10% (p=0.02). No significant changes were seen in the GV measures. The findings showed a significant improvement in the median percentage of effective bolus insulin injections (based on the glucose response to the insulin), with an increase of 17% from 69% (IQR, 62-73) to 86% (IQR, 82-93) (p<0.001). In some participants there were large reductions in the total daily insulin dose, in five participants these were 25, 20, 9, 8, 6 units respectively; of the remainder most had minimal dose changes, with two having a modest increase of 3 and 4 units. The LH characterisation study involved the US assessment and digital palpation of 74 participants. The US images showed that the LH was heterogenous in its morphology, with the main presenting features being: LH nodules; and areas of diffuse (dense) subcutaneous tissue. Additional observations included hypoechogenic tissue within nodules which could indicate necrotic tissue; inflammatory changes; and disruption to dermal tissue. The US screening identified 740 LH nodules in the participants, with 304 diffuse areas. The most common areas where LH were observed where in the lower abdominal and thigh areas. LH areas were graded from 1-5 (1 = diffuse LH; 2 = nodules <6mm; 3 = nodules $\geq$6mm to <8mm;

4 = ≥8mm to <10mm; and 5 = ≥10mm). Palpation was shown to have a moderate level of accuracy (Cohen's kappa 0.44) in detecting LH compared to US, missing over half of the areas detected via US.

Conclusion

The finding from this study provide some important new insights into the morphology of LH and its impact on clinical management. Overall, the study identified that the interaction between LH and glucose regulation is complex and challenging to study. The current data shows that changing injection sites in a small sample of participants with T1DM led to improvements in TIR for some participants and reductions in insulin doses. The study has also revealed that LH is complex and heterogenous in presentation, and a potential method for grading LH has been presented which could be adopted clinically with further validation. As the study was unable to identify a clear estimation of the association between LH and GV, determining the clinical significance of LH remains an important objective for future studies.

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Abbreviations list

Abbreviations	
ADA	American Diabetes Association
ATTD	Advanced Technologies & Treatment for Diabetes
BG	Blood Glucose
BMI	Body Mass Index
DCCT	Diabetes Control and Complications Trial
CGM	Continuous Glucose Monitoring
CINAHL	Cumulative Index to Nursing and Allied Health Literature
CONGA	Continuous Overlapping Net Glycaemic Action
CSII	Continuous Subcutaneous Insulin Infusion (insulin pump)
CV	Coefficient of Variation
DAFNE	Dose Adjustment for Normal Eating
DDS	Diabetes Distress Scale
DSN	Diabetes Specialist Nurse
EADS	European Association for the Study of Diabetes
EDIC	Epidemiology of Diabetes Interventions and Complications
EMBASE	Excerpta Medica dataBASE
FIT	First Injection Techniques
GDPR	General Data Protection Regulation

Abbreviations	
GP	General Practitioners
GSTFT	Guy's & St Thomas' NHS Foundation Trust
GV	Glucose Variability
HbA1c	Haemoglobin A1c
HRA	Health Research Authority
Hyper	Hyperglycaemia (episode of high blood sugar)
Hypo	Hypoglycaemia (episode of low blood sugar)
IDF	International Diabetes Federation
ITSQ	Insulin Treatment Satisfaction Questionnaire
IQR	Interquartile Range
KCL	King's College London
LH	Lipohypertrophy
MAG	Mean Absolute Glucose
MAGE	Mean Amplitude of Glucose Excursions
MDI	Multiple Daily Injections
MEDLINE	Medical Literature on-line
mmol/L	Millimoles Per Litre (the unit of measurement for blood sugar levels)
MODD	Means of the Daily Differences

Abbreviations	
NICE	National Institute for Health and Care Excellence
NIH	National Institutes of Health
PPI	Patient and Public Involvement
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analysis
QoL	Quality of Life
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
SC	Subcutaneous
SD	Standard Deviation
SMBG	Self-Monitoring of Blood Glucose
SOP	Standard Operator Procedure
SPSS	Statistical Package for Social Sciences
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
TDI	Total Daily Insulin
TIR	Time In Range
TBR	Time Below Range
TAR	Time Above Range

Abbreviations	
UK	United Kingdom
US	Ultrasound
WoS	Web of Science
1,5-AG	1,5-Anhydroglucitol

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Chapter 1. Introduction

Type 1 diabetes mellitus (T1DM) is an autoimmune disease that results in destruction of beta-cells in the pancreas with subsequent loss of insulin production and hyperglycaemia (Atkinson et al. 2014, Eisenbarth 1986). The only treatment for the condition is to compensate for the loss of endogenous insulin supply with insulin therapy. Most people with T1DM either follow a multiple daily injection (MDI) regimen involving ≥ 4 injections per day or use a continuous subcutaneous insulin infusion (CSII) device (insulin pump). Hence, people with T1DM are exposed to high levels of subcutaneous insulin. The development of lipohypertrophy (LH) due to the anabolic effect of insulin on the local tissues is a potential consequence of this exposure. This can lead to significant dense subcutaneous lesions, which may impact on and impede the absorption of insulin, potentially leading to erratic blood glycaemic control. While LH is a well-recognised phenomenon in the context of insulin use, currently there is a lack of knowledge about its effects on glucose regulation and the extent to which this contributes to clinically important events, such as excess hyperglycaemia or hypoglycaemia.

This thesis presents an exploratory study designed to contribute new knowledge on: the morphology of LH lesions using ultrasound technology as a means of characterising their presentation in people with T1DM; and to estimate the impact of LH on glucose regulation by comparing Time in range and glucose variability in 2 conditions (1. insulin is injected into LH affected area; and 2. participant avoids LH affected injection sites). This chapter introduces some of the key elements relevant to the conduct of the study, with consideration given to the following areas:

- Type 1 Diabetes mellitus
- Insulin therapy in people with type 1 diabetes mellitus
- Lipohypertrophy in association with insulin exposure

1.1 Type 1 Diabetes Mellitus

T1DM is a condition characterised by hyperglycaemia due to an absolute deficiency of endogenous insulin production (Daneman 2006) caused by the autoimmune destruction of insulin-producing beta cells in the pancreas (Bluestone et al. 2010, Maahs et al. 2010, Atkinson & Eisenbarth 2001). The factors that precipitate this process are not fully understood, but a combination of genetic susceptibility and environmental triggers, such as a viral exposure or dietary factors, have been postulated (Peng & Hagopian 2006). The incidence of T1DM onset peaks in childhood and adolescence, although 42% of cases occur in adulthood (Thomas et al. 2018). The prevalence of T1DM is increasing across the globe and it currently accounts for 10% of the total diabetes population (International Diabetes Federation [IDF] 2017a). There are national variations in the prevalence; Finland currently has the highest incidence with 57.2 cases per 100,000 people each year (IDF 2017b), and the lowest levels are reported in China and Venezuela (0.1 cases per 100,000 people each year) (Karvonen et al. 2000). In the UK, based on estimates from IDF report, a rate of 25.9 cases per 100,000 among children (aged <20) each year (IDF 2017b). It is also estimated that 29,000 children and up to 370,000 adults have T1DM in UK (The National Institute for Health and Care Excellence [NICE] 2015).

The hyperglycaemia that is a consequence of T1DM drives a range of potential microvascular and macrovascular complications that include: retinopathy, nephropathy; neuropathy; vascular disease and increased risk of mortality (Chawla et al. 2016). A landmark study, the Diabetes Control and Complications Trial, showed that intensifying insulin therapy to achieve near normal glucose levels (with a glycated haemoglobin target measured by HbA1c of 46 mmol/mol [6.4%]) led to a significant reduction in the development and progression of these complications (Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications [DCCT/EDIC] 2005, Diabetes Control and Complications Trial [DCCT] 1995). The intensive insulin treatment involved the administration of insulin (long and quick acting) up to four times a day or via a continuous subcutaneous insulin infusion (CSII), with dosage adjustments according to the results of the self-monitoring of blood

glucose (SMBG), adjusting for food intake and activity levels. Hence, the findings of this trial and the subsequent follow-up of participants indicates that intensive insulin management with tight glucose control over a prolonged period can significantly reduce diabetes complications and mortality hazard. However, it has also been observed that intensive insulin therapy can lead to problems, such as: weight gain (Russell-Jones & Khan 2007); hypoglycaemia (Diabetes Control & Complications Trial Research Group 1993, Reichard et al. 1990); and local tissue changes, such as the formation of LH (Blanco et al. 2013). Therefore, it is important to understand how to reduce these potential problems in the use of insulin in people with T1DM, such that insulin will have the maximum effect without these hazards. While there have been significant investigations on strategies to manage and reduce hypoglycaemia and, to a lesser extent, weight gain related to insulin use, there has been much less investigation of managing local tissue changes such as LH.

1.2 Insulin therapy in people with Type 1 Diabetes Mellitus

Prior to the discovery of insulin in 1921, T1DM was a fatal disease (Rosenfeld 2002). Since the discovery of insulin and its clinical application in the treatment of T1DM, there have been many advances in both the formulation of insulin and the method of delivering it. Initially, animal insulins were the main source of insulin; subsequently, human and more recently analogue insulins have become more popular (Shah et al. 2013). In terms of insulin delivery, there are multiple potential models for delivery, this includes using twice-daily mixed insulin, MDI models or CSII methods (with or without glucose sensors) (Shah et al. 2016). The most common model in the UK is MDI followed by CSII, although the latter is increasing particularly in paediatric diabetes (Olsen et al. 2015, Danne et al. 2007).

The advantage of the MDI and CSII models is that they provide more flexibility in intensifying insulin delivery in order to try and mimic normal insulin physiology. While insulin is the most important hormone in reducing hyperglycaemia systemically, it also has a significant localised effect. While historically animal insulins could cause atrophy

of local tissue through an immunological reaction to the animal proteins (Schernthaner 1993), the most common reaction observed in exposure to human and analogue insulin is an increase in local tissue synthesis and density in response to the anabolic effect of the insulin on fat and protein (Chowdhury & Escudier 2003, Hauner et al. 1996). Therefore, the most important and common localised tissue effect of insulin is the development of LH lesions which may have potentially detrimental effects on insulin delivery and absorption, with adverse consequences for managing diabetes.

1.3 Insulin related Lipohypertrophy

LH is characterised by the enlargement (hypertrophy) and to lesser extent proliferation (hyperplasia) of adipocytes in the subcutaneous tissue arising from the anabolic effect of exogenous insulin exposure (Hambridge 2007, Chowdhury & Escudier 2003, Richardson & Kerr 2003, Hauner et al. 1996). The anabolic effect of the insulin is related to the effect of the insulin on insulin growth factor (IGF) receptors (Clemmons 2012). It has also been suggested that local inflammation due to insulin exposure and injections may also increase the density of the subcutaneous tissue by stimulating an inflammatory response with the accumulation of macrophages into dense plaques of tissue (Anderson et al. 2008, Anderson & Shive 1997), although this hypothesis has not been confirmed in studies directly studying insulin exposed tissue. LH has also been associated with sub-optimal glycaemia control (Al Hayek et al. 2016). The prevalence of LH in the diabetes population has been varyingly reported with ranges from 30-64.4% (Blanco et al. 2013, Pavlovic et al. 2007) reflecting the methods used for LH case definition, although the pooled prevalence from a meta-analysis of 26 studies estimated it to be 38% (Deng et al. 2018), suggesting that it is a common problem.

According to the *First Injection Techniques* guidelines (FIT 2016) (which was founded by a group of diabetes nurses and sponsored by a medical technology company that makes insulin needles) on the management of injection sites, LH can be detected through clinical examination either palpation or through visual assessment (FIT 2016,

Frid et al. 2010a). However, this approach is focussed on detecting defined nodules rather than the wider tissue disruption that can occur with LH (Seyoum & Abdulkadir 1996). More recently, the use of ultrasound has been used to detect LH and has been shown to have higher diagnostic sensitivity both in terms of the frequency and the extent of the LH detected (Ghazaleh et al. 2018). Consequently, the prevalence of LH has been reported as being significantly higher (56%) when compared to clinical examination (Volkova et al. 2015). However, it is possible that this disparity reflects the fact that ultrasound is more sensitive in detecting LH at a sub-clinical level, as there is no currently recognised threshold for clinically significant LH. Clinical significance should reflect the point at which the LH's impact on glucose levels lead to extend hyperglycaemia and increased frequency of hypoglycaemia. Hence, while both clinical and ultrasound based studies suggest that LH is a common problem (Gupta et al. 2018, Kapeluto et al. 2018, Gentile et al. 2016a, Volkova et al. 2015), it is yet to be established at what level the LH becomes problematic in terms of glucose management and glycaemic control. Therefore, studies are needed to: (1) classify LH, identifying clinically relevant grades or levels where glycaemia becomes disrupted; and (2) identify and test strategies to prevent, minimise or manage the LH.

In terms of the clinical effect of LH, injections into the LH regions can attenuate insulin absorption and activity (Famulla et al. 2016, Johansson et al. 2005, Young et al. 1984, Thow et al. 1990). The adipose tissue in LH-affected tissue can become more fibrous, resulting in reduced blood vessels and flow around and through the tissue, leading to the decreased absorption of insulin and an altered insulin action curve (Heinemann 2010, Young et al. 1984). This effect has recently been investigated in glucose clamping studies to quantify the levels of insulin action and glucose response when insulin exposure is mediated by LH. Famulla et al. (2016) used a glucose clamp to maintain blood glucose concentration for 24 hours in the study participants, followed by regular insulin injections into the LH or normal tissue. Insulin absorption and action were found to be substantially diminished and showed increased response variability when the insulin was injected directly into LH areas in both studies (Famulla et al. 2016), In another study (Hovelmann et al. 2015) participants were fed the same meals

and evaluated using a mixed meal tolerance test, the study showed a net reduction in the impact of insulin on glucose when injected into an LH region of 20-25%. Slower absorption and decreased insulin action were identified when the insulin was injected into LH tissue rather than normal adipose tissue, with considerably greater post-meal glycaemic excursions (Hovelmann et al. 2015). It was strongly suggested by this study that insulin was not effectively absorbed when injected into LH tissue, leading to erratic glucose control with both high to low glucose variations. It should be noted, however, that such studies are conducted in very controlled conditions. So while they may show the effect of LH on insulin action in these conditions the extent to which this translates to the 'real world' context where multiple extraneous factors (injection behaviours, insulin requirement, carbohydrate estimation, exercise/activity and stress levels) influence glucose levels and insulin action is unknown. Nevertheless, these unpredictable blood glucose responses to insulin, may be very frustrating for people with diabetes, potentially undermining their self-confidence in optimally adjust their insulin therapy and increasing the risk of hypoglycaemia if they overcompensate with insulin doses or inject in areas not affected by LH (Vora & Heise 2013). The net impact of LH on patient behaviours may lead them to alter their self-management behaviours in a way that increases their overall glucose exposure to avoid hypoglycaemic events (Gradel et al. 2018, Gonder-Frederick et al.1997).

Hence, it is very likely that LH may contribute to glucose variability in people with T1DM. While there are multiple interpretations of the term 'glucose variability', it can be generally understood as a deviation of blood glucose from a mean or ideal value over time (hours and days) - a phenomenon that has also be described as 'glucose fluctuation' (Vora & Heise 2013). Glucose variability (GV) is also observed as day-to-day differences in glucose values obtained at set time points, or in the 24-hour blood glucose profile, and such variability goes under the term 'predictability' (Vora & Heise 2013). In people with diabetes using insulin therapy, the term 'within-subject variability' usually describes differences in the blood glucose response from one injection to another in the same individual (Vora & Heise 2013). This variability contributes to GV and is the sum of two components: 1) A *pharmacokinetic component*, determined by

the extent and rate of absorption, distribution and clearance of insulin and 2) a *pharmacodynamic component*, determined by insulin's metabolic effects (Heinemann 2002). Since the variability in insulin pharmacokinetics - which is commonly understood as variability in exposure between injections or 'within-subject variability' - for most insulin preparations is largely determined by the absorption profile of insulin, an understanding of the factors influencing insulin absorption is necessary in terms of improving glycaemic control and the long-term prognosis in people with diabetes.

Some studies have explored the correlation of GV and LH severity (Strollo et al. 2016, Blanco et al. 2013, Ibarra & Gallego 1998). However, the methods used to determine this association in these studies were varied and none identified the clinical relevance of the observations made (A full review and critique of these studies presented in the next chapter). Therefore, more data are needed to confirm the effects of LH on variations in glucose levels and to establish a system with which to classify the level and significance types of LH.

Factors with insulin use other than the anabolic effect of the insulin have also been postulated as contributing to develop LH development, many of which relate to patient level behaviours. These risk factors include the reuse of needles, failure to rotate the insulin injection sites and the utilisation of incorrect injection techniques (Grassi et al. 2014, Blanco et al. 2013, De Coninck et al. 2010, Vardar & Kizilci, 2007). In addition, the prevalence of LH has been associated with patient characteristics including low education levels, the duration of insulin use, younger age, and low socio-economic status (Al Hayek et al. 2016, Al Ajlouni et al. 2015, Ji & Lou 2014, Blanco et al. 2013, Hajheydari et al. 2011, De Coninck et al. 2010, Vardar & Kizilci 2007, Ibarra & Gallego 1998, Hauner et al. 1996). If LH is going to be prevented in the future it will be important to gain a greater understanding of these patient level factors in addition to establishing the clinical impact of LH. However, the focus of this study is to consider the clinical effect of LH on glucose regulation as a platform for further inquiry to inform their clinical management.

1.4 Summary

People with T1DM are exposed to high levels of subcutaneous insulin, potentially leading to the development of LH lesions. LH can significantly impair the rate of insulin absorption, thereby increasing variability in glucose levels and the risk of hypoglycaemia. The factors responsible for the development of LH are multifaceted and are related to individual patient behaviours, preferences and injection technique. It has also been established that clinical examination may not be adequately sensitive to detecting LH reliably, whereas ultrasound techniques while more sensitive may lack clinical specificity. In the absence of an objective model for determining when LH is clinically significant in respect of GV, it is difficult to determine what is the optimal method for screening LH. Without a clear clinical definition of LH thresholds and hazard it is likely that LH will remain an under recognised and under-managed problem. Consequently, it is important to develop a valid grading scale for LH, validated against measures of GV, which could then be used to determine the true extent of LH in diabetes populations, and according to which more robust strategies could be developed to prevent, screen and manage LH. This study will undertake some important preliminary work to help fulfil these objectives by exploring the relationship between different ultrasound detected LH profiles, glucose regulation (time in range), GV, glycaemic control and the incidence of severe hypoglycaemia in participants with T1DM.

1.5 Organisation of the thesis

The thesis is organised into five chapters, as follows:

- Chapter 1: Introduction;
- Chapter 2. Literature Review (incorporating a systematic review of glycaemic control and glucose variability in the context of LH);
- Chapter 3. The study methodology, including sample selection, standard operating procedures and the processes used for data analysis;
- Chapter 4. Study findings;
- Chapter 5. Discussion and conclusion.

Chapter 2. Background

This chapter presents the current theoretical and empirical knowledge relevant to this study, together with an overview of current practices for the management of LH in relation to people with diabetes treated with insulin. The chapter also presents a systematic review of current evidence in relation to the impact of LH on glycaemic control and variability. The chapter is organised as follows:

- Aetiology, pathophysiology of LH;
- Factors contributing to the development of LH;
- Current clinical guidance, policy and practice associated with LH;
- Current methods for the assessment and characterisation of LH;
- Systematic review of the impact of the LH on glycaemic control and variability.

2.1 Lipohypertrophy

LH is a form of lipodystrophy which is characterised by the accumulation of extra subcutaneous fat and protein. Lipodystrophy also encompasses lipoatrophy which is a loss of subcutaneous fat (Handelsman et al. 2013, Reeves et al. 1980). While lipodystrophies are associated with a number of different conditions (Monajemi et al. 2007, Mandal et al. 2006), they are most commonly observed in diabetes in the context of subcutaneous insulin administration. The relationship between lipodystrophy and diabetes is long-standing, with reports of the lipodystrophic effects of insulin going back to the 1930s, where it was observed that frequent injections of insulin into the same area of skin may lead to changes in subcutaneous tissue, sometimes presenting as a swelling (LH) or as a dissipation of tissue observed as dips and hollows in the subcutaneous tissue at the site of injection (lipoatrophy), (Richardson & Kerr 2003, Rowe & Garrison 1932, Eeg-Olofsson 1930). The incidence of lipoatrophy has diminished over recent years as this was largely caused by an autoimmune response to the now rarely used, animal insulins (Hussein et al. 2007, Richardson & Kerr 2003), and hence following the introduction of human and analogue insulins lipodystrophy in diabetes is almost exclusively seen as LH (Richardson & Kerr 2003).

While the data on the pathology of LH are limited, it is generally accepted that LH involves hypertrophy and hyperplasia of adipocytes in area of the subcutaneous tissue with frequent insulin exposure (Vardar & Kizilci 2007, Hauner et al. 1996). The pathophysiology of LH results from the consequence of the localised anabolic effect of insulin on adipocyte growth (Singha et al. 2016, McNally et al. 1988). The physiological mechanisms that drive the tissue synthesis behind LH are quite complex. In normal physiological conditions adipocyte growth is in part mediated by insulin growth factor in particularly insulin growth factor 1 (IGF1) (Boucher et al. 2016). As IGF1 and insulin are molecularly very similar, insulin can bind on to the insulin growth receptor on the adipocyte thereby stimulating cell growth. Hence, it is almost inevitable that tissues that are over exposed to insulin will become hypertrophic. Indeed, histologic examination of LH tissues show an increase in the adipocyte size, with insulin exposed cells being nearly double the volume of non-exposed cells (Fujikura et al. 2005).

It has also been hypothesised that a mild inflammatory process may contribute to the development of LH lesions. It has been suggested that the introduction of material into the body during the injection process can cause an inflammatory reaction that may increase the density of the effected tissue (Anderson et al. 2008, Anderson & Shive 1997). Insulin antibodies have also been associated with LH in children and adolescents with T1DM (Raile et al. 2001), although their direct pathogenic role in this condition has not yet been established. Indeed, there is no evidence that insulin antibodies are promote the growth of adipocytes (Vardar & Kizilci 2007, Hambridge 2007, Hauner et al. 1996).

The phenotypical characteristics of LH are heterogeneous, and LH can be complex in presentation. LH areas usually present either as a nodule, formed of hypertrophic adipocytes, or as fibrocollagenous scar tissue within the dermis (Wallymahmed et al. 2004) and can vary in size from relatively small and undetectable lesions to lesions that are extremely large in size (Hambridge 2007). While these lesions are mostly firm,

they can occasionally present as a soft lesion which maybe more difficult to detect in standard physical examination (Gentile et al. 2016a). A more detailed characterisation of LH can be gained from ultrasound scans. The scanning of the injected subcutaneous tissues can show nodular areas of concentrated dense tissue, as well as more general areas of increased echogenicity suggesting some increased density to the subcutaneous tissue presenting as diffuse areas of disrupted tissue. The nodules and diffuse areas often present together (Kapeluto et al. 2018, Bertuzzi et al. 2017, Mulnier et al. 2017, Perciun & Mihu 2014). Kapeluto et al. (2018) defined this change further as the nodules not having a capsule or vascularity, which differentiates the US signature of LH from haematomas or fluid filled cysts, which do have capsules (Kapeluto et al. 2018). In some cases, LH nodules contain hypoechogenic areas which may be fluid (oedema) or areas of necrosis (Bertuzzi et al. 2017, Perciun 2010). Thickening of the dermal layer and loss of a clear delineation between the subcutaneous and dermal layer at an injection site has been identified as a potential inflammatory response to repeated injections of insulin (Bertuzzi et al. 2017, Perciun 2010). Fluid examination from nodules has sown high insulin content within the intra-nodular fluid (Gentile et al. 2018).

There are very few histological studies of LH. Fujikura et al (2005), presented a case-study with a detailed histological investigation of an extensive LH area which was surgically excised. The tissue was analysed using Hematoxilin-cosin tissue staining and electron microscope examination. The analysis showed significant hypertrophy of the adipocytes in the insulin exposed area, the cells were double the size of those observed in the unexposed tissue. They also observed some evidence of hyperplasia, with infiltration of adipocytes into the dermal tissue and an increase number of smaller less mature adipocytes being observed in the LH affected area amongst the enlarged adipocytes. As an explanation for the latter observation, they point to studies showing that adipocyte hyperplasia is known to occur in obesity, when adipocytes volume is doubled; and the fact that insulin is known to mediate the proliferation of preadipocytes. In another case-study, a tissue sample of a large LH lesion was taken (Wallymahmed et al. 2004). Tissue biopsies of the central core of the lesion showed

hypovascular collagen with fibroblasts, suggestive of tissue damage. They also reported small areas of necrosis. Overall, these limited data concur with the assumption that LH is largely mediated by the anabolic effect of insulin leading to adipocyte hypertrophy and hyperplasia, alongside some general inflammatory changes which may be related to intensive tissue invasion and possibly trauma.

2.2 Factors associated with the development of LH

The risks for developing problematic LH are multifactorial and accumulative. Patient behaviours and insulin delivery systems have all been considered as risk factors, the most commonly cited risk factors in the literature are: lack of site rotation; needle reuse; a high or low body mass Index (BMI); frequency of injections; incorrect injection technique; a low level of general education; longer duration of diabetes and longer duration of insulin use; female gender; younger age; needle length; and low socio-economic status (Sürücü & Arslan 2018, Al Hayek et al. 2016, Al Ajlouni et al. 2015, Ayad et al. 2014, Ji & Lou 2014, Blanco et al. 2013, Hajheydari et al. 2011, De Coninck et al. 2010, Vardar & Kizilci 2007, Ibarra & Gallego 1998, Hauner et al. 1996, Seyoum & Abdulkadir 1996). A lack of site rotation was consistently reported to be the most significant risk factor in these studies, although it was also the most frequently studied. The evidence for each of the factors that have been implicated in LH development are outlined below.

2.2.1 Insulin injection behaviours

Failure to rotate insulin injection sites effectively or size of rotation area for injections has been identified as a strong contributing factor to the development of LH. Despite extensive education and training on self-management, injection technique and insulin administration, in general people treated with insulin still do not rotate between injections sites (De Coninck et al. 2010). A survey of insulin site rotation in 201 people with T1DM, found that 22% of respondents used only one site, with the most common reason for not changing the site being a fear of pain (Patton et al. 2010). LH occurs because people with diabetes tend to inject into the same site repeatedly. LH frequently occurs in areas that are convenient to inject in, and where the individual's

hands reach most naturally. In Gentile et al.'s (2016b) study of 60 people with diabetes using insulin and having LH lesions, participants' injection techniques were assessed using a questionnaire and 37% (n=22) of the participants report that injecting into LH affected areas is less painful, reinforcing their preference for the LH site.

Needle reuse is another commonly reported factor influencing the development of LH. The use of the same needle more than once can cause damage to the tip of the needle and lead to a loss of needle lubrication, making the injection more traumatic to the injection area and with the subsequent tissue damage potentially accelerating the development of LH (Waddingham 2008, Vardar & Kızılcı 2007, Teft 2002). Blanco et al. (2013) assessed the frequency of LH in 430 people with diabetes using insulin-injecting (the study included both T1DM and T2DM) and found that more than 56% (n=240) of the participants reused needles at least once. Another cross-sectional study of 174 people with T1DM (52%, n=91 with LH and 48%, n=83 without LH) showed that infrequent needle changing was associated with a near eight-fold increase risk of developing LH (Adjusted OR= 7.47, p= 0.001) (Al Hayek et al. 2016).

2.2.2 Body mass Index

While studies have identified BMI as a factor related to LH, the evidence is contradictory. A cross sectional study of people with diabetes treated with insulin (223 people with T1DM and 56 people with T2DM) found that people with higher BMI were less likely to have LH (estimated OR= 0.82, p< 0.01) (Hauner et al. 1996). The authors argue that people with higher BMI have a larger surface area to inject into, potentially reducing the concentration of insulin exposure (Hauner et al. 1996). While Al Ajlouni et al. (2015) in a study of 1090 people with T2DM diabetes treated with insulin, found that LH was significantly higher among those with high BMI (between 35 and 39.9 kg/m2) (p= 0.034). This finding may be related to the fact that increased BMI is associated with insulin resistance and hence the potential for higher insulin dosages, thereby increasing subcutaneous insulin exposure. However, when BMI was modelled alongside other factors in a multivariate logistic regression it was not significantly associated with LH (Al Ajlouni et al. 2015). A further factor to consider in relation to

the studies reporting a lower incidence of LH in obese people may be that the LH is harder to detect in people who are overweight. Overall, evidence around the relationship between BMI and LH is weak, as the studies follow different methods and were conducted in different samples so the conclusions that can be drawn are equivocal. Most importantly, it is very difficult to extrapolate from either of these studies to the T1DM population as the insulin exposures and phenotype of T1DM and T2DM are very distinct.

2.2.3 Diabetes duration

The duration of diabetes is also suggested to have a link with the presence of LH (Wallymahmed et al. 2004). In a study by Al Ajlouni et al. (2015) of 1090 people with T2DM diabetes treated with insulin, LH was identified in 71% of people with a duration of 10 or more years, compared to (29%) in those <10years duration. Another study of 65 people with T1DM diabetes with LH showed that duration of diabetes was associated with a one-fold increase risk of developing LH (OR =1.16, 95% CI 1.05-1.32, p< 0.01) (Omar et al. 2011). None of these studies were prospective, so while length of insulin use would seem to be logically related to the development of LH, neither study provides insights into the length of exposure and the presentation of LH.

Therefore, from the current evidence available it would seem that the most important and modifiable factors contributing to LH are behavioural and relate to insulin injecting practices.

2.3 Effect of LH on insulin absorption

Insulin absorption has been shown to be attenuated in LH affected sites compared to other sites without LH in the same individual (Gradel et al. 2018). In the case of advanced LH, the subcutaneous tissue is reported to be fibrous. The rate of insulin absorption—which depends on diffusion of insulin molecules across the endothelial barrier into the blood stream—may be reduced due to the lack of blood vessels in the area (Heinemann 2010). It has been shown that metabolic control in people with diabetes with LH is improved when they are instructed to apply the insulin into other sites, indicating that insulin absorption may be impaired when they repeatedly use sites with LH (Blanco et al. 2013). A recent crossover study of 13 people with T1DM, using a euglycemic glucose clamp with deliberate injections into LH showed significant blunting of insulin absorption profiles and markedly increased variability when compared to injections into adjacent normal tissue (Famulla et al. 2016). People with diabetes and LH have been reported to have higher average insulin requirements as well as higher rates of unexplained hypoglycaemia and GV (Blanco et al. 2013). Despite these data, the full extent to which LH impacts on insulin absorption and subsequent glucose regulation has not been studied clinically. It is important, therefore, to consider whether LH is associated with increased risk of adverse clinical outcomes by impeding insulin adsorption thereby potentially generating fluctuations in glucose levels. Hence, further studies are required to consider the clinical significance of LH and its impact on glucose regulation.

2.4 Assessing and Managing LH in Clinical Practice

Increasing awareness of the importance of LH in diabetes care has led to the development of several international guidelines for managing injection areas and for detecting LH (Frid et al. 2016a, FIT 2016). The two main international guidelines/recommendations for the assessment of LH are those from the American Association of Diabetes Educators (AADE) (Siminerio et al. 2011) and the Forum for Injection Technique (FIT 2011). These guidelines recommend that LH must be assessed annually, and that site rotation of injection sites be emphasised at each routine care visit. A clinician should ask people with diabetes how they are managing

their injection sites and should aim to use open-ended questions to elicit as much information about injecting behaviours as possible as these practices can be quite complex (Teft 2002). The assessment should also include a physical examination and palpation of the injections sites to look for any signs of problems or signs of LH (Pledger et al. 2012). The clinician is advised to palpate the tissue using their fingers in the areas that the individual identifies as their injection sites (Siminerio et al. 2011, FIT 2011). Any nodules that are visible and/or palpable should be marked and recorded so that recovery can be monitored and compared at future appointments (FIT 2011). The current FIT guidelines recommend that the clinician uses an examination lamp and lubricating jelly to improve the sensitivity of the palpation technique (FIT 2016). One recent multi-centred UK study examining the implementation of one of FIT guidelines (n=75 participants, 55 with T2DM and 20 with T1DM) reported that two thirds of those assessed demonstrated improved injecting behaviours (increased site rotation and LH avoidance), they also reported: reductions in the palpated size of nodules; decreased insulin doses (a mean of 5.6 units) and metabolic improvement (a mean reduction of 4.4 mmol/mol [0.4%] in HbA1c) (Smith et al. 2017). However, much of the data collected in this studies was based on self-report, assessmnet fidelity was not reported and there were was no control group.

Furthermore, the extent to which these guidelines are implemented in routine clinical care is unknown, nor is it known how frequently or rigorously LH is assessed in general (Gentile et al. 2016a). A limitation of the guidelines is that they are not based on empirical evidence, or supported by a validated screening method for the detection of clinically important problematic LH; instead they rely on clinical examination by health professionals, which will be variable and may underestimate or fail to detect significant LH. A recent observational study considering how lesion features (size, location and shape) influence the ability of trained and non-trained health professionals to identify LH, based on physical examination of typical injection sites, found that the trained health professionals were unable to identify some lesion types particularly the flat small type and arm-localised LH lesions (Gentile et al. 2016a). Furthermore, an observational study using ultrasound to examine the injection sites of a 215 people

with diabetes has shown that smaller areas or less dense LH areas may be missed with the palpation method (Volkova et al. 2013). Therefore, more specific screening techniques are required to ensure that most clinically relevant LH is detected.

2.5 LH prevalence by screening methods

2.5.1 Physical Examination

LH prevalence based on physical assessment suggest that LH is common in people with T1DM, although there is some variation between studies in the levels of prevalence, which ranged from 30 to 64% (Table 1).

Table 1 LH prevalence based on physical examination

Author (Year)	Diabetes Type	Sample size (%T1DM)	Duration of Insulin exposure mean±SD, median (range) years or n=(%)	LH prevalence
Hauner et al. (1996)	T1DM & T2DM	279 (80% T1DM)	NS	28.7% (T1DM)
Ibarra & Gallego (1998)	T1DM & T2DM	150 (75% T1DM)	11.4 ±7.9	52%
Kordonouri et al. (2002)	T1DM	282	3.7 (0.1-18.8)	47.8%
Vardar & Kizilci (2007)	T1DM & T2DM	215 (14.4% T1DM)	Years n (%) 0–5 66 (30.7) 6–10 59 (27.4) 11–15 57 (26.5) 16–20 33 (15.4)	48.8%
Omar at el. (2001)	T1DM	119	NS	54.9%
Blanco et al. (2013)	T1DM & T2DM	430 (41% T1DM)	(6-13 T1DM) (1-5 T2DM)	64.4%
Cunningham & Mckenna (2013)	T1DM & T2DM	55 (75% T1DM)	19.2 ±13.6 with LH 10.6 ±10 without LH	51%
Grassi et al. (2014)	T1DM & T2DM	346 (not stated the T1DM)	13.0 ±9.8	49%
NS, Not Stated				

This variability may be explained by a number of factors, most notably the lack of a standardised and validated (in terms of accuracy) method for detecting the LH. These studies used a range of procedures including visual inspections and palpation of injection sites. Furthermore, the studies lacked details as to the level of training the examiners received and were there were multiple examiners inter-rater assessment reliability was not reported. Only one study followed a published protocol and detailed that the examiners were trained (Grassi et al. 2014), However, in this study they did not differentiate participants with T1DM and T2DM, so it is difficult to relate the prevalence specifically to those with T1DM. Indeed, only two studies were exclusive to T1DM with one study giving a separate prevalence for the T1DM participants (Hauner et al. 1996). While most of the studies reported duration of insulin use and insulin doses; none of the studies adjusted for duration and volume (daily insulin dose) of insulin exposure, which may be a relevant factor in considering the prevalence of LH. It may also be that pathogenesis of LH is different in T1DM and T2DM; while the former may have longer duration of insulin exposure, the latter are generally more obese and insulin resistant which means there insulin doses can be quite large compared to those with T1DM. Therefore, without a more objective screening method it is difficult to form a true estimate of the incidence of LH. Hence, in the next section consideration is given to studies using ultrasound to identify and characterise LH reveal.

2.5.2 Ultrasound Detection of LH

Ultrasound (US) provides the opportunity to look in more depth at the extent to which LH is present in insulin exposed areas as well as providing a more detailed insight on the characteristics of LH affected areas. US can provide a much more detailed perspective on the nature of LH compared to palpation in respect of the size and depth of tissue changes observed following repeated insulin exposure (Bertuzzi et al. 2017, Perciun & Mihu 2014, Blanco et al. 2013, Volkova et al. 2013, Perciun et al. 2012, Volkova & Davidenko 2011, Perciun 2010, Kasperska-Czyzyk et al. 2000).

A study of 215 people with diabetes (type not specified) treated with insulin (Volkova et al. 2013) reported that US scanning detected 56% more LH lesions than with palpation alone. Bertuzzi et al.'s (2017) study identified overall equivalence in the detection of LH using US and palpation, although they found that US was able to detect more sites in the arm and gluteus regions than palpation. This study also reported high precision in the US-assessed LH region in relation to the size and distribution of the affected areas (Bertuzzi et al. 2017). The LH extensions were noted to be 5cm^2 bigger with US (~35 ± 10 cm^2) compared to those recorded by palpation and inspection (~30 ± 15 cm^2).

This suggests that US may be a more sensitive method for detecting LH compared to palpation. However, no standard protocol for using US to detect LH has yet been established. Furthermore, it could be that using US as a method lacks specificity in relation to detecting clinically important LH, as some levels of LH may be non-problematic, and it is likely that all people using insulin will develop LH to some level. This lack of clinical specificity as a problem in the management of LH in general, as with most screening-based diagnostic it is important to have some means of estimating the clinical risks to patients so that these can be graded in respect of the size and distribution of the problem. In the context of diabetes this might best be considered in respect of glycaemic control and GV as they may confer hazard for short- and long-term diabetes complications.

2.6 Grading of LH

Attempts have been made to develop grading systems for LH. Based on physical examination, Kordonouri et al. (2002) offered a scale with four grades: Grade 0 = no changes; Grade 1 = visible hypertrophy of fat tissue but palpably normal consistency; Grade 2 = massive thickening of fat tissue with higher consistency; and Grade 3 = lipoatrophy. Conwell et al. (2008) used a slightly different scale also with four grades: Grade 0 = absent; Grade 1 = mild; Grade 2 = moderate; and Grade 3 = severe. However, neither of these studies actually provided any measure of size to qualify LH as mild, moderate or severe. Nor did they report data on the clinical validity of these grading systems in relation to GV, poor glycaemic control of the presence of problematic hypoglycaemia. A study by Hauner et al. (1996) used a ruler to scale the size and the height of the LH above the skin; the researcher also classified LH as 'discrete' when tissue swellings did not exceed a size of 3 cm in diameter and of 0.5 cm in height. However, again the relationship between the degree/different sizes of LH and changes in glycaemic control was not studied. This has led to the assumption that larger palpable nodules cause more glucose fluctuation than smaller lesions, and if the nodules cannot be seen or felt, then they are not causing a problem. The latter element of this assumption may lead to an underestimation of the impact of LH on glucose regulation.

Four studies attempted to classify LH into types or grades using US. Perciun (2010) included five levels for LH grading: 1) nearly-normal, 2) diffuse echogenicity (fibrous tissue) with no well-defined delineation between dermis and subcutis, 3) focal areas within this tissue (nodules within diffuse areas), 4) focal areas with hypoechogenic halos within the nodules, a thickened dermal layer and loss of delineation between the dermis and subcutis layers, 5) Nodules with a hypoechogenic necrotic or liquid-filled areas and thickened dermis (Perciun 2010). Mulnier et al. (2017) further identified a four-level grading scale of LH based on: the presence of diffuse areas; nodule size and nodule number; and inflammatory changes. Bertuzzi et al. (2017) characterised LH on the basis of hyperechogenic regions with prevailing fibrosis, hypoechogenic areas and mixed hypo/hyperechogenicity. Kapeluto et al. (2018) provided a more

granular assessment of LH, reporting whether the nodules were capsulated or had evident vascularity to provide five levels of classification: 1) well circumscribed either by hyperechoic foci with defined borders or a nodular shape with a hypoechoic halo, 2) heterogeneous in echotexture compared with surrounding tissue, 3) associated with distortion of surrounding connective tissue with 4) absence of vascularity and 5) absence of capsule (Kapeluto et al. 2018) .

US has additional advantages over palpation as it can better assign the nature and severity of LH in much more detail compared to palpation enabling greater granularity in grading the LH (size, distribution and density); thereby giving clinicians the opportunity to give more detailed advice to patients. Therefore, further studies are needed to determine an optimal grading systems and measurement method to determine LH. Such studies need to formulate and validate these systems and methods in relation to the GV.

2.7 Prevention and management of LH

Primary prevention refers to prevention of a condition prior to its occurrence and secondary prevention relates to minimising the effect of a problem once identified/diagnosed. In terms of primary prevention of LH the emphasis in current guidelines has been on optimal injection technique, including site rotation and avoidance of needle reuse (FIT 2016, Kalra et al. 2016). However, the effectiveness of the injection site rotation protocol advocated in the current guidelines has not been extensively studied (Ibarra & Gallego 1998), with the exception of one study of insulin-treated people with diabetes which suggested that this method may have some protective value against LH (Blanco et al. 2013). Of the 430 participants who took part in the study, 64% (n= 277) had LH; only 2% (n=6) of them had used the rotation technique correctly and 92% (264) were not using rotation or the technique correctly (Blanco et al. 2013). The low rate of LH in the group using the correct technique may be related to the fact that these participants were more recently diagnosed and may not yet have developed significant LH. Overall, the high prevalence of LH in people

with diabetes suggests that primary prevention is currently ineffective. Therefore, optimal strategies for site rotation and their impact on LH development are yet to be identified. Furthermore, any method of site rotation is dependent on the willingness of the patient to observe the rotation protocol and there may be many reasons why patients may find following the rotation difficult. Including remembering to change sites or that for social and psychological reasons patients may develop a preference for certain sites.

Another factor that can impact on patient behaviours in managing their injection sites is the health education received from health care professionals. In a systematic litreature review by Frid et al. (2010b), of studies that asked people with diabetes about the information and training they had received on insulin injection techniques, most of the studies reported that people with diabetes could not identify having received any education on insulin injection technique or site management. This was also the case even when people with diabetes had attended education sessions that included site rotation training; this implies that either people with diabetes do not retain the information provided at education sessions or they chose not to follow the suggested advice (Pledger et al. 2012). It may be that current advice is too complicated or not practicable for people with diabetes to follow in the course of their daily life routines (Pledger et al. 2012); or that there is a lack of awareness within the community of health professionals and insulin-treated people with diabetes on the prevalence and significance of LH.

Once LH develops, the management is to change the injection sites and avoid the affected areas in the hope that regression will occur. Recommendations as to the length of time that insulin injection into LH tissue should be avoided varies between studies (Hauner et al. 1996, Wallymahmed et al. 2004). The FIT guidelines recommend not injecting into sites close to LH site and that some areas may need to be rested for more than one year (FIT 2016), although they provide no objective data to support these recommendations. The study of Perciun and Mihu (2014), which

included 53 children with T1DM, after a six months period of avoiding the LH areas, the clinical examination diagnosed the remission of some hypertrophies and nodular aspects. Also, the US technique identified more residual images and certified which types of echostructures had recovered or not. The US showed reduced echogenicity when the sites had been rested, suggesting dissipation of the LH, but not in all cases and particularly not in those showing greater fibrosis of the fat tissue (echogenicity), or in those with possible necrosis at baseline scan. However, as the study was conducted in children who are still growing and developing and may have different rates for repairing tissue damage, these observations may not be common to adults with diabetes.

If avoidance of affected areas or any of the other therapeutic options fail (such as switch from multiple daily injections to CSII), some clinical groups have reported on the use of more invasive techniques such surgical extraction or liposuction (Barak et al. 1996, Samdal et al. 1993). However, while such procedures may result in good cosmetic outcomes (Hardy et al. 1993, Samdal et al. 1993) their impact on the capacity of the remaining tissue in insulin dispersal and glucose regulation is unknown (Richardson & Kerr 2003).

In the next section a detailed systematic review of the literature addressing the impact of LH on glycaemic control and GV in people with diabetes who are treated with insulin.

2.8 Systematic review of the impact of LH on glycaemic control and variability

A systematic review of the empirical literature examining the association between LH and GV and/or glycaemic control in insulin-treated people with diabetes was undertaken to inform the conduct of the study. The review was designed to fulfil the following objectives:

- to identify and retrieve all primary studies examining the relationship between LH and GV and/or control;
- to critically appraise the identified studies to determine their quality;
- to consider the methods used to determine LH and measure GV;
- to extract data on the relationship between detected LH and GV and glycated haemoglobin (HbA1c);
- to synthesise the collective evidence from the identified studies to estimate the observed association between LH and GV and HbA1c.

2.8.1. Review method

This section details the methods used to perform the systematic review, consideration is given to study identification; study inclusion criteria; data extraction; critical appraisal; and data synthesis. The Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) statement was followed for the conduct and reporting of this review (Moher et al. 2009).

2.8.1.1 Study Identification

Appropriate studies were identified primarily through a protocol-based search of electronic databases, supplemented by citation searching, secondary references, key author searching and hand-searches of relevant journals to enhance the sensitivity of the search. Given that the review aimed to identify observational studies examining the association between LH and GV and HbA1c with potentially a high level of heterogeneity in respect of study types, a protocol was developed with high sensitivity, comprised of two facets: population (terms and synonyms for diabetes and LH) and outcome (terms and synonyms for HbA1c or GV). Following consultation with a library information specialist scoping searches of electronic bibliographic databases representing nursing, medicine and social sciences were conducted to refine the search. Each search was adapted to reflect the indexing and search guidelines for each database. These scoping searches and key articles were used to refine the selection of keywords and index terms for the protocol. The scoping searches suggested that the inclusion of the outcome filter (HbA1c and GV) was too specific excluding potentially relevant studies, hence the final protocol focused exclusively on diabetes and LH terms. Furthermore, the scoping review identified that many studies include both participants with T1DM and T2DM, and in many cases these were not differentiated in the studies. However, because the likely mechanism and impact on GV is common, in term of how it may impede the absorption and action of insulin, it is likely that there will be some commonality between the effects of GV and insulin in both (people with TIDM and T2DM). Therefore, it was deemed to be appropriate for the overall review to consider both types. However, it is acknowledged that the differences between T1DM and T2DM might bring some confounding elements in the association between LH and HbA1c and GV. Therefore, consideration will be given in sub-analysis to determine whether or not there was any distinction in variability reported in articles that were exclusively on T1DM. The full search terms used are described in Appendix (1).

To facilitate the search process, the thesaurus of terms was checked to identify additional relevant terms. Index terms were focused or exploded according to relevance of specific terms. Truncation symbols and Boolean operators were used

when appropriate to create a more concise search. Limitation by database filters such as “humans” was also employed. The syntax of the search terms was then customised for each specific database. The selected databases were searched individually, and the citations from each database were electronically exported to Endnote version 9.0, checked for duplicate references, and then combined into a single EndNote reference library. Duplicate references were identified using EndNote automated duplicates finder and by manual sorting, then recorded and removed prior to screening.

LH is studied in multiple research and clinical contexts; hence a wide range of bibliographic databases were chosen; MEDLINE (Medical literature on-line) which contain medical literature, published in medical, nursing and health journals (Polit & Beck 2013, Bruce et al. 2008); EMBASE (Excerpta Medica dataBASE) containing biomedical articles (Polit & Beck 2013, Bruce et al. 2008); CINAHL (Cumulative Index to Nursing and Allied Health Literature) containing studies from other relevant disciplines including nursing (Grove et al. 2012); and WoS (Web of Science database) includes conference abstracts (Chadegani et al. 2013). The databases were searched from inception until 18th March 2019. Table (2) presents an example of the search strategy for EMBASE database. Detailed search strategies for each database are reported in Appendix (1).

Table 2: An example of the Searching Strategy for EMBASE database

Index Term	**P** (Diabetes Mellitus)	**O** (Lipohypertrophy)
Key words Mesh terms	exp diabetes mellitus/ exp insulin dependent diabetes mellitus/ exp diabetic patient/	exp lipohypertrophy/ exp dystrophy/ exp lipodystrophy/ exp hypertrophy/
Free text	Diabetes Mellitus.mp. Diabetes mellitus type 1.mp. Insulin dependent diabetes mellitus.mp. Insulin-dependent diabetes patients.mp. Insulin-dependent diabetic patients.mp. Type 1 diabetes.mp. Type 1 diabetic.mp. T1D.mp. T1DM.mp. DM type 1.mp. IDDM.mp. Juvenile diabetes.mp. Diabetes mellitus type 2.mp. Type 2 diabetes.mp. Type 2 diabetic.mp. T2D.mp. T2DM.mp. DM type 2.mp. Diabetic patients.mp. Diabetes.mp. Diabetic.mp. Diabetes*.mp. Diabetic*.mp. Diabetie$.mp. Insulin treated patients.mp. Insulin-treated patient$.mp.	lipohypertrophy.mp. lypohypertrophy.mp. Diabetic lipohypertrophy.mp. Diabetes lipohypertrophy.mp. lipohypertrophic.mp. Lipohypertrophied.mp. lipohypertrophies.mp. Insulin dystrophy.mp. Dystrophy.mp. Dystrophies.mp. Subcutaneous Dystrophy.mp. Subcutaneous dystrophies.mp. Subcutaneous tissue dystrophies.mp. Lipodystrophy.mp. Lipodystrophies.mp. Lipodystrophic.mp. hypertrophy.mp. Fat hypertrophy.mp. Fat lump.mp. Fatty lump.mp.

The citations yielded from this search were screened for eligibility. The titles of the citations were read and excluded if they clearly did not meet the research criteria. Subsequently the abstract for the remaining citations were reviewed, including citations where it was not possible to determine from the title whether they met the search criteria. When duplicate conference abstracts were presented, the most recent was chosen. Pertinent abstracts were selected from the review and full text articles retrieved and read to determine whether they met the inclusion criteria. Full texts were also retrieved to check for relevance in the case of citations where the eligibility was ambiguous from the abstract alone. Authors were contacted when the abstract was deemed to meet the inclusion criteria, but the full text was not available. The cited references of the relevant articles identified were then screened to search for any unidentified studies (Greenhalgh & Peacock 2005). The citation was scrutinised, and full text articles read. The reference list of the *First Injection Technique* (FIT) guideline (FIT 2015, FIT 2016) was searched to check for potentially eligible articles not retrieved by the computer search. The following conferences abstract websites were searched for unpublished studies: American Diabetes Association (ADA); Diabetes UK; European Association for the Study of Diabetes (EADS); and Advanced Technologies & Treatment for Diabetes (ATTD). The final decision to include studies was based on the explicit inclusion criteria, differences were resolved with discussion by the research supervisors.

2.8.1.2 The **Inclusion criteria** were:

- Participants with T1DM as per study criteria or people with diabetes treated with insulin therapy (including both T1DM and T2DM)
- Studied the relationship between LH and HbA1c and/or GV in insulin-treated people with and without LH
- Studies where the population comprised both adult and paediatric with diabetes were included if separated data were provided for the adult participants
- Observational studies using quantitative methods (cross-sectional or cohort studies), experimental studies were included if they provided a baseline estimation of LH and GV and HbA1c at baseline.

2.8.1.3 Studies and reports were **excluded** as follows:

- Case studies, clinical audits, non-systematic literature reviews, guidelines, editorials and opinion pieces
- Studies in which the study population comprised only T2DM or paediatrics, or gestational diabetes or people with human immunodeficiency virus (HIV)
- Participants receiving insulin via a CSII (insulin pump).

The search was not limited by language, in order to maximise identification of relevant literature; however, only articles published in the English Language were included. The Publication date was not a limiting criterion to enhance the sensitivity of the search strategy. The results of this search and the selection criteria are summarised in the PRISMA flow diagram in Figure 1 (Moher et al. 2009).

Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)

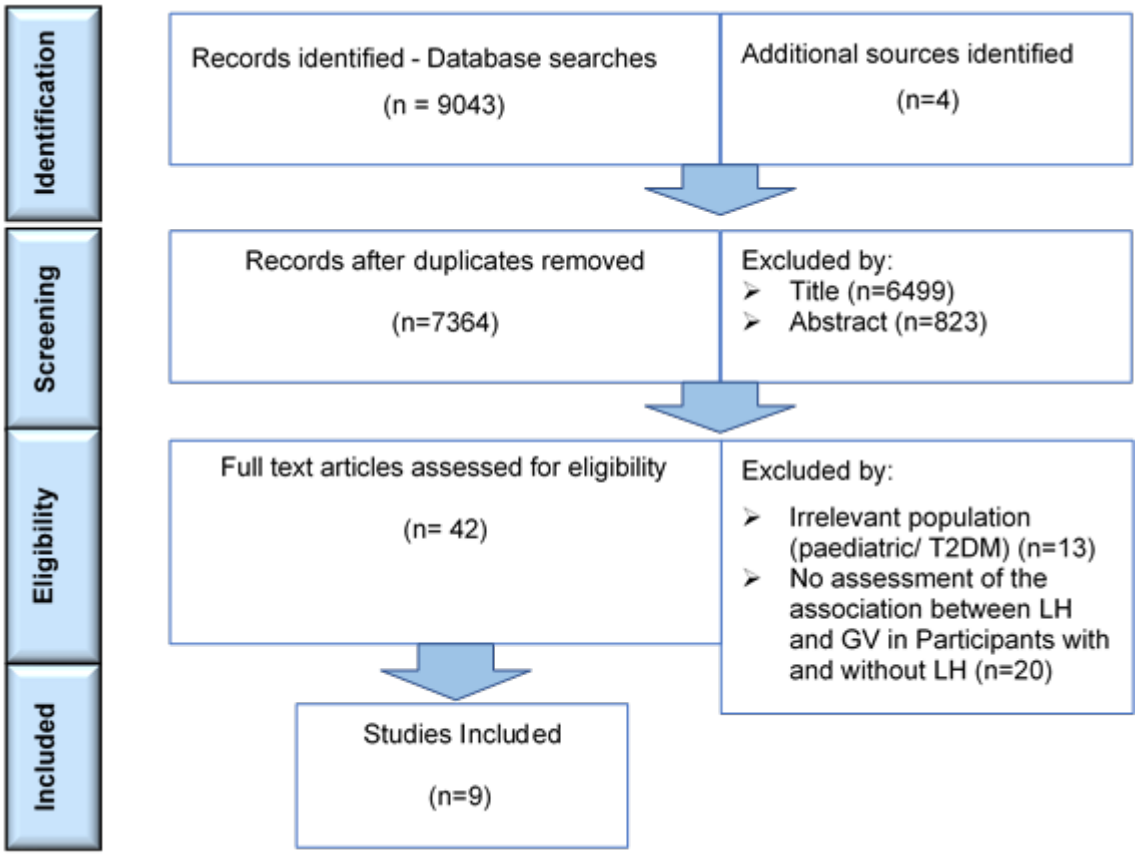


Figure 1: PRISMA flow chart of database searches

2.8.1.4 Data extraction

Studies meeting the inclusion criteria were obtained and reviewed for inclusion. Data were extracted using spreadsheet (Excel), based on the review objectives (Appendix (2). Items were developed, and the data extraction sheet was piloted and refined on three studies (Blanco et al. 2013, Ibarra & Gallego 1998, Hauner et al. 1996). The extraction sheet covered the following topics:

- study characteristics (first author's last name, year of publication and location);
- Study objectives and study design;
- participant characteristics (age, gender, diabetes type, duration of diabetes, duration of insulin use, injection sites);
- method of detection, definition, grading and classification of LH;
- the examiner who undertook detection and the procedure that followed to detect the LH;
- HbA1c and GV;
- prevalence of LH.

2.8.1.5 Quality Assessment

To establish the methodological quality of the selected articles and their strengths and limitations, each article was critically appraised to determine the overall quality of the research. As anticipated in the research inclusion criteria, most of these studies were either cross-sectional or cohort observational studies. Therefore, the relevant studies were assessed using the Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies devised by the National Institutes of Health (NIH 2014). This tool measures 14 different criteria included; study design, selection of participants, validity and reliability of study instrument (LH detection methods)/, and statistical analysis. Each items of the tool were evaluated as "yes," "no," "not applicable," "cannot determine," or "not reported." This was then used to guide the overall rating for the quality of each study as good, fair, or poor. Included articles were scored based on

their fulfilment for each item in the appraisal tool and the checklist of the identified tool is presented in Appendix (3). All quality assessments were performed by a single assessor.

2.8.1.6 Data synthesis

The purpose of data synthesis is to present a valid interpretation of the collective bodies within the work of the studies included in the review. Three different methods of data synthesis were used: tabulative summary, narrative synthesis and meta-analysis. The narrative synthesis was used to summarise findings collectively in respect of observations that did not aggregate to form a consistent data presentation, but which offered interpretation to a common point. In this review, the extracted data from studies were grouped according to study characteristics and then tabulated to summarise in a standardised way the study participants, methods and findings. In seven studies that reported HbA1c values for those with and without LH, a statistical meta-analysis was undertaken using the RStudio version 1.1.456 software (RStudio 2015). The meta-analyses were performed using a random effects model to combine the results of individual studies allowing for study heterogeneity. Forest plots showing the point estimate and confidence intervals for each study were created. The heterogeneity was quantified by the inconsistency test I^2, which quantifies the percentage of total variation across studies that is due to heterogeneity rather than chance (Higgins et al. 2003), where 0%, 25%, 50% and 75% indicate, respectively, absent, low, moderate and high heterogeneity among studies (Higgins et al. 2003).

2.8.2 Review Findings

2.8.2.1 Search outcomes

The electronic search identified 9043 studies published from 1950 to 18th March 2019, 6499 of which did not meet the inclusion criteria at title screening and there were 1683 duplicates. After reviewing the abstracts, 42 studies were selected for further evaluation, the full text was obtained, read and screened against the eligibility criteria and nine studies were deemed eligible to be part of the review. Studies that did not meet the eligibility criteria were excluded and the reason for excluded is detailed in the PRISMA flow chart (Figure 1).

The characteristics of the studies, the sample, the detection method of LH and GV /glycaemic control data are presented in Table 3 and 4. All studies were observational; most of the studies utilised a prospective approach except one which used a retrospective approach. Seven studies presented data on HbA1c in participants with and without LH (Deeb et al. 2019, Pozzuoli et al. 2018, Ji et al. 2017, Strollo et al. 2016, Hajheydari et al. 2011, Hauner et al. 1996, McNally et al. 1988) and three studies presented data on GV in participants with and without LH (Strollo et al. 2016, Blanco et al. 2013, Ibarra & Gallego 1998).

2.8.2.2 Quality assessment

The NIH quality assessment tool for observational cohort and cross-sectional studies was used to assess the quality of included studies (NIH 2014). A study scored a point when it fulfilled a criterion with the scores displayed in Appendix 3.1, a maximum score of 14 is possible. Five studies had an overall rating of good (score 9 to 12) and four studies were rated fair (score 7 to 8). In general, studies lacked sample size justification and level of follow-up, three studies (Hajheydari et al. 2011, Ibarra & Gallego 1998, McNally et al. 1988) did not provide information about the reliability and validity of the examination criteria or the procedure of LH detection. Despite the overall diversity of the studies' quality, they all yielded data of relevance to the review objectives.

2.8.2.3 Study and participant characteristics

Six of the included studies were conducted in Europe (United Kingdom, Germany, Italy and Spain) and three further studies were carried out in Asia (China, United Arab Emirates and Iran). Three of the included studies were older than ten years (Ibarra & Gallego 1998, Hauner et al.1996, McNally et al.1988).

A total of 2565 participants treated with insulin were included in the review, out of these 48% (n=1239) participants had LH. The participants had a mean age of 47.56 years (± 23) and include 46% (n=1140) male and 54% (n=1353) female participants, and one study did not report the gender. In the studies identifying type of diabetes 32% (n=713) were T1DM and 68% (n=1506) were T2DM. One study (Deeb et al. 2019) included exclusively people with T1DM. Four studies reported duration of diabetes and this ranged from 3 months - 41 years (Strollo et al. 2016, Blanco et al. 2013, Hajheydari et al. 2011, Ibarra & Gallego 1998).

2.8.2.4 Prevalence, detection method and anatomical distribution of LH

In studies using visual inspection and digital palpation to detect LH the reported prevalence ranged from 14.5% to 77.1%. Four of the most recent studies (Deeb et al. 2019, Pozzuoli et al. 2018, Ji et al. 2017, Strollo et al. 2016) described the detection method procedure of LH, further three studies (Deeb et al. 2019, Pozzuoli et al. 2018, Strollo et al. 2016) followed a standardised approach “the pinch manoeuvre” as described by Gentile et al. (2016) and one study took into consideration the body positions of participants when they evaluated the injection sites and examination environment (Ji et al. 2017). None of the studies reported any grading method of LH, apart from one study, which classified LH as “discrete” when tissue swellings did not exceed a size of 3 cm in diameter (Hauner et al. 1996). Eight studies examined the anatomical distribution of LH sites, with the abdomen, thighs and arms being the most frequently identified, with the gluteal area being the least commonly used (Deeb et al. 2019, Pozzuoli et al. 2018, Ji et al. 2017, Blanco et al. 2013, Hajheydari et al. 2011, Ibarra & Gallego 1998, Hauner et al. 1996, McNally et al. 1988).

2.8.2.5 Measurement of GV in the included studies

In the studies that reported GV, in participants with and without LH, the GV was crudely assessed using methods based on self-monitoring of blood glucose. The GV definitions varied between the studies: both Strollo et al. (2016) and Blanco et al. (2013) defined GV as 'fluctuations of blood glucose values from between < 3.3 mmol/L (60 mg/dL) to > 13.9 mmol/L (250 mg/dL) on at least three occasions per week in an unpredictable manner, for at least six months (Blanco et al. 2013 p. 446 – 447). While Ibarra & Gallego (1998) study defined GV as 'irregular or unstable when more than two glycaemic fluctuations per week were unexplained' (Ibarra & Gallego 1998 p. 9). Clearly, both methods were not well characterised and with a high risk of measurement error and inconsistency.

2.8.2.6 Association between LH and GV

Strollo et al. (2016) study demonstrated that participants with LH 70.1% (n=255) had at least a fourfold increased risk of developing GV compared with those who have not had LH 29.9% (n=109), (OR=4.43 (95%CI 3.11-6.33), p< 0.001. While the Blanco et al. (2013) study, found that participants with LH 93% (n=136) had nearly a fourteen-fold increased risk of developing GV compared to those without LH 7% (n=10), (OR=13.79 (95 % CI 6.97 to 27.31), p < 0.001. In the Ibarra & Gallego (1998) study of 128 participants, of which 43% (n=55) had glycaemic profile fluctuations, the study showed that participants with LH 75% (n=41) had nearly a fivefold increased risk of developing GV compared with those who have not had LH 25% (n=14), (OR=4.71 (95 % CI 2.18 to 10.15), p< 0.001. (Table 3: presents the reported relationships identified between LH and GV for each study).

2.8.2.7 Association between LH and glycaemic control

Seven studies reported observation on the association between LH and glycaemic control. All but one study reported that participants with LH had higher HbA1c levels compared to those without LH (see Table 4), which reports the HbA1c levels observed in those with and without LH. It is important to note that there were some differences in the characteristics of the comparison or reference samples without LH in the studies that are summarised in Table 5.

Table 3: Reviewed studies examining the association between LH and GV

References location	Study design	Population characteristics				Assessment and prevalence of LH %(n)	GV % (n) with and without LH (OR (95%CI)
		n (T1DM)	n with GV	Gender % (n)	Mean age (SD)		
Strollo et al. (2016) Italy	Retrospective observational study	387 (81)	364	M45.5(176), F 54.5(211)	61(±16)	Visual inspection & palpation 77.1 (298)	LH present: 70.1 (255) LH absent: 29.9 (109) OR = 4.43 (3.11-6.33) p< 0.0001
Blanco et al. (2013) Spain	Cross-sectional	430(177)	146	M 51.4 (221), F 47 (202)	49(±22.8)	Visual inspection & ultrasound was used in 78 64.4 (277)	LH present: 93 (136) LH absent: 7 (10) OR = 13.79 (6.97 - 27.31) p < 0.0001
Ibarra & Gallego (1998) Spain	Cross-sectional	150 (113)	55	M 38 (57), F 62 (93)	36.9 (±17.9)	Visual inspection & palpation 52 (78)	LH present: 75 (41) LH absent: 25 (14) OR = 4.71 (2.18 - 10.15) p = 0.0001
GV, Glucose Variability; LH, Lipohypertrophy; n, Number; M, Male; F, Female							

Table 4: Reviewed studies examining the relationship between LH and glycaemic control

References location	Study design	Population characteristics			Assessment and prevalence of LH %(n)	HbA1c with and without LH (mean±SD) mmol/L [%]
		N (T1DM)	Gender %(n)	Age (Mean± SD) Year		
Deeb et al. (2019) United Arab Emirates	Cross-sectional	65	NS	54.65 ±16	Visual inspection & palpation Examiner: trained team 32.3 (21)	LH present: 69 ±9.1 [8.5 ±0.83] LH absent: 54 ±9.8 [7.1 ±0.9] p= 0.001
Pozzuoli et al. (2018) Italy	Cross-sectional	352 (36)	M 43.2 (152), F 56.8 (200)	68 ±12	Visual inspection & palpation Examiner: well-trained healthcare 42.9 (151)	LH present: 61 ±15.3 [7.7 ±1.4] LH absent: 61 ±14.2 [7.7 ±1.3] p=0.83
Ji et al. (2017) China	Cross-sectional	401 (27)	M 49.9 (200), F 50.1 (201)	59.6 ±11.5	Visual inspection & palpation Examiner: Trained study staff 53.1 (213)	LH present: 66 ±19.7 [8.2 ±1.8] LH absent: 61 ±16.4 [7.7 ±1.5] p=0.003

References location	Study design	Population characteristics			Assessment and prevalence of LH %(n)	HbA1c with and without LH (mean±SD) mmol/L [%]
		N (T1DM)	Gender %(n)	Age (Mean± SD) Year		
Strollo et al. (2016) Italy	Retrospective observational study	387 (81)	M 45.5 (176), F 54.5 (211)	61 ±16	Visual inspection & palpation Examiner: medical staff 77.1 (298)	LH present: 67 ±13.1 [8.3 ±1.2] LH absent: 58 ±12.0 [7.5 ±1.1] p< 0.001
Hajheydari et al. (2011) Iran	Cross-sectional	220 (56)	M 27.3 (60), F 72.7 (160)	49 ±17.9	Visual inspection & palpation Examiner: one specialist physician 14.5 (35)	LH present: 80 ±24.0 [9.5 ±2.2] LH absent: 72 ±20.8 [8.7 ±1.9] p= 0.03
Hauner et al. (1996) Germany	Cross-sectional	279 (223)	M 44 (123), F 56 (156)	41.4 ±13.3	Visual inspection & palpation Examiner: Trained physician 32.3 (90)	LH present: 74 ±19.7 [8.9 ±1.8] LH absent: 73 ±18.6 [8.8 ±1.7] p=0.68
McNally et al. (1988) United Kingdom	Cross-sectional	281 (NS)	M 54 (151), F 46 (130)	45.5 ±19.75*	Visual inspection & palpation Examiner: two researchers 27.1 (76)	LH present: 81 ±28.4 [9.6 ±2.6] LH absent: 77 ±26.2 [9.2 ±2.4] p=0.24
LH, Lipohypertrophy; n, Number; M, Male; F, Female; NS, Not Stated; SD, Standard deviation; * Estimate SD from range						

Table 5: Characteristics of participants with and without LH

References	(n (%); or mean ±SD and /or range)	
	LH present	No LH
Hajheydari et al. 2011	Gender: M 17 (28.3), F 18 (11.2) Education Level: Elementary/Secondary school level: 31 (86) University level: 4 (11.4)	Gender: M 43 (71.7), F 142 (88.8) Education Level: Elementary/Secondary school level: 172 (93) University level: 13 (7)
McNally et al.1988	Mean age: 40 (11-82) Gender: M 40 (52.6), F 36 (47.4) Mean duration of insulin use: 14.3 (±9.8) Injection site/LH aware: 43 (56.6)	Mean age: 46.3 (7-86) Gender: M 111 (55), F 90 (45) Mean duration of insulin use: 9.4 (±9.7) Injection site/LH aware: 117 (58.2)
Hauner et al.1996	Total Daily Insulin (Units): 49 (±16)	Total Daily Insulin (Units): 45 (±15)
Strollo et al. 2016	Mean age: 61 (±10) Gender: M 100 (34), 211 (66) Duration of insulin use: 17 (±9)	Mean age: 63 (±12) Gender: M 87 (98), F 2 (2) Duration of insulin use: 20 (±8)
Pozzuoli et al. 2018	Mean age: 68.2 (±12.6) Duration of insulin use: 11.6 (±9.6) Total Daily Insulin (Units): 48.8 (±25.9)	Mean age: 67.3 (±11.9) Duration of insulin use: 7.2 (±7.3) Total Daily Insulin (Units): 37.8 (±21.4)

A meta-analysis was performed to assess the overall difference observed in the collected studies in HbA1c values for those with and without LH. Participants with LH had clinically significantly higher HbA1c values than participants without LH. The estimated difference for the studies was 6.1 (95% CI 2.1 -10.2) mmol/mol [0.56 (95% CI 0.19-0.93) %], which represents a clinically important difference in respect of the risk of diabetes complications. A high heterogeneity among studies was observed (I^2 = 83%, p for heterogeneity <0.01) (see figure 2).

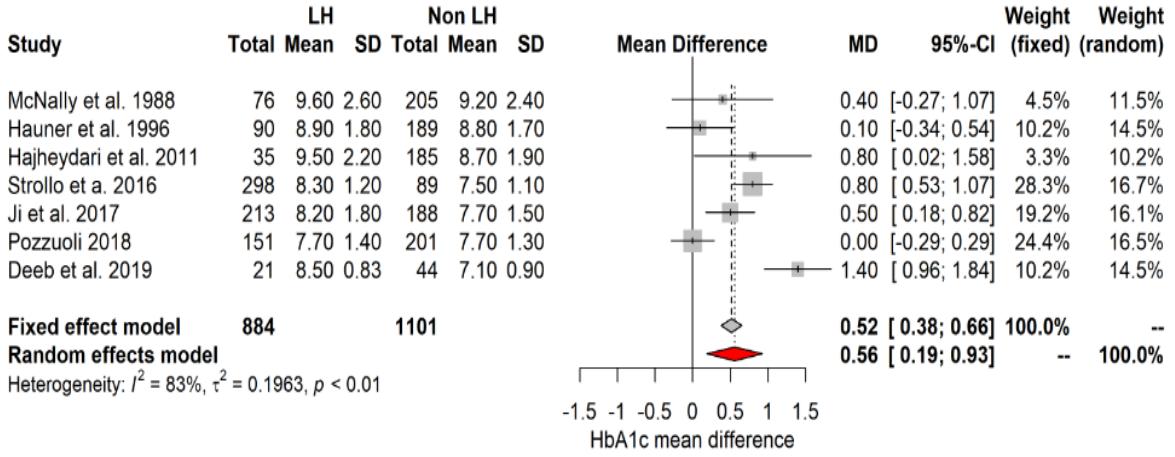


Figure 2: Forest plot mean difference (95% confidence interval) in HbA1c for participants with LH versus participants without LH.

A sub-analysis excluding studies that included animal insulin was also undertaken. Excluding these studies increased the estimated difference in HbA1c levels between the LH and non-LH participants to 7.4 mmol/mol [0.68%] (see Figure 3).

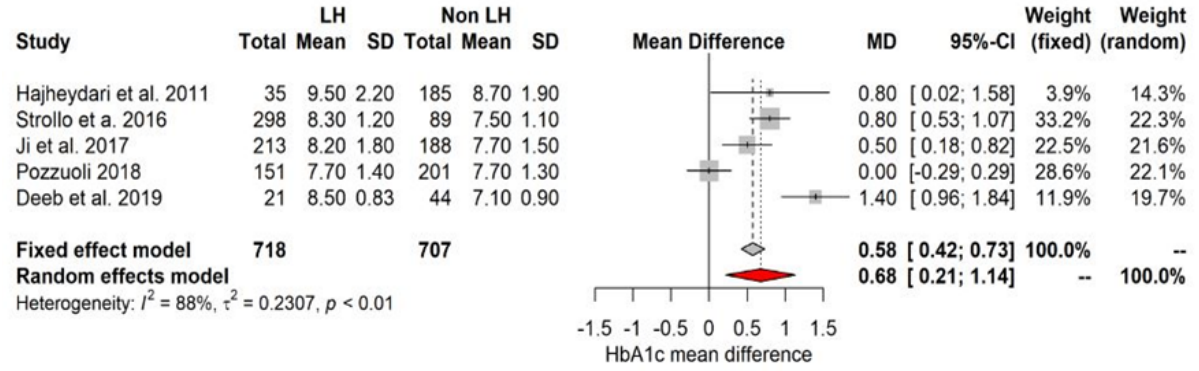


Figure 3: Forest plot showing mean differences (95% confidence interval) in HbA1c for participants with LH versus participants without LH in studies using human insulin and/or insulin analogues.

2.8.3 Review Discussion

This systematic review and meta-analysis identified nine studies, evaluating the association between LH and GV and glycaemic control in insulin-treated people with diabetes. The majority of these studies reported findings relating to glycaemic control and only three were related to GV. The glycaemic control studies suggest that participants with LH had higher glycaemic levels compared to those without LH. The limited data available on the association between GV and LH was inconclusive and limited by the methods used to assess the GV.

The prevalence of LH varied across studies by some margin. This in part maybe explained by the variation in the methods used for detecting LH. While most of the studies reported that they used visual inspection and palpation, they did not specify any quality assurance or fidelity protocols. As result of the inconsistency the estimations of LH prevalence may be either under or over estimated.

The association between glycaemic control and LH is difficult to interpret from these studies. While the LH may have been a contributing factor, it could also be inferred that people who are prone to develop LH due to limited insulin site management may have deficits in other areas of their diabetes self-management (such as glucose monitoring and carbohydrate adjustments). Ji et al. (2017) reported that in a study of 401 adult participants injecting insulin, correct site rotation was more common in participants without LH (92.3%) compared to those with LH (67.6%), $p < 0.0001$. The same observation was seen in the study of Pozzuoli et al. (2017), the participants with LH were less likely to rotate the site of injection (45.7 vs. 59.7%; $p = 0.028$). Therefore, it is not possible to identify from these studies the extent to which the LH contributed to the levels of glycaemic control observed.

The data do suggest, however, that people with diabetes using insulin need regular reviews of their insulin injecting behaviours and some guidance on avoiding LH, although there is no current best evidence on site-rotation models. In one survey with >1,000 participants performed in Europe, less than 50% of the participants reported that they were taught about LH (Strauss et al. 2002), and there were no details on the quality or validity of the advice provided. The study of Smith et al. (2017) which reported some improvements in glycaemic control with the introduction of LH assessment and standardised injection education suggests that some LH intervention may lead to improved control.

The relationship observed in these studies is not explanatory; it is associative. Therefore, we do not know whether the relationship is causative. It is known, for example, that psychological problems can mediate self-management performance in people with diabetes, psychological distress has been associated with higher glycaemic levels (Snoek et al. 2015). Therefore, it may be that people with diabetes who have problems such as depression or are less attentive to their diabetes self-management may be less diligent in relation to their injection behaviours. Perhaps psychological factors may be important in relation to the development of LH. It could also be that having LH may contribute to diabetes distress by making glucose management more unpredictable and frustrating, hence further studies are required to consider the association of psychological factors on LH. In addition, as highlighted in Section 2.2 (factors associated with the development of LH) injection technique, the number of injections performed, the reuse of injection needles, the size of the injection area usually used, and other related factors all seem to contribute to the development of LH (Ji et al. 2017, Blanco et al. 2013). This again would suggest that people with diabetes need more support in increasing their awareness of LH and how to avoid it.

However, before any recommendations can be made on how to improve the management of LH, the review findings suggest that there are many uncertainties and

unknowns in respect of LH and its impact on metabolic control and glucose regulation that need further consideration. A significant area of weakness in the current knowledge base is the lack of consistency in how LH detected and characterised. It is also not clear from the previous studies on LH at what point they become clinically problematic in terms of diabetes control. Consideration below is given to the limitation of the review and what its findings relay for the conduct of this study and future research in general.

2.8.3.1 Strengths and Limitation of the review

1. Limited source material for the review- Multiple electronic databases and other search strategies were applied in order to decrease the chance of missing relevant articles. Additionally, the reference lists for each study were checked and turned to citation tracking to identify many relevant articles. However, despite using these methods only nine studies were included, although it is likely that this was a fairly complete capture of the studies that have consider LH and GV and/or glycaemic control.

2. Heterogeneity and lack of clarity on LH detection methods and GV assessment- While the included studies reporting using similar detection methods for LH (visual inspection and palpation), there was no clear definition of the protocol observed. Neither did the studies give details of the characteristics of the LH observed. The GV methods were also very weakly defined, in the Strollo et al. (2016) and Blanco et al. (2013) studies, GV was defined as ‘fluctuations of blood glucose values from between < 3.3 mmol/L (60 mg/dL) to > 13.9 mmol/L (250 mg/dL) on at least three occasion per week in an unpredictable manner. While Ibarra & Gallego (1998) study defined GV as ‘irregular or unstable when more than two glycaemic fluctuations per week were unexplained’ (Ibarra & Gallego 1998 p. 9). The clinically specificity of these definition and the interpretation of unpredictable/unexplained is unclear. Hence, the review was

not able to offer any meaningful interpretation of the relationship between GV and LH. Looking forward to future more vigorous methods for the assessment of self-monitored blood glucose results to standardised criteria are required. The optimal method for assessing GV is continuous glucose monitoring (CGM). CGM measures interstitial fluid every 5-10 seconds and an average glucose value is calculated every five minutes, although there is some variability between CGM systems (Funtanilla et al. 2019, Secretariat 2011). This gives a more complete picture of daily glucose fluctuations by providing a continuous trend of the glucose level. In tandem with individual level data inputs (food intake, insulin use and activity) it is possible to consider how these different glucose affecting events impact on the glucose level. In the context of studying LH this would allow for these factors to be assessed when considering the impact of LH on the GV and to estimate the impact of injected insulin on the glucose level.

Following these limitations, we recommend that future LH studies need much more robust methods for assessing GV, that also allow for the assessment of some of the main extraneous factors that can influence glucose levels. There should also be standard reporting of LH (including grading system) and optimally this should be undertaken using US. For observational studies, it is also necessary to minimise potential information or classification biases by using appropriate participant selection methods adjusting for differences in the comparison groups (age, gender, duration of diabetes). Prospective studies are also needed, involving enough participants to consider the effect rather than the association between LH and GV. Before and after studies with participants changing injection sites to estimate the impact of LH affected areas on GV should be considered, prior to larger trials where participants with a standardised levels of LH can be randomised to usual injection areas versus injecting into areas not affected by LH to consider differences in GV and insulin requirement. Although a big challenge to overcome is how to change individual behaviours in the intervention groups and how to avoid behaviour change in the control groups once participants have had their LH identified (this also raises some ethical dilemmas too).

These factors were, as far as was feasible, consider and adopted within the study design as outlined in the next chapter.

2.9 Summary

This chapter has set out the empirical data available regarding LH, highlighting the prevalence of LH and detailing some of the risk factors associated with it. The review has also identified that there may be some associations between LH and GV and LH and glycaemic control, although these observations are limited by the paucity of methods used in the studies. Particular attention has been drawn to the lack of standardised procedures to detect LH and to define GV. Hence, this study will undertake some preliminary work to explore the association between LH and GV; and develop a process for assessing and characterising LH in a more robust way. The following chapter sets out the aim and objectives for this study and the adopted study design for this project, which was developed with reference to the findings of the literature review.

Chapter 3: Methods

This chapter details the method and instruments used in the conduct of the study. The chapter discusses the design adopted by the study and outlines the rationale for the adopted measures and the model for the analysis. Consideration is given to the following:

- Study aim and objectives
- Study approach and theoretical perspectives
- Study design and hypotheses
- Study setting, population and sample
- Study measures
- Data collection and management
- Data analysis
- Process evaluation
- Validity and Reliability
- Ethical issues in the conduct of the study

3.1 Aim and objectives

The study aims and objectives for the study were developed in response to the issues identified in the previous chapter. The primary aim of the study was to explore the association between ultrasonographically characterised LH lesions with time in range (TIR 4 to 10 mmol/L) and glucose variability (GV) in people with T1DM. The secondary aim was to identify a process for assessing and characterising the LH. The study objectives were to:

1. Characterise (location, morphology and distribution) different presentations of LH tissue in participants with T1DM using ultrasound.
2. Investigate the effect of observed LH presentations on GV; glycaemic control; and/or incident severe hypoglycaemia.
3. Study the relationship between LH and participant insulin-injecting behaviours, and type of insulin.
4. Compare physical clinical assessment of LH (palpation) to ultrasound detected LH.
5. Consider participants' experiences of changing their injection sites.
6. Assess: study procedures; recruitment, retention and completion rates; and adverse events.

3.2 Study approach and theoretical perspectives

In the previous chapter the underpinning clinical issues were identified, together with a review of current knowledge on the study topic. This review revealed that there is currently a lack of standardised methods for assessing and characterising LH, and the clinical impact of LH on glucose levels and variability is poorly understood. Clearly, these are important considerations for an intervention to minimise the impact of LH on glucose management in people with T1DM. Hence, what is required is a study that can begin to identify optimal methods for characterising LH and assessing its impact on glucose regulation in people with T1DM. Therefore, this research was undertaken

as a preliminary study to consider how LH can be characterised and to explore its potential impact on glucose regulation and GV, to inform the development of a future intervention. In this section, consideration is given to different methodological strategies for undertaking such a study and the rationale for the study approach.

The first step in identifying a study approach should be to consider the level of theory to be addressed. Dickoff and James' (1968) classic taxonomy of theory progression in clinical inquiry provides a useful point of reference in determining the level of theory for the study. Their taxonomy identifies four levels of theory development:

1. Factor isolation—at this level the focus is on identifying a phenomenon or an activity that has clinical relevance. This level in relation to LH, has to some extent been addressed in previous studies, as we know that LH is a clinical problem with some data to show that it has a clinical impact on glucose regulation. However, what is not known is how the problem might be best addressed. It is also evident that more understanding of the nature of LH is required, particularly in how it should be characterised and assessed.

2. Factor relating—at this level the focus is on building a theoretical context for the original observation considering what activities or strategies might be useful in addressing the problem. In the context of this study this would be related to determining whether LH impacts on glucose levels, as this has only partially been addressed in previous studies. It would also be important to consider other factors such that may be important for a future intervention, such as how do people with diabetes find changing their injecting behaviours.

3. Situation relating—at this level the focus is on identifying factors that might inform an intervention and see if they can make a clinical impact (questions of efficacy). In

the context of this study that would be to test the impact of an intervention (avoiding LH areas) to estimate its impact on glucose levels.

4. Situation producing—at this level the focus is on assessing the probability that an intervention has a predictable impact on a specific outcome (questions of effectiveness). This level would be typically addressed by a larger study, usually a clinical trial, with the adequate statistical power to establish with confidence whether changing an injection site to avoid LH (following an explicit reproducible process) would improve glucose levels and GV.

Considering this taxonomy, it would seem that the level of theory desired from this study is largely Level 2., although there is also some primary knowledge required in respect of characterising LH. The characterisation of LH is important as the size and distribution of the LH might influence the effect the LH has on an individual's glucose levels. It is also clear from the literature review that there are potentially multiple components and behaviours involved in assessing and managing LH, which introduces a degree of complexity. Addressing this complexity will be kept to nay future intervention. Therefore, as the ultimate purpose of this study would be to inform a future intervention to help reduce the impact of LH on people with diabetes, it is useful to consider the study with reference to the Medical Research Council's (MRC) complex evaluation framework. The MRC have defined complex research as follows: "the greater the difficulty in defining precisely what exactly are the 'active ingredients' of an intervention and how they relate to each other, the greater the likelihood that you are dealing with a complex intervention" (Campbell et al. 2000). The MRC complex evaluation framework is similar to the taxonomy of Dickoff & James (1968), as it follows a process for developing theory and translating it into an intervention. At its inception the framework was comprised of four clinical phases (following a pre-clinical theory-building phase):

- Phase I- the modelling phase, in which potential mechanisms of action are explicated and intervention components are modelled in relation to the outcomes of interest.
- Phase II- exploratory studies, these studies focus on the acceptability, feasibility and efficacy of an intervention.
- Phase III- requires a definitive randomised controlled trial designed to assess the complexity of the intervention.
- Phase IV- long-term follow-up and replication studies.

The MRC complex evaluation framework has subsequently been updated into a more iterative process that involves: defining and understanding the clinical problem; conceptualising the problem using established theory to identify the argument for the relationship between the intervention and the problem; collecting diverse evidence to enable more expansive assessments of confounding variables; and optimising the intervention and outcomes selected to ensure a valid assessment of the intervention (Campbell et al. 2007). The proposed LH study will require some modelling in respect of the characteristics of the observed LH and the relationship with GV, but it will primarily sit within the exploratory phase, by exploring the impact of avoiding LH areas on GV. In keeping with the modified version of the MRC guidelines for evaluating complex interventions, the study will also consider: the relationship between the problem and the intervention (LH and GV); collecting different data sources that may be relevant to the impact of LH and avoiding LH affected areas (insulin type and injecting behaviours); and in estimating the potential outcomes that could be useful in studying the impact of LH on glucose regulation.

In the exploratory phase of the MRC framework, the principle approach is to undertake feasibility or pilot studies prior to the main study, to refine/consider: data collection processes, outcome selection; and potential intervention components. Distinguishing between feasibility and pilot studies can be confusing; a recent international

consensus meeting has produced guidance on how to distinguish the two (Eldridge et al. 2016). The guidance states that: “A feasibility study asks whether something can be done, should we proceed with it, and if so, how. A pilot study asks the same questions but also has a specific design feature: in a pilot study a future study, or part of a future study, is conducted on a smaller scale.” (Eldridge et al. 2016). These guidelines specify that pilot studies should be viewed as a type of feasibility study, which tests out a basic assumption (such as the change that would form part of an intervention) to inform a future study- pilot studies can be either randomised or non-randomised. As one objective of this study is to test the assumption that GV will be reduced if insulin is injected into an area not affected by LH, then this element would be considered a pilot-study. The study also aims to address some additional feasibility questions such as: identifying an approach for characterising and assessing the LH, and practical issues in relation to a future study such as recruitment and retention.

In relation to developing insights from feasibility studies that will inform the conduct of a future study, particularly in respect of areas of complex intervention, a process evaluation should be conducted. The current MRC guidance on process evaluations recommends that process evaluations should begin with feasibility studies (Moore et al. 2015). Process evaluations study the setting, the implementation, and the mechanisms of an intervention to support the interpretation of the findings (Oakley et al. 2006). In the MRC process evaluation framework there are three areas: context (the impact of contextual factors on the intervention); implementation (fidelity, dose, reach and adaptation); and mechanisms of impact (considering participant experiences, mediating factors and unexpected occurrences). In the context of this study, it is important therefore, to assess some of these processes, to inform the development of an intervention to reduce the impact of LH; and to provide information on how to conduct a future study assessing such an intervention. As this study will assess the impact of changing injection behaviours on GV, it will be important to consider the participants’ experiences of this change as well as finding ways of

assessing adherence. In terms of a future study it will also be important to estimate recruitment and retention (reach). There is also a need to consider the fidelity of the LH assessment procedures and to decide whether it is necessary to adapt the study in anyway.

A recent international consensus project, classified feasibility and pilot studies as exploratory studies (Hallingberg et al. 2018). The study group behind this project, defined the purpose of *exploratory* as to decide whether and how to proceed with full-scale evaluations. In terms of this study the *how* part relates to the need to understand some of the issues that need to be considered in any future research: methods for assessing LH and characterising it; procedures for the conduct of the study such as supporting people with diabetes in changing injection behaviours; and how to optimise study recruitment and retention. In relation to *should,* the key consideration is to establish whether avoiding LH affected areas will be beneficial in terms of glucose regulation and estimating that potential benefit (effect).

Therefore, this study follows an exploratory research approach to examine some preliminary parameters that would be important in considering the intervention components and design for a future study to assess whether supporting people with diabetes in avoiding areas affected by LH would improve their glucose management. The approach is grounded in current MRC guidance for complex evaluations for assessing feasibility questions and process evaluation. Hence, the study objectives and methods have been designed to incorporate questions of feasibility and process evaluation as detailed in Table 6.

Table 6: Methodological approach to study objectives

Objective	Exploratory Approach	MRC Framework level	Dickoff & James level
1. Characterise LH	Feasibility	Modelling	I
2. LH effect GV	Pilot	Exploratory	II
3. Injecting behaviours	Feasibility	Modelling	I
4. US vs. DP assessment LH	Pilot	Exploratory	NA
5. Participants experiences	Feasibility	PE	NA
6. Procedures/recruitment	Feasibility	PE	NA
DP, Digital palpation; PE, Process Evaluation; NA, Not applicable; Vs., Versus			

3.3 Study Design

The study was designed to address the identified objectives for the study and following the study approached identified in the previous section. Following from the previous section there were a number of different objectives and approaches to integrate within the design. An experimental model was required to estimate whether there was an effect of LH on GV; and more descriptive data were required in respect of characterising the LH and for the process evaluation. Hence, the study followed an integrated design, with experimental study focussing on the impact of LH on GV (*the GV study*) and a parallel *LH characterisation study*, with an embedded *process evaluation*. This section presents the details of that integrated design.

3.3.1 The GV study

This element of the study was designed to estimate the effect of LH on GV, by considering the difference observed when participants injected insulin in areas unaffected by LH. While there have been previous studies (as outlined in the previous chapter) that have demonstrated this effect with insulin clamp studies, such studies

are conducted in extremely controlled conditions. Therefore, in this study the intention was to consider the effect in a clinical context as would occur if LH avoidance were to be tested in larger clinical study. Studying the effect in a “real world” rather than lab-based context does bring with it a number of challenges, particularly given the multiple factors that might mediate the effect of interest: injection behaviours and adherence; variations in underlying insulin requirements; and the multiple factors that can impact on GV (daily food and activity variations). As an exploratory study, it was important to try and capture these parameters in the data, to try and develop an understanding as to how they might be considered in a future study.

The exposure (independent variable) of interest in the design was the administration of insulin into areas without LH, in people with diabetes who had been identified as having significant areas of LH affected tissue. Designing a study to test the effect of this exposure introduced some important challenges, these included: maintaining internal validity in the exposure condition; and the challenge of addressing the relativity of GV. The former relates to the need to compensate for the potential hazard in respect of increasing the insulin effect when injecting in non-LH areas. This demanded that the research incorporated a period of insulin adjustment in a way that would minimise any effect GV while also protecting participant safety. In relation to the latter, the issue was the multitudinous factors that can contribute to GV (diet, exercise, correction factors). These factors can vary between people and within people based on their day-to-day activities and decision making. One solution to this problem could have been a randomised controlled trial design (RCT). However, while an RCT may have reduced some of the individual person variability, it would not overcome the issue of reducing and managing insulin doses in the transition from LH to LH free injection sites. It would also not be feasible to recruit sufficient participants to adjust fully for the patient-level GV factors. Hence, the study was designed using the participants as their own controls, using a case-crossover study.

The case-crossover design is an extension of the crossover design to observational studies. It was introduced in 1991 as a new technique to examine the effects of exposure outcomes with rapid sensitivity (Maclure 1991), such as the interaction between insulin and glucose. In a case-crossover design each participant contributes their own control and on-treatment conditions. In this study this reflects a comparison of: recorded glucose levels while the participants injected in their usual way - with no site changes; to recorded glucose levels after a five-week washout period injecting exclusively into areas not affected by LH. The case-crossover design is based on subject-matched sampling (Maclure 1991), in this study the presence of LH and GV (defined by the standard deviation of their recent self-monitored blood glucose readings). Therefore, GV study is based on the case-crossover design.

The case-crossover element of the study has been designed to examine the relationship between ultrasonographically characterised LH lesions and GV in people with T1DM. GV will be measured in two conditions: Condition 1- will be normal injecting practice (which will include injecting into LH areas) and Condition 2- injecting exclusively in areas not affected by LH. The participants will be screened (including clinical and ultrasound examination for LH) and then monitored using blind continuous glucose monitor (CGM) for one week to measure GV in Condition 1. Following this baseline observation period, it will be necessary for an insulin adjustment phase as a safety measure to avoid the potential for hypoglycaemia when participants inject insulin into areas not affected by LH, as the amount and speed of the delivered insulin may be affected. Importantly, no changes will be made to the participants type of insulin or frequency of injections (details of the insulin adjustment phase are provided in the study procedures). Then participants will move to Condition 2, where undergo repeat monitoring with blind CGM for another week while continuing to inject exclusively into areas not affected by LH.

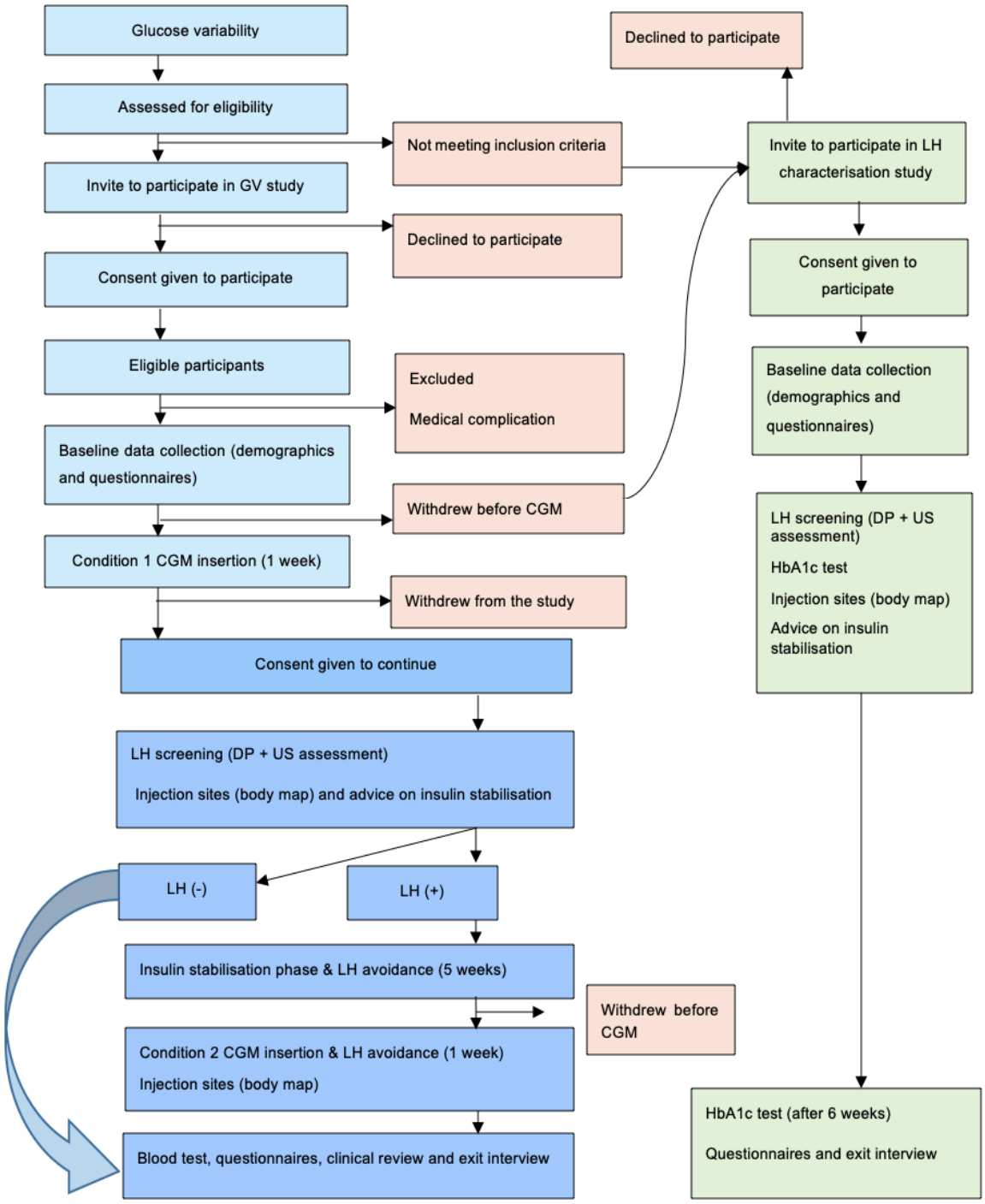
3.3.2 LH Characterisation study

The objective of this study was to generate knowledge in relation to characteristics of LH. This is important as there are currently no standardised or validated systems for assessing or grading LH and developing models for this would be useful for future studies. It would also be useful to estimate the level of LH observed in participants in the GV study to consider this as a mediating factor. Another consideration for this study, was to consider the method for determining LH. As outlined in Chapter 2. The current clinical model for assessing LH is through visualisation and digital palpation. This approach, however, is difficult to standardise and shows much lower sensitivity compared to ultrasonic methods of assessment. Hence, this part of the study was also designed to compare current best-practice for clinical examination of LH with ultrasound detected LH.

The LH characterisation study was designed as a pragmatic observational study built out of the GV study. It was anticipated that a proportion of people with LH who were eligible for the GV study may decline participation. These people were invited to participate in LH characterisation study, where they would have their LH assessed with digital palpation and ultrasound. They were also given advice on avoiding their LH affected sites and invited to return so we could assess their experiences of changing sites. This observational study was also used to compare digital palpation examination of LH using a current gold-standard approach to ultrasound detected LH.

Hence, the study overall study design comprised; a case-crossover study to assess impact on GV (the GV study); and an observational study for characterise LH regions in a larger sample of people and to compare digital palpation with ultrasound for LH detection (the LH characterisation study). Figure 4 shows the recruitment flow for the studies and how they were integrated.

Figure 4 . Flow diagram to illustrate design (TITANIC Studies Design flowchart):



3.3.3 Process Evaluation

The process evaluation was used to consider the feasibility objectives of the study. As an exploratory study, it is important to identify factors that could help optimise study procedures for a future study. The process evaluation was used multiple data collections points to: monitor adherence with study procedures; assess recruitment and retention; and collect data on participants' experiences of the study.

3.4 GV study measures

The MRC framework for complex evaluation studies, recommends that in exploratory stage feasibility studies the aim is to optimise outcome selection, for a future study (Eldridge et al. 2016, Moore et al. 2015). This requires testing or modelling outcomes to identify this which may be most sensitive to the intervention mechanism (in this case changing injection sites to LH free areas of tissue). The outcomes of interest for the GV study were, time in range, indices of GV and insulin requirements. Time in range and GV were measured in two conditions (before and after injection site change) for each participant using a blinded CGM device [The Medtronic iPro2 CGM System]: Condition 1 - participants injected in their usual way (with no changes to their injection sites, insulin dose or behaviour); and Condition 2 - a second week of glucose recording after a five-week washout period were they were advised to inject exclusively into areas not affected by LH. The other study measures were also assessed following each condition. The selection of measures used for the study are detailed below.

3.4.1 CGM measures

The CGM sensors generate a large volume of glucose data, the variability of which can be assessed in multiple ways. The primary variability measure for this study was the proportion of time spend in range based on a target glucose range of 4-10 mmol/L (Danne et al. 2017). While time in range is not a measure of GV, it is an important indicator of overall glucose exposure. Given the exploratory nature of the inquiry and

the uncertainty of the impact of LH on glucose regulation, time in range was identified as potentially the most sensitive indicator for the impact of changing injection sites on overall glucose exposure, it would also provide insights into the amount of hypoglycaemia experienced by participants following the change of sites. A minimum threshold for improvement of an increase of ≥10% for time in range was set as a reasonable clinical threshold to indicate a transferable benefit in reducing risk of complications, a change of 10% in people who spend <40% of time in range reduces the risk of microvascular complication by 30% with benefits increasing when the proportion is lower as there is an inverse correlation between time in range and complications (Beck et al. 2019). To further consider the impact of changing sites consideration was also given to time spend below range (4 mmol/L) and time above range (10 mmol/L), as this also is clinically important when assessing insulin action and sensitivity when the injection sites were changed in Condition 2 (Danne et al. 2017).

In addition to time in range it was particularly important in assessing the impact of LH on glucose regulation to measure GV. As identified in the literature chapter previous studies that have assessed the relationship between LH and GV have used crude methods for this assessment, such as the standard deviation of self-monitored glucose readings. As the data from the limited clamp-study suggested that LH was associated with altered insulin activity curves and glucose responses (Famulla et al. 2016), assessing the impact of GV would help test this assumption in a real-world clinical context. Hence, a number of GV measures were assessed, with each measure contributing slightly different information on the glucose profiles generated by the CGM data (assessing the volume and magnitude of glucose excursions). These measures included:

- Standard Deviation (SD) of mean glucose - The SD is a widely used measurement of variability used in the assessment of glycaemic profiles. It

shows how much variation or dispersion there is from the average (Hill et al. 2011).

- Coefficient of Variation (CV) - The CV (which is the SD divided by the mean) has the advantage of being a metric relative to the mean, which makes it more descriptive of hypoglycaemic excursions than the SD alone (Rodbard 2012). Stable glucose levels are defined as a CV <36%, and unstable glucose levels are defined as CV ≥36% (Monnier et al. 2017).

- Mean Amplitude of Glucose Excursions (MAGE) - The MAGE is a measure of within-day GV to assess the degree of glucose excursions (Service et al. 1970). The MAGE is calculated as the mean height of excursions >1 SD from the mean.

- Mean Absolute Glucose (MAG) calculates the sum of the differences between successive glucose values divided by the total time measured in hours (Hermanides et al. 2010).

- Means of the Daily Differences (MODD) - The MODD is a measure of the blood glucose changes resulting from day-to-day variation and is calculated on the absolute difference between paired CGM values obtained during two successive days (Molnar et al. 1972).

- Continuous Overlapping Net Glycaemic Action (CONGA) - CONGA assesses GV within a predetermined time window (McDonnell et al. 2005), it represents the SD of all valid differences between a current observation and an observation (n) hours earlier. The longer the time interval the wider the window of variation considered, generally 1, 2 or 4 hour intervals are used (Rawlings et al. 2011). In this study the 4 hour interval was used to provide and a more general estimate of the observed GV over the monitoring period.

3.4.2 Glycaemic control

Glycaemic control was assessed using a measure of glycated haemoglobin (HbA1c) and 1,5-Anhydroglucitol (1,5-AG). While HbA1c was primarily collected as a reference value in defining the characteristics of the participants, and it was also measured again following the Condition 2 exposure as an outcome, to ascertain whether there had been any change in glucose exposure. However, HbA1c is not an optimal measure for assessing short-term changes in glucose exposure as it can take 2-3 months before it fully reflects the glucose levels (Parrinello & Slevin 2014).1,5-AG is a relatively novel marker for glycaemic control, measuring activation of glucose channelling into the polyol-pathway (Yamanouchi & Akanuma 1994). It has the advantage of being more sensitive to glucose exposures in the short term (Parrinello & Selvin 2014, Dungan 2008). 1,5-AG is highly responses to changes in glucose levels, when the glucose level rises it inhibits reabsorption of 1,5-AG in the renal tubule leading to a drop in plasma level of 1,5-AG- these changes are detectable within a 24hr period and when assessed over 14 days it provides a short-term estimate of glucose exposure (McGill et al. 2004, Buse et al. 2003). Therefore, given the short period of observation adopted in the study and the need to estimate overall glucose exposure, 1,5-AG maybe a more sensitive marker of intra-cellular glucose exposure compared to HbA1c. While it is acknowledged that this study would not be able to provide a definitive estimate of its sensitivity to glucose changes in respect of avoiding LH affected areas, it would be possible to estimate whether it would be useful to include it as a marker in future studies addressing LH avoidance and its impact on glycaemia.

3.4.3 Additional outcomes

As injecting insulin to regulate glucose levels is a complex behaviour which can be demanding for people living with T1DM, consideration was given to other outcomes reflecting the participants: psychological orientation to diabetes; satisfaction with insulin; and their general quality of life. To capture the effect of changing injection sites on these areas, the following measures were used:

- The Insulin-Treatment-Satisfaction-Questionnaire (ITSQ) - ITSQ consist of 22 items with five subscales assessing treatment satisfaction for persons with diabetes on insulin over the past month, the subscales are: Inconvenience of Regimen (IR-5 items), Lifestyle Flexibility (LF-3 items), Glycaemic Control (GC-3 items), Hypoglycaemic Control (HC-5 items) and Insulin Delivery Device (DD-6 items). All items are scored on a seven-point Likert scale, which ranged from "not at all" to "extremely satisfied". The 22-item ITSQ provides an overall score and for each subscale, the sum score is divided by the number of items and the higher score indicates higher treatment satisfaction (Anderson et al. 2004).

- Diabetes distress was measured using the 17-item Diabetes Distress Scale (DDS-17) - The DDS measures diabetes-related emotional distress across four subscales: regimen distress, emotional burden, physician-related distress, and diabetes-related interpersonal distress (Polonsky et al. 2005). The scale has been widely used and validated with good internal reliability ($\alpha > 0.87$) and shows a discriminant association with measures of diabetes disease management and depression (Polonksy et al. 2005). Items are scored on a on a 6-point Likert scale with each item scored from 1 (no distress) to 6 (serious distress) concerning distress experienced over the last month (Appendix 7). A mean item score of ≥3 (which indicates moderate distress) is used to distinguish high from low distress for the total score and subscales (Fisher et al. 2012).

- Health-related quality of life was assessed using the EQ-5D-5L (Herdman et al. 2011). The EQ-5D-5L is a generic instrument for describing and valuing health. It is based on a descriptive system that defines health in terms of five dimensions including: Mobility, Self-Care, Usual Activities, Pain/Discomfort and Anxiety/Depression and an EQ-Visual Analog Scale (EQ-VAS) (Herdman et al. 2011). Each dimension is represented by one question, which all have five response options, where 1 is having no problems and 5 is being unable to do the activity or experiencing extreme pain or anxiety/depression. The EQ-5D-5L index score, is calculated from the five questions, scores can range from −0.28 (a state worse than death) to 1.000 (best possible health state) (Devlin et al. 2018). The EQ-VAS, which measure the overall health on that day, ranges from 0 to 100 indicating worst to best possible health.

3.4.4 Demographic and clinical characteristics

Additional data were collected to characterise the participants, these data included demographic and biometric characteristics, as well as clinical parameters such as insulin type and doses. Demographic and biometric data include age; gender; ethnicity; education level/degree, duration of diabetes; diabetes complications; current medicines; past medical history; weight and body mass index (BMI), (BMI = weight (Kg)/height $(m)^2$).

In terms of clinical data an assessment of hypoglycaemia and insulin requirements/use was undertaken. Awareness and frequency of hypoglycaemia was measured using the Gold score, which asks the participants to rate whether they are aware that they are experiencing hypoglycaemia on a 7-point Likert scale, with 1 representing ‘always aware’ and 7 representing ‘never aware’, and a score of ≥4 implies impaired awareness of hypoglycaemia (Gold et al. 1994). Participants also indicated any severe hypoglycaemic episodes in the previous year. These data were important in relation to the safety of the study as it was possible that when changing injection sites, the

participant may have been at increased risk of hypoglycaemia, if the insulin's action was potentiated.

A range of data on the type, doses and methods of administration used were collected, these included: type of insulin, total daily dose (proportion of basal and bolus insulin); and needle length. To consider whether the participant may be taking more or less insulin than might be anticipated, a physiological total insulin requirement was estimated based on this formula - 0.6units per KG of body weight (Rubin et al. 2011). While insulin requirements vary between individuals depending on the amount of residual endogenous insulin supply and factors related to insulin resistance, it provides a reasonable estimate for considering dose requirements in T1DM.

3.4.5 Feasibility measures

In order to consider feasibility aspects of the study (reach and fidelity), data were collected on the conduct of the study. These data included study recruitment, retention and attendance rates. The researcher kept a record of the total number of people who were screened for eligibility, the number of people who were eligible to participate, the total number who were approached to participate and total number of those who agreed to participate during the recruitment period. The total number of participants who completed the GV and LH elements of the study, and the total number of participants with missing data was also recorded.

The participants fidelity with study requirements were recorded at Condition 2 to establish if they had maintained avoidance of LH affected areas and followed the insulin advice they had been given. These data were collected through a diary that participants maintained through the CGM monitoring periods in Conditions 1 and 2 (see appendix 10). Further data were collected via the exit interviews, which assessed the participants adherence to the study protocol.

3.5 Study setting

The study was conducted in the diabetes' clinics of two large teaching hospitals (Guy's and St Thomas' NHS Foundation Trust (GSTFT) in London, UK). These clinics provide care to approximate 2000 people with Type 1 diabetes, with around 100 people attending every day. People attending these services are managed by a multi-disciplinary team of diabetes professionals. These diabetes services also provide structured education programmes for people with T1DM. The US scanning and physical palpation (digital palpation) took place in the diabetes clinic at St Thomas' Hospital site, with access to bed space which provided privacy for confidentiality.

3.6 Study participants (sampling and recruitment)

This section outlines how the participants in the study were identified and recruited.

3.6.1 Eligibility criteria

Adults with T1DM were recruited to the study using the following eligibility criteria, which were agreed following the discussion with healthcare professionals and people living with T1DM:

- Diagnosed T1DM and using insulin for >3 years.
- Taking multiple daily injections ≥4 per-day.
- Performing self-monitoring blood glucose testing an average of ≥4 tests per day or taking 3 tests per-day and willing to increase to 4 tests per day for the duration of the study.
- GV with a SD≥3.5 mmol/L of mean glucose readings in the past 4 weeks.
- Stable diabetes medication regimen (using the same insulin type and delivery method) six months before study entry.
- Ability to speak, read, and write English.

3.6.2 Exclusion Criteria

- Age <20 years
- T2DM or Gestational diabetes mellitus (GDM)
- Have a condition or receiving therapies, other than insulin, associated with lipodystrophies such as amyloidosis.
- Have another medical condition or take medicines that may influence blood glucose control (including currently active cancer; uncontrolled endocrine disorder; eating disorders; coeliac disease; and cystic fibrosis)
- Any recent acute intercurrent illness impacting on blood glucose readings.
- Have a serious medical or mental health condition that could limit adherence to required study tasks.
- Using other injectable treatments in diabetes such as growth hormone or glucagon-like peptide-1.
- Using continuous subcutaneous insulin infusion (CSII).
- Not undertaking blood glucose tests ≥4 tests per day.
- Unable to give consent.
- Unable to speak English, as providing language support was beyond the scope of the recourses for the study.

3.6.3 Study withdrawal criteria were as follows:

Consenting participants who met the inclusion criteria were withdrawn from the study following baseline data collection, if:

- They no longer met the inclusion criteria.
- Their physician changed the insulin type or method of delivery.
- Participant was unwell or was unable to comply with the study protocol (e.g. an adverse reaction to CGM sensor adhesive).
- Loss of capacity to give informed consent.

3.6.4 Sample size

The target was to recruit 34 participants to detect a 10% difference in the time spent in range between Conditions 1 and 2. This estimation was based on a power calculation (using G-power) for analysis of covariance (ANCOVA) with an estimated effect size of 1.3, with power at 95% and alpha @5%, with a 25% inflation to allow for dropouts and/or CGM sensor failure. The effect estimate was based on a previous crossover study (n=47) with CGM and percentage time in range (4-10 mmol/L) where a 10% (95%CI 8-11.2) increase in time in range (an absolute change) was observed (Van Beers et al. 2016).

3.6.5 Expected recruitment rate

To plan the study a recruitment was estimated, to ensure there was enough time to complete the recruitment within the time available. This identified that a recruitment rate of one to two potential participants a week minimum was needed over a four-month period to recruit the target of 34 participants to the study. This was assessed as feasible given the high volume of people with diabetes attending the clinical sites and feedback from the public involvement (PPI) group. However, despite best efforts, the two months recruitment review suggested that this target would not be achieved, hence the study recruitment period was extended by a further two months. Unfortunately, recruitment remained low and at the end of this period (seven months from the start), therefore, a decision was made to close recruitment prior to achieving the required sample.

3.6.6 Procedures to maximise retention

Improving the retention rates of participants who are required to undertake multiple hospital visits is a challenge. A number of strategies that have been linked to increased study retention were utilised (Turner 2013):

- Advanced appointment scheduling,
- Consistent follow-up through email,

- Scheduled visit phone call or SMS text reminders,
- Flexible appointment scheduling,
- £50 gift voucher was available for each participant that completed the study.

3.7 Preliminary work and Ultrasound training

As the study involved using US as a tool to detect LH, two researchers had undertaken six 2-hour long training sessions with Mrs.Susan Halson-Brown a senior sonographer and US trainer. Mrs. Halson-Brown is an international expert in US and a Senior Lecturer and Program Director for Specialist Ultrasound Practice at King's College London (KCL). She provided training to the researchers involved in data collection, with an individualised condensed version of the full training course offered by KCL "Specialist Ultrasound Practice". The training included how to use the US machine, how to identify different forms of LH, and how to take measurements of skin depth and nodule dimensions. One researcher undertook further practice for four days assessing the LH conditions for 11 volunteers to achieve a consistent level of competence, the volunteers had signed the training and education consent form. In addition to training the researcher and assessing their competence, Mrs. Halson-Brown also reviewed the scans undertaken to assess the LH measurements made on the images for accuracy.

During this preliminary work, a standard operator procedure (SOP) for using US to detect and measure LH was developed by the researcher in order to enhance the reliability of measurement and ensure inter-examiner consistency (see appendix 13.1). The SOP was approved by Mrs. Halson-Brown. The SOP detailed the equipment required for scanning, the scanning procedure (anatomical sites to be reviewed) and the technique for using the US equipment. The Sonosite X-Porte US machine with a high-frequency linear probe (6-13 MHz) was used to collect all data for the study. The Sonosite X-Porte incorporates proprietary beam-forming technology: XDI (Extreme Definition Imaging). This signal analysis algorithm shapes X-Porte's US beam to pinpoint precision resulting in very precise images in superficial regions, such as subcutaneous tissue (Fujifilm SonoSite 2017, Kaplan et al. 1990). The distance

from the skin surface to muscle fascia was measured at the all identified injection sites as well as the area around the injection sites. The skin measures included: the dermis layer, and subcutaneous tissue (SC). LH tissues where identified as areas of hyper-echogenicity which indicates greater tissue density. Any other abnormal changes to the tissue were also identified and labelled on the images and later interpreted with Mrs. Halson-Brown. In addition to areas potentially affected by LH (i.e. where insulin exposure occurred), a reference scan of normal tissue from an area not generally exposed to insulin, was undertaken for comparative purposes.

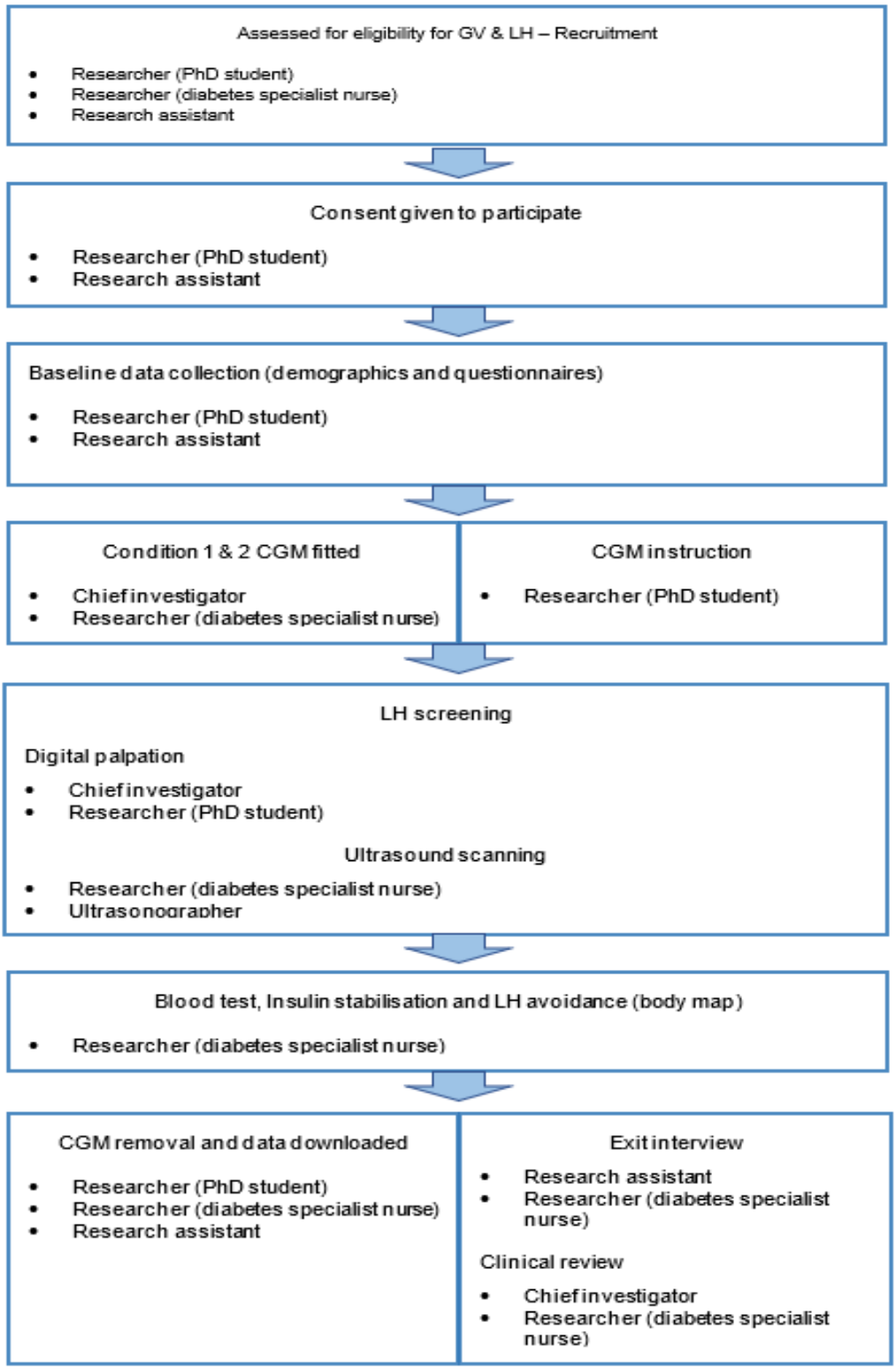
3.8 Patient and public involvement

It is increasingly recognised that public and patient involvement (PPI) can enhance the quality and relevance of health and social science research (Staley 2009), including PPI can provide valuable input in the health research during all phases of the study (Brett et al. 2014) Therefore, during the formative stage of the study, two people living with T1DM were involved in developing and reviewing the study design. The PPI members were identified from the local clinical diabetes service by one of the PhD supervisors who worked in the clinical service. Their input was very helpful in determining the data collection processes that were employed to help ensure they would be more acceptable to potential participants. The two PPI group members gave their feedback on the draft questionnaires for the study, following which modifications were made to the content and format of the questionnaires. While these two PPI group members made a very important contribution to the study, a larger reference group should have used and given the recruitment issues mentioned above this would be an important consideration for any future study. Unfortunately, it was not feasible for this study to have had a larger PPI group due to resource and time constraints.

3.9 Project personnel

Five people were involved in the conduct of the project; the chief investigator, ultrasonographer, two researchers (diabetes specialist nurse and PhD student) and a research assistant. All held honorary contracts with Guy's and St Thomas' NHS Foundation. A research assistant was employed for a one-year period to assist with data collection in the clinical setting and to conduct some of the exit interview at the end of the study. Figure 5 shows the project personnel roles in relation to the study design.

Figure 5: Flow diagram to illustrate design and the role of project personnel



3.10 Data collection tools and study documents

Standard operating procedures (SOPs) and data collection tools were designed and refined by the researcher with the support of experts in US and the study supervisors. Validated tools were used as previously indicated to assess the impact of injection site changes on insulin satisfaction and diabetes distress, using the: Insulin Treatment Satisfaction Questionnaire (ITSQ: Anderson et al. 2004); and the Diabetes Distress Scale (DDS: Polonsky et al. 2005). The EuroQol EQ-5D-5L was used to assess health-related quality of life (Herdman et al. 2011) (see section 3.4.3 *Additional outcomes* on page 85 and 86). The demographic and clinical questions were researcher-developed. Following refinement by the study team, the tools were sent to the PPI representatives for feedback and amended.

Participant information sheets and consent forms were designed specifically for the research project; refined by the researcher, supervisory team, PPI and the Health Research Authority approval process. Table 7 details the appendices for research tools, participant information sheets and consent forms.

Table 7 Data collection tools

Item	Appendix
Participant Information Sheet: GV	Appendix 5.1 & 5.2
Participant Information Sheet: LH	Appendix 5.3
Consent forms	Appendix 6
Participant questionnaire	Appendix 8
Clinical Data Forms: Digital Palpation & Ultrasound Examination	Appendix 9.1 & 9.2
Continuous Glucose Monitor Diary	Appendix 10
Injection Site and Dose Calculation Form	Appendix 11
Participants' interviews guide	Appendix 12
SOP 1 Physical examination and ultrasound scan technique	Appendix 13.1
SOP 2 Insertion Technique for the Ipro2 Continuous Glucose Monitor	Appendix 13.2
SOP 3 Identification of lipohypertrophy free injection sites and safety issues regarding use of these new injection sites	Appendix 13.3

3.11 Data collection process

3.11.1 Study Process and Field Activities

After receiving the ethical approval from the Health Research Authority (HRA) on behalf of the NHS in England, pre-testing was undertaken to model participants recruitment. The aim of the pre-testing was to understand the organisation of diabetes outpatient clinics in the targeted hospitals to determine the appropriate method for approaching potential participants. Initial observations were conducted to familiarise the researcher with the outpatient diabetes clinic setting within GSTFT. These included outpatient diabetes clinics working environment, patients' appointment system, consultations, referral process, and all activities relating to the operation of diabetes clinic. The observations enabled the patient journey within the clinic to be mapped, identifying opportunities for screening and recruiting potential participants.

3.11.2 Recruitment process

Recruitment to the study took place from end of September 2017 once the local confirmation of capability and capacity permission was issued by the research site. Potentially eligible participants attending out-patients' diabetes clinics were approached and given some information about the study, either in the waiting room or directly after they had finished their appointment. They had the opportunity to discuss the study with the researcher and consider participation, they were given at least 48 hours to consider their decision with family, friends and their clinical care team. A member of the research team would then contact them by telephone, text message or email and answer any additional questions, verify their understanding of what was involved and confirm their interest in study participation. A first appointment was arranged to gain written consent, and participants were informed that their demographic and clinical data would be collected through a review of their medical records and questionnaires. CGM insertion was facilitated for those who agreed to take part, either on the same day or arranged for the following Friday or time convenient for the participant.

Clinicians were also briefed about the study and referred people with T1DM who they felt could benefit from the study for consideration. Clinicians were given a study identification card, including the eligibility criteria for both studies to identify potential participants (see Appendix 4). The number of eligible participants not recruited was recorded.

3.11.3 Study procedures

Data collection from consenting participants, who met the inclusion criteria, were organised over a series of visits to the clinical research facility at St Thomas' Hospital, as detailed below.

3.11.3.1 Section A. The study process for the GV study participants.

Visit 1 - Consenting participants were required to bring their blood glucose meters to clinic at each visit and the researcher used Diasend™ software to download glucose values into the study database. At the beginning of visit 1 participants completed a structured questionnaire comprising questions on: socio-demographic characteristics; insulin use (insulin type, doses and needle length), insulin to carb ratios; diabetes education received; carbohydrate counting; diabetes complications; and incidents of severe hypoglycaemia. They also completed the DDS, ITSQ, EQ5D_5L questionnaires and the Gold score, and anthropometric assessment (weight/BMI) was conducted by the researcher. The questionnaires were self-completed and checked for completeness by a researcher during the visit (Appendix 7 schedule of procedures). Participants were also allocated a unique study identification number and their General Practitioners (GP) were notified by letter of their participation.

Participants were fitted with CGM device (Medtronic Ipro2) by trained member of the research team. The iPro2 device calculates and stores glucose readings every five minutes (Medtronic MiniMed 2016). This type of sensor is "blinded" to the participant so there is no possibility of instantaneous reading of the blood glucose (BG) values

which may have influenced the participant to adjust their insulin doses according to the CGM reading. The sensor was placed in the anterior abdominal wall according to manufacturer's Instructions and following the procedure detailed in SOP2 (Appendix 13.2).

During that week, participants were asked to continue measuring their blood glucose levels using their meter for a minimum of four times a day, and were given a diary to record details of their daily activities: all insulin injections (including dose), food and carbohydrate estimates per meal, physical activity, and hypoglycaemia (symptomatic or severe). Further, they were asked to indicate on a body map where they gave their insulin injection (Appendix 10). At the end of visit 1 participants were advised not to change their self-care habits and to maintain their normal injection behaviours, no changes in diet and exercise habits were required and they were not told the intention of the study was to identify LH.

Visit 2 - One week after the CGM insertion, participants returned to have their CGM and blood glucose monitoring data downloaded and return their completed diary. The sensor was removed and plugged into a docking station to upload the readings into a computer that contains dedicated software, called Carelink iPro Software. The calibration data (at least two capillary blood glucose values per day) was entered and the results reviewed with the participants. CGM data were considered valid if at least four days of recording were obtained. Participants were then informed about the study purpose in relation to LH and re-consented. Consenting participants then had a digital palpation examination for LH following the standard operating procedure (SOP) (see Appendix 13.1 SOP1), which was documented anatomically. Digital palpation is the current clinical best-practice method for assessing injections sites and the protocol in the SOP1 followed the latest clinical guidelines and tested method for assessing LH (FITTER 2016, Frid et al. 2016a, Gentile et al. 2016a). The digital palpation was performed by two study nurses trained in using the protocol from the SOP1, digital

palpation was conducted blind to the US and vice versa and participants were advised not to discuss each examination with the researcher.

The US examination of the injection sites was carried out with reference to the previously described SOP (SOP1 Appendix 13.1). The US investigation was carried out by an expert ultrasonographer/or an US trained diabetes educator. A high frequency linear probe set at between 6 and 13 megahertz (MHz) was used for the scanning. The SOP1 specified probe placement and recording of 'normal' non-injected tissue and tissue in injection sites at anatomic positions. Measurements were included skin thickness (epidermis and dermis) and subcutaneous tissue depth. In all injected areas images were recorded for signs of changes including morphology (nodular or diffuse) of LH. Following this assessment, it was revealed to the participants whether they had clinically significant LH.

During the US scan the participants had an opportunity to see where their LH areas were, and what possible new area (LH free areas) they can use for their future insulin injection. After the scanning, the participants were advised to avoid injecting insulin into LH identified areas and were advised on adjusting their insulin dose following the change from the LH areas. This advice did not change their insulin model (mode of injecting) but did reduce doses to physiologic requirement (1unit per 0.6Kg body weight) to avoid risk of hypoglycaemia. While this is a population level estimate, it is an established clinical method for calculating an initiating dose of insulin and in the case of the study provided a reasonable margin of safety in reducing potential hazard of severe hypoglycaemia. This calculation gave the total daily insulin dose which was then divided by two to give the basal dose. If the participant had a carbohydrate to insulin ratio > 1 unit: 10 grams of carb they were advised to reduce back to that level (see SOP3 Appendix 13.3). At the end of this visit, the participants had venous blood samples collected to assess: HbA1c, 1,5-anhydroglucitol and insulin antibodies.

Washout Phase-Insulin Stabilisation- Following any reductions in insulin doses, the participants had a washout or insulin stabilisation phase, where they were advised to follow their normal practice for dose intensification based on their blood glucose readings to compensate for any deficit or excess in the dose. During this period participants had ongoing monitoring from a diabetes specialist nurse to reduce any risk of hypoglycaemia (see SOP3 Appendix 13.3). The insulin stabilisation phase had been purely designed as a participant safety measure. Participants without LH left the study at this point with clinical advice from a diabetes specialist nurse on reducing their insulin variability.

Visit 3- In this visit the participants returned after five weeks and were fitted with the second blind CGM sensor. The diary was also provided for them to complete, detailing: all insulin injections (including dose and sites); and food and carbohydrate estimates per meal for the next six days. It was emphasised that during this CGM phase they must only inject in their LH free areas.

Visit 4- One week after visit 3 the participants returned to have their CGM sensors removed. At this visit, participants were given a clinical consultation involving a review of both CGM readings and given supportive advice on how to best manage their insulin injections going forward. The participants also completed the study questionnaires again and repeated blood test (HbA1c and 1,5-anhydroglucitol). At the end of this visit**,** a short exit interview was conducted to consider the participants' experiences of injecting into LH free sites, and an appropriate clinical follow-up by the diabetes team was arranged for the participants if needed.

Exit interview procedure

Short structured interviews were held following completion of the study. The topics focused on experiences of changing the injection sites, as well as their views on any previous injection site education and examination (see Appendix 12). All participants

were interviewed face-to-face. The interviews were undertaken by one researcher and interview durations were variable, ranging from 20 to 40 minutes each.

3.11.3.2 Section B. The study process for the LH characterisation participants.

Participants, not eligible for or who declined the GV study were given the opportunity to participate in the LH characterisation study. This study progressed as follows:

Visit 1 Consenting participants completed a face-to-face structured questionnaire comprising questions on: socio-demographic characteristics; insulin use (insulin type and doses, injection behaviours, delivery system (including needle length), insulin to carb ratios); diabetes education received; carbohydrate counting practice and competence; diabetes complications; and incidents of severe hypoglycaemia. They also completed the DDS, ITSQ, EQ5D_5L questionnaires and the Gold score and underwent anthropometric assessment (weight/BMI). Their injection sites were examined with digital palpation and with US as with the GV study. Unlike the GV study they did not have CGM or measurement of 1,5-anhydroglucitol, however, HbA1c was assessed.

Visit 2 After six weeks' participants returned, completed the study questionnaires again and had a repeat HbA1c collected.

3.12 Data management and entry procedures

3.12.1 Data protection and storage

Participants were informed, both verbally and through the Participant Information Sheet (Appendix 5), that the research team would access their medical records during the study, in order to collect clinical information about them. Participants provided written informed consent form to confirm their willingness for the research team to access their personal information. Each participant was assigned a unique study identifier, and this was used throughout the study. Personalised information with the unique study identifier were securely stored in restricted access, lockable containers at King's College London (KCL). No personalised data were stored electronically; all data were anonymised stored on password protected university computers. In line with the university's research framework, research records will be kept for a period of 10 years on completion of the study, archived in line with KCL policy. Where possible, paperwork was scanned and stored electronically on a secure server. The researcher and the study supervisors met regularly to ensure high standards of data collection and analysis were maintained throughout. The study was later registered with KCL Data Protection Register to ensure compliance with the introduction of the General Data Protection Regulation (GDPR) (Regulation G.D.P 2016) which came into effect on the 25th May 2018.

3.12.2 Data entry procedures

The collected data (questionnaires and clinical data) were entered onto an encrypted database by the researchers. The blood test results were also entered onto the database from paper records or from Microsoft Excel datasheets provided by the laboratories used. The CGM data for Conditions 1 and 2 were downloaded by the researcher from the sensors, generating excel files. US images were categorised in terms of LH patterns and anatomical distributions for each participant and entered onto a Microsoft Excel and Microsoft Access data files then entered onto the main database. Data were then transferred to Statistical Package for Social Sciences

(SPSS) version 25.0 statistical software (IBM 2017) for analysis. The exit interview data were coded from the interview schedules and also entered into an excel spread sheet for analysis.

3.13 Data analysis

The data were analysed in three phases:

3.13.1 Phase 1 Preparatory analysis

The preparatory element involved: checking data for completeness and accuracy prior to entry into databases; Microsoft Access, Microsoft Excel and SPSS, undertaking any necessary data transformations or adjustments and exploring data distributions and to identify and investigate any outliers.

The EasyGV version 10 software was used to calculated the Time in range (TIR), Time below range (TBR), Time above range (TAR), SD, MAGE, CONGA-4, MODD, and MAG.

The LH assessment data from US and clinical exam in line with SOP1 (Appendix 13.1) were used to characterise the LH profiles. The LH patterns observed on the scans of each participant were described and characterised with reference to the presentation type (diffuse and/or nodular formation); location and distribution; and the width of the LH. These data were used to grade and map the LH regions. Grading was determined as follows: Grade 0 (no evident nodules or diffuse areas); Grade 1 (diffuse lipohypertrophy); Grade 2 (diffuse areas of the injected subcutaneous tissue containing clearly defined nodules with circumscribed margins sized 1-5.9mm); Grade 3 (diffuse areas of the injected subcutaneous tissue, with clearly defined nodules of different sizes 6-7.9 mm); and Grade 4 (diffuse areas of the injected subcutaneous tissue, with clearly defined nodules of different sizes 8-9.9mm); Grade 5 (diffuse areas

of the injected subcutaneous tissue, with clearly defined nodules ≥10mm). Descriptive data on the prevalence of these different types, grades and locations of LH was generated. The severity of LH nodules was calculated using the size and number of nodules, using the following formula:

$$\text{Severity} = (\text{max grade}^2 \times \text{nLH nodules})/100$$

The ITSQ, DDS and QoL scores were calculated according to the developers' instructions. The frequency of the bolus injection was counted from participants diary and CGM daily summary for Condition 1 and 2 and presented as percentage.

3.13.2 Phase 2 Primary analysis

The primary analysis tested the study hypothesis using nonparametric test (Wilcoxon's signed rank test) to assess the change in GV between Condition 1 and 2 including; time spent in target range, time spent below range and above range; the other GV measures.

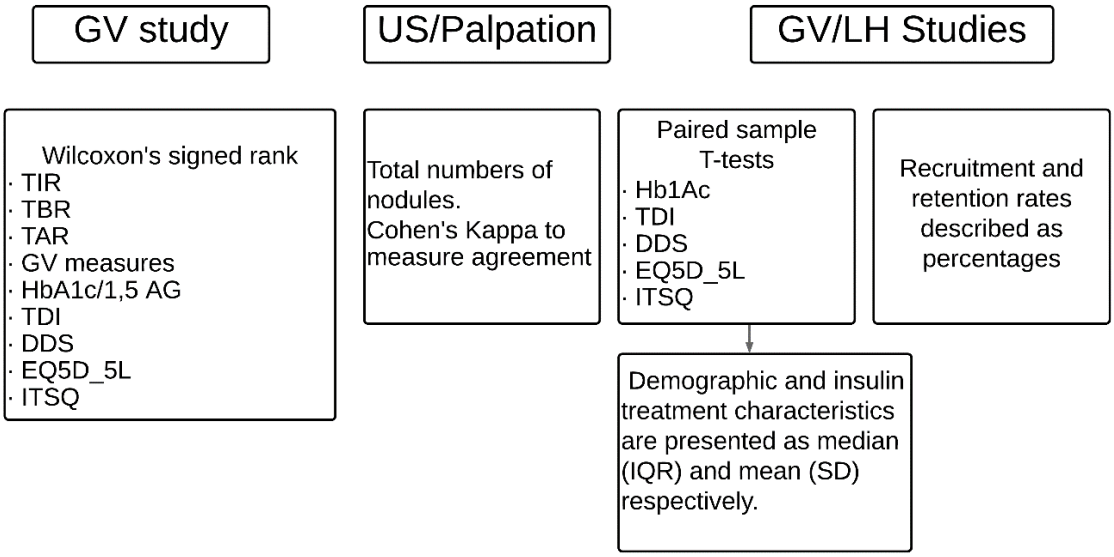
The change in glycaemic control, 1,5-Anhydroglucitol, study questionnaires and the total daily insulin (estimated insulin requirement, basal and quick acting insulin doses) was also assessed using the same test (Wilcoxon's signed rank test).

A paired sample T-tests was utilised to assess the glycaemic control and total daily dose for all participants (GV study and LH characterisation study). The same test was utilised to assess the psychosocial measures (DDS, EQ5D_5L and the ITSQ).

A descriptive analysis of the demographic and insulin treatment characteristics (GV and LH studies variables), presented as median (IQR) and mean (SD) respectively. The recruitment and retention rates described as percentages.

To compare the procedure performed by palpation against the US to detect LH, these procedures where performed independently (blind), though they shared common anatomical map into which the identified LH areas were located. The total numbers of the identified nodules by both methods were calculated. The Cohen's Kappa was used to measure the agreement between the two methods using US as reference standard (McHugh 2012).

Figure 6: Flow chart of phase 2 data analysis



3.13.3 Phase 3 Exploratory analysis

As indicated in section 3.6.5 (*Expected recruitment rate)* the study did not complete full recruitment, potentially undermining the power of the study to be able to estimate the impact of site changes as hypothesised. To compensate for this limitation additional exploratory analyses were undertaken at the individual case level. This analysis gave an added depth of interpretation to consider some of the variables that

seemed to mediate the effects observed. Each participant was ranked by the level of impact seen on their time in range. This ranking enabled case to case comparisons and identified mediating factors that explain the observations made on GV. The studies provide an integrated analysis looking at the LH profiles of each case, a detailed analysis of glucose levels on the CGM considering how responsive the glucose levels were to quick acting insulin. Information from the questionnaires and exit interviews was also used to add explanation considering how adherent participants had been to observe their new injection areas.

3.13.4 Missing data

The researcher attempted to minimise missing data by checking different source information (electronic/paper) in the participants medical records to ensure data completeness as far as possible. For the questionnaire scales a minimum of 50% item completion was required before these were calculated. For the SPSS analysis missing were left blank and the “exclude cases analysis by analysis” function for missing data was used.

Regarding the CGM missing data, periods without glucose values, in this study the data of participants who had a missing value >50 gap of readings ‘the defined Max Gap in EasyGV 10’ were excluded. In cases where the amount of missing values was <50 gap of readings the Glucose Interpolation function in CGM was used.

3.14 Process evaluation data

In this study the process evaluation assessed the implementation process and delivery of study intervention (included: insulin stabilisation and injection sites examination and recommendation regarding the avoidance of LH areas), fidelity with insulin adjustment and LH avoidance and reach/recruitment (Moore et al. 2015).

The process evaluation used a mixed-method approach of data collection:

- Quantitative data included: a report of the number and characteristics of eligible participants that decline participation in GV and LH characterisation studies; the body map in the CGM diary to monitor fidelity and compliance with injection site advice in the GV participants group.

- The qualitative element involved brief semi-structured interviews with all participants at the end of the study. The interview was conducted face-to-face and explored with participants their experiences (positive and negative) with their respective injection site; LH knowledge and their motivation/intention to sustain their current injection behaviour.

3.15 Validity and Reliability

The validity of the study refers to the degree to which a concept is accurately measured in a study (Price et al. 2018). There are two main types of validity internal and external. Internal validity relates to how well the methods and measures used are in considering the research question; and external considers the extent to which the findings can be generalised (Gravetter & Forzano 2018). As the study was designed as an exploratory study the emphasis was on internal validity (Seale 2012). To enhance this validity, previously validated structured questionnaires were used to measure constructs of relevance to the study. A gold standard technique for digital palpation examination was used to assess the LH areas and this was blinded to the US to avoid bias, a second researcher observed the digital palpation to ensure adherence to the SOP.

The US examination followed a SOP to ensure consistent and valid measurements of the LH affected areas were conducted.

The reliability refers to the consistency of the measurements (Price et al. 2018). There are several methods that can be used to test the reliability of measurements tools for example test-retest reliability and inter-rater reliability (Gravetter & Forzano 2018). Test-retest reliability requires that successive measurements be taken while the inter-rater reliability refers to the extent to which two or more examiners agree (Lange 2011). In this study, the US scan and digital palpation examination were performed by examiners with different level of experiences however to enhance the study outcome and measure a designed SOP was utilised by the examiners and verified by another researcher observing adherence to the SOP.

3.16 Ethical Approvals Process and Research Governance

3.16.1 Ethical approvals process

Prior to accessing the participants and starting the fieldwork, ethical approval was obtained from the Health Research Authority (HRA), and no additional ethical issues were raised by the ethics committee. Ethics was obtained after submitting the full research protocol through the integrated Research Application System (IRAS), along with the participant information sheets, participant consent forms, and appropriate study documents. This application was also considered and approved by the Research and Development (R&D) at GSTFT. King's College London acted as sponsor for this study. The HRA ethical approval to conduct the study was granted on the 02/08/2017, and permission to conduct data collection in GSTFT was sought from the R&D on the 15/09/2017. Confirmation of this is included in Appendix 14. The data collection activities began only after getting permission from the above authority body.

3.16.2 Research Governance

The researcher followed the recommendations of the 'Good Clinical Practice' training undertaken and throughout the study complied with University and NHS Trust research guidelines. Appropriate sponsorship, ethics and R&D approval was in place prior to any data collection. The study is registered at ClinicalTrials.gov, number NCT03669770.

3.16.3 Ethical issues in the conduct of the study

The principle risk for participation in the study related to hypoglycaemia when injecting in LH free areas. Participants may have inflated their insulin doses to compensate for poor absorption and without adjustment this could cause hypoglycaemia when injecting in LH free sites. To mitigate against this risk, participants were asked to reduce their insulin levels as stipulated in study procedure section *(3.11.3.1 Study process A Visit 2 on page 99).* Prior to data collection, all the potential clinical hazards associated with this study were discussed with clinical colleagues not directly involved in the study and in meeting with diabetes team at GSTFT. This resulted in the engagement of health professional, and further support for conducting the study. In addition to that, most of the study visits took place on Fridays ensuring adequate safety monitoring over five consecutive days. Participants had 24-hour access to an on-call diabetes consultant and had written instructions in reducing insulin doses.

The study had also the following safety monitoring procedures: an incident log for every participant to record any adverse events; an incident log for all clinical research areas to monitor adherence to study protocol and SOP and for recording any incidents that may occur in the conduct of the study; and weekly team meetings to consider any difficulties or potential hazards.

3.17 Contribution of the researcher to the study

The researcher reviewed the outpatient diabetes clinic lists for potential participants and approached participants at their routine clinic appointment to invite them to take part in the study. The researcher administered the questionnaires, performed 25% of digital palpation examination, downloaded all CGM and SBGM data, was responsible for the transportation of US machine and organising shipping of the blood sample to the laboratory. The researcher undertook the double-entry of data (questionnaires, clinical examination forms, participants information sheets) onto Excel and SPSS databases. Consent forms and GP notification letters were printed and compiled by the researcher on a weekly basis. All statistical analyses were performed by the researcher under the direction of the main supervisor.

3.18 Chapter summary

This chapter provided a detailed explanation of the method used in this study. It demonstrated how the participants were recruited, and how the data were collected and analysed. Furthermore, the processes used to manage the data was outlined, and important ethical aspects considered and discussed. The next following chapters present the findings of the study.

Chapter 4 Findings

4.1 Introduction

This chapter presents the findings of the study in three sections, detailing: the GV study analysis; the LH characterisation study data; and data on participant perspectives in relation to their injecting behaviours. The chapter is organised as follows:

- GV study- findings
    - Sample characteristics
    - Distribution and morphology of the LH observed
    - Time in range and GV findings (baseline and follow-up)
    - Secondary outcome data – insulin satisfaction, diabetes distress and quality of life questionnaires
- LH characterisation study- findings
    - Sample characteristics
    - Characterisation of LH in non-participants
    - Comparison of digital palpation and ultrasound in detecting LH.
- Participant perspectives on injecting behaviours

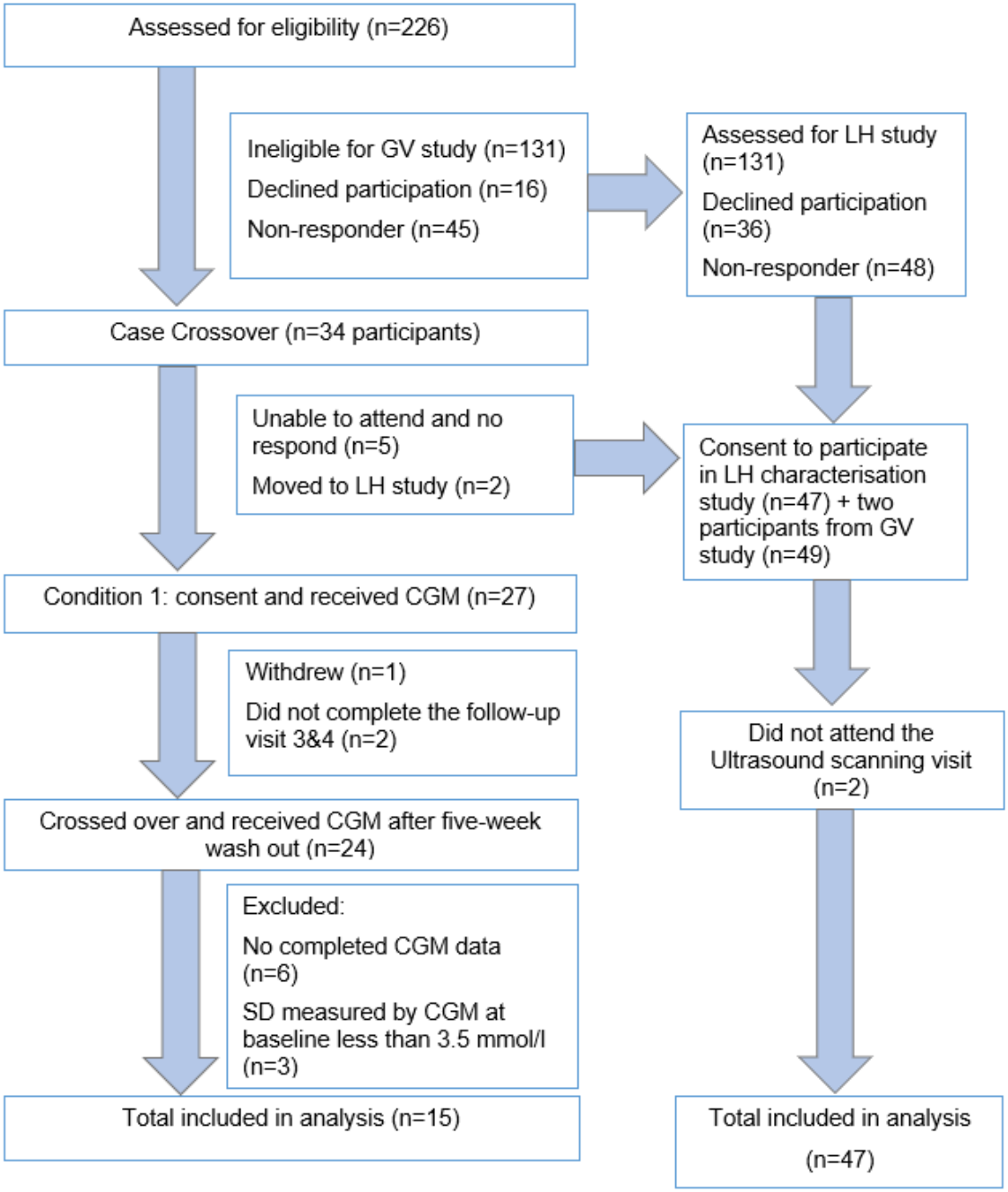
4.1.1 Participants

Data were collected over a seven-month period, commencing in September 2017 and concluding in April 2018. A total of 226 people with T1DM were screened by the researcher to confirm eligibility, based on the study eligibility criteria. Ninety-five (42%) people with T1DM met the eligibility criteria, of whom 34 (36%) agreed to participate in the GV study, while 61 (64%) did not respond or declined to participate. The main reasons for declining participation were time investment and a lack of perceived benefit in participating.

Of the 34 participants who entered the GV study, five did not attend their baseline assessment visit. Of the remaining 27 participants, 12 did not complete the study for the following reasons: inadequate baseline variability on CGM download (SD<3.5mmo/l) (n=3); insufficient CGM data (n=6); and being lost to follow-up (n=3) assessment. In respect of those who were excluded due to low GV, these people revealed that they had altered their insulin injecting behaviours or dose prior to their baseline assessment following advice from clinical staff, thereby making them unsuitable for inclusion. Hence, full data were available only for 15 participants, all of whom were included in the analysis.

The participants who did not meet the GV study criteria because of inadequate blood glucose testing, but who had potential GV or LH, were offered the opportunity to take part in the LH characterisation study (58%, n=131). Of that group 47 (36%) agreed to participate in the LH characterisation study, and 84 (64%) did not respond or declined to participate. Details of these participants are presented in Section 4.8. An overview of participation in the GV and characterisation studies is presented in the flow chart below (see Figure 7).

Figure 7: Flow chart of participation and compliance in the study



4.2 GV study Findings

4.2.1 Participant characteristics

The median age of the participants (n=15), was 32 (IQR, 25-60) years (range 20-71 years), with a median duration of T1DM of 14 (IQR, 10-23) years. The majority of the participants were female (67%, n=10), of White ethnicity (87%, n=13) and had a degree or equivalent educational qualification (67%, n=10). English was the first language for 14 (93%) participants and for one it was a second language. All of the participants, with one exception, were right-handed. The median baseline HbA1c value of the participants was 65 (IQR, 55-70) mmol/mol [8.1 (IQR, 7.2-8.6) %]. The median body mass index (BMI) was 24.7 (IQR, 21.4-26.5) kg/m^2. In addition to insulin injection, nearly half of the participants (47%, n=7) were taking other medication, including: cholesterol-lowering agents (n=4), Metformin (n=2), antihypertensive therapy (n=2), antidepressants (n=2), thyroxine (n=1), nonsteroidal anti-inflammatory drugs (n=1), and hormonal contraception (n=1). The level of comorbidity was low, with only two participants reporting other physical conditions; one participant reported hypothyroidism, breast cancer in remission, and brachial plexus syndrome. The other participant reported a history of depression. Only three participants reported diabetes complications, which included retinopathy (n=1), nephropathy (n=1) and a foot complication (n=1).

The median age of the participants who did not complete (n=12) the study was 40 (IQR, 30.3-49.5) years (range 22-65 years), with a median duration of T1DM of 22 (IQR, 13.5-25.3) years and median baseline HbA1c value 64 (IQR, 56-70) mmol/mol [8 (IQR, 7.3-8.6) %]. Study withdrawal was more common in males (67%, n=8). A summary of the characteristics of the completed and non-completed participants is presented in Table 8.

Table 8: Participant characteristics

	n=(%); or median (IQR)	
Characteristic	Participants (n=15)	Non-completers (n=12)
Age in years		
Median (IQR)	32 (25-60)	40 (30.3-49.5)
Range (years)	20-71	22-65
<30	7 (46.7)	2 (16.7)
30-50	4 (26.7)	8 (66.7)
>50	4 (26.7)	2 (16.7)
Gender		
Male	5 (33)	8 (67)
Female	10 (67)	4 (33)
Ethnicity		
White	13 (87)	9 (75)
Black	2 (13)	1 (8.3)
Asian	-	1 (8.3)
Mixed	-	1 (8.3)
BMI n (%)		
Median (IQR)	24.7 (21.4-26.5)	26 (23.2-33.2)
Range	20.4-32.2	19.3-38.6
<20	-	1 (8.3)
20-24.9	10 (66.7)	4 (33.3)
25-29.9	4 (26.7)	4 (33.3)
30>	1 (6.7)	3 (25)
T1DM Duration (years)		
Median (IQR)	14 (10-23)	22 (13.5-25.3)
Range (years)	3-38	8-40
1-4 years	1 (6.7)	-
5-9 years	-	1 (8.3)
10-14 years	7 (46.7)	2 (16.7)
15-19 years	2 (13.3)	2 (16.7)
20-24 years	2 (13.3)	4 (33.3)
≥25 years	3 (20)	4 (33.3)
HbA1c at baseline		
Median (IQR) mmol/mol	65 (55-70)	64 (56-70)
Range mmol/mol	46-103	54-91
Median (IQR) %	8.1 (7.2-8.6)	8 (7.3-8.6)

	n=(%); or median (IQR)	
Characteristic	Participants (n=15)	Non-completers (n=12)
Range %	6.4-11.6	7.1-10.5
HbA1c categories		
42-52 mmol/mol [6.0-6.9 %]	1 (6.7)	-
53-63 mmol/mol [7.0-7.9 %]	5 (33.3)	5 (41.7)
64-74 mmol/mol [8.0-8.9 %]	6 (40)	6 (50)
75-85 mmol/mol [9.0-9.9 %]	1 (6.7)	-
≥ 86 mmol/mol [≥10 %]	2 (13.3)	1 (8.3)
Education level		
Secondary school level	5 (33)	4 (33)
University level or above	10 (67)	8 (67)
English		
as first language	14 (93)	12 (100)
as second language	1 (7)	-
Dominant hand		
Right-handed	14 (93)	11 (92)
Left-handed	1 (7)	-
Unknown	-	1 (8%)

N, Number; SD, Standard deviation; BMI, Body mass index; %, Percentage; T1DM, Type 1 diabetes mellitus

4.2.2 Insulin treatment characteristics

Basal-bolus therapy was used by all participants, for bolus insulin: 60% (n=9) were taking insulin aspart; 33.3% (n=5) insulin lispro; and one participant used glulisine. Basal insulins were: glargine (40%, n=6); detemir (53.3%, n=8); and isophane insulin (n=1). The median total daily dose of insulin at baseline was 43 (IQR, 35.5-55) units. Prior to the study all the participants, except one who did not provide the data, reported testing their blood glucose four or more times per day. Two-thirds (67%, n=10) of the participants had attended a structured education programme incorporating dose adjustment and carbohydrate counting, while one participant observed carbohydrate counting without having attended a structured programme. The needle sizes used by the participants were as follows: 4-mm needles, 47% (n=7); 5-mm needles, 20% (n=3); 6-mm needles, 27% (n=4); and 8-mm needles, 7% (n=1). A summary of the insulin treatment characteristics of the completed and non-completed participants is presented in Table 9.

Table 9: Insulin treatment characteristics

	(n=(%); or median (IQR)	
Characteristic	Participants (n=15)	Non-completers (n=12)
Insulin requirement*		
Median (IQR)	43.4 (36.3-49.3)	48.6 (43.8-54.2)
Baseline total insulin dose	43.0 (35.5-55)	44.0 (33.5-68.5)
Baseline total basal Insulin	25.0 (18-31)	20.0 (18.5-34.5)
Baseline total bolus insulin	18.0 (14.5-20.5)	21.3 (13.9-36)
Long-acting insulin n (%)		
Glargine	6 (40)	3 (25)
Detemir	8 (53.3)	8 (66.7)
Degludec	-	1 (8.3)
Isophane Insulin	1 (6.7)	-
Quick-acting insulin n (%)		
Aspart	9 (60)	9 (75)
Lispro	5 (33.3)	3 (25)
Glulisine	1 (6.7)	-
Needle length n (%)		
4 mm	7 (46.7)	3 (25)
5mm	3 (20)	5 (41.7)
6mm	4 (26.7)	3 (25)
8mm	1 (6.7)	1 (8.3)
Blood glucose monitoring test/day n (%)		
<4	0	1 (8.3)
4	6 (40)	4 (33.3)
>4	8 (53.3)	7 (58.3)
Median number of tests (IQR)	5 (4-5)	5 (4-5)
Attended structured education n (%)	10 (67)	7 (58.3)
Carbohydrate counting n (%)	11 (73)	8 (67)
*0.6 units/Kg body weight		

4.2.3 Hypoglycaemia awareness and frequency

The participants' awareness of hypoglycaemia is summarised in Table 10. In respect of the Gold score, 73% of participants (n=11) had normal awareness of hypoglycaemia (Gold score of 1-2), one participant had moderate awareness (scored 3), and 20% of participants (n=3) were noted to have impaired awareness (Gold score >4). Six participants had previously experienced a hypoglycaemic episode requiring third party assistance, with three participants experiencing such an episode in the previous 12 months.

Table 10: Awareness of hypoglycaemia

	n (%)	
Characteristic	Participants (n=15)	Non-completers (n=12)
Hypoglycaemia awareness (Gold score)		
1-2 (Aware)	11 (73)	9 (75)
3	1 (7)	2 (17)
4-7(Unaware)	3 (20)	1 (8)
Hypoglycaemia assistance	6 (40)	6 (50)
Frequency of severe hypoglycaemia last year		
None	12 (80)	9 (75)
1	2 (13.3)	1 (8.3)
2	-	1 (8.3)
3	-	-
4	-	-
>4	1 (6.6)	1 (8.3)

4.2.4 Baseline glucose levels

The standard deviations of the mean blood glucose levels, based on a download of one month's data from participants blood glucose meters, indicated that both the participants and non-completers had elevated variability in their glucose levels (see Table 11). While these data do not adjust for variations in testing in respect of food intake, as the median number of tests per day was 5 (IQR, 4-5) and by considering the daily patterns for the glucose testing times, it would be reasonable to assume that the majority of the tests were pre-meals (in accordance with the standard recommendation for testing in structured education programmes). The table shows that the glucose standard deviation observed at the recruitment screening visit had reduced in some participants by the time of the CGM insertion visit. The time gap between participants being screened and having the CGM fitted varied from 0 to 49 days (median=8 (IQR, 0-23)).

Table 11: Median standard deviation of the mean blood glucose levels in the last 28 days (pre-recruitment and pre-CGM)

median (IQR) mmol/L			
Participants (n=15)		Non-completers (n=12)	
Pre-recruitment	Pre-CGM	Pre-recruitment	Pre-CGM
4.9 (4.8-5.2)	4.8 (4.4-5.4)	5.4 (4.8-6.1)	5.4 (4.9-5.8)
CGM, Continuous glucose monitoring; IQR, Interquartile range			

4.3 LH characterisation by ultrasound assessment

All participants underwent US assessment of their injection sites following the baseline period (Condition 1). In this section the LH lesions observed are characterised, considering: grading; anatomical distribution; frequency; and severity.

4.3.1 Grading

US scanning identified heterogeneous areas of LH tissue at all injection sites in comparison to normal tissue seen in areas adjacent to injection sites that had not been used as an injection site. The affected subcutaneous tissue displayed areas of increased echogenicity or reflectivity in comparison to the normal tissue, suggestive of increased tissue density associated with hypertrophic fat tissue. This varied from diffuse patches to diffuse patches with reflective 'nodules' of differing sizes within them. The size of the nodules varied and in some instances they were very large. A few of these larger nodules had areas with reduced echogenicity within them, which may indicate low blood flow and necrotic tissue (across the whole study including those in the LH characterisation arm of the study), 42 areas of reduced echogenicity within nodules were identified in 30% (n=22) of the participants). These characteristics and the size of the nodules were used to grade the LH tissue into a grading scheme containing five grades: 1 = diffuse LH; 2 = nodules <6mm; 3 = nodules ≥6mm to <8mm; 4 = ≥8mm to <10mm; and 5 = ≥10mm. The dermal layers also exhibited changes at the injection sites. The delineation between the dermal layer and the subcutaneous tissue is usually demarked by a clear margin between the tissues, in most of the LH affected areas this margin was less clearly defined or disrupted on US. The extent of the disruption to the margin varied between the areas affected by LH. In participants with marked disruption to the dermal layer, the dermal layer was also observed to be thicker in comparison to adjacent areas not being used as an injection site. These changes are potentially suggestive of inflammation being present in the skin of some people who inject insulin. Examples of each LH grade (0-5), inflamed and disrupted dermal layers, and necrotic patches are presented below:

Grade 0 - Normal tissue

Areas not exposed to insulin without any evident LH were graded 0 (see Image 1).

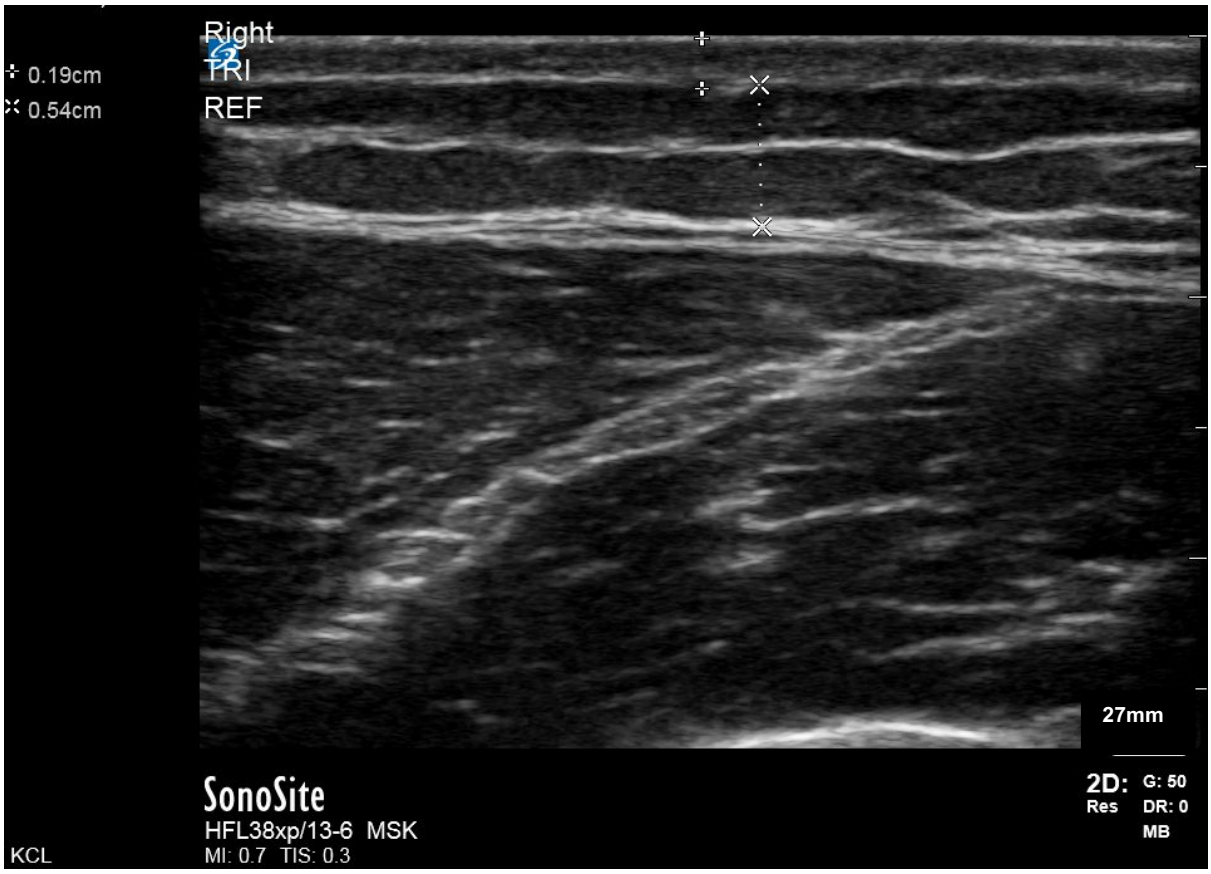


Image 1: Grade 0 - Normal tissue

This image is of a right triceps area which was not exposed to insulin injections. The image shows a clearly defined dermis and dermal margin, without disruption at a thickness of 1.9mm. The subcutaneous tissue exhibits normal tissue with a depth of 5.4mm to the muscle sheath. The subcutaneous tissue has normal appearance and echogenicity.

Grade 1 - Diffuse

Insulin exposed areas, with diffuse changes to the subcutaneous tissue, were graded 1 (see Image 2).

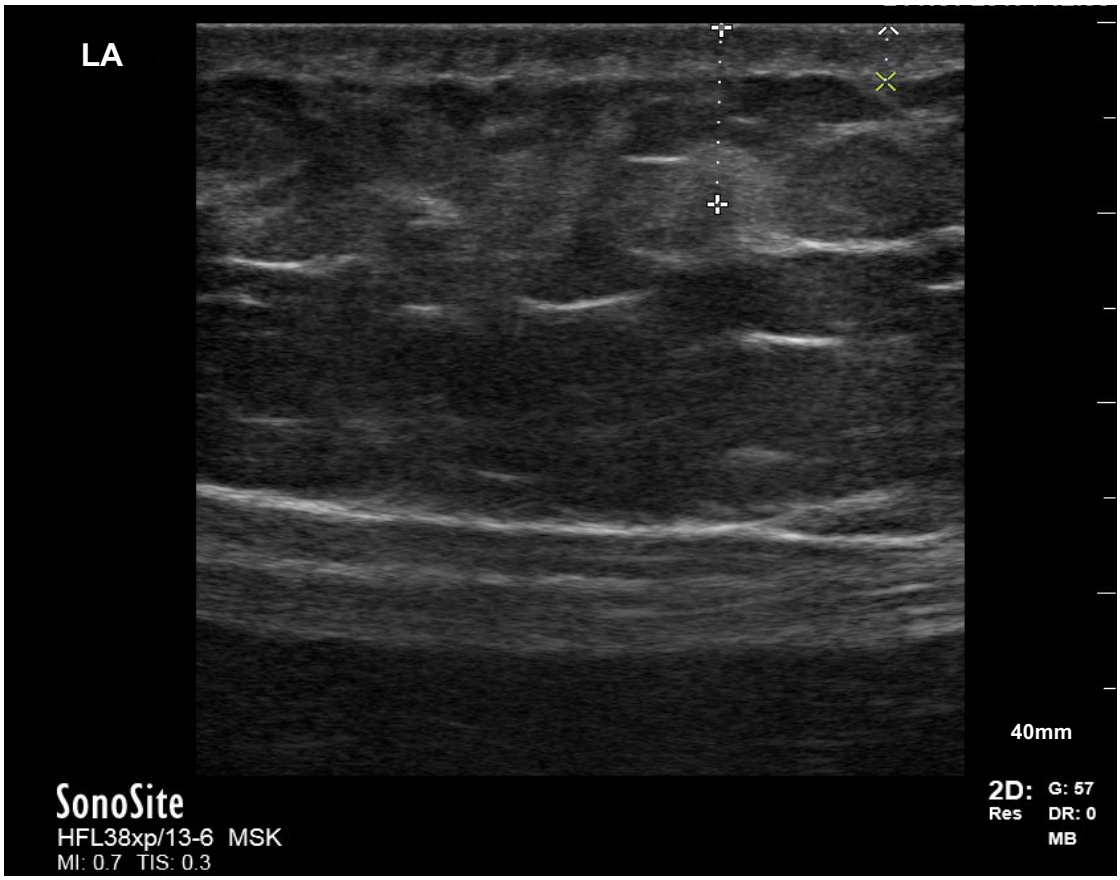


Image 2: Grade 1 - Diffuse

This image shows an abdominal injection site. The image reveals an area of increased reflectivity at around 9mm depth across the whole image. Nodules are starting to form within the site. The dermal layer is thickened (3mm) and disrupted. This participant used 8mm needles.

Grade 2

Diffuse areas of the injected subcutaneous tissue containing clearly defined nodules with sized 1–5.9mm (see Image 3).

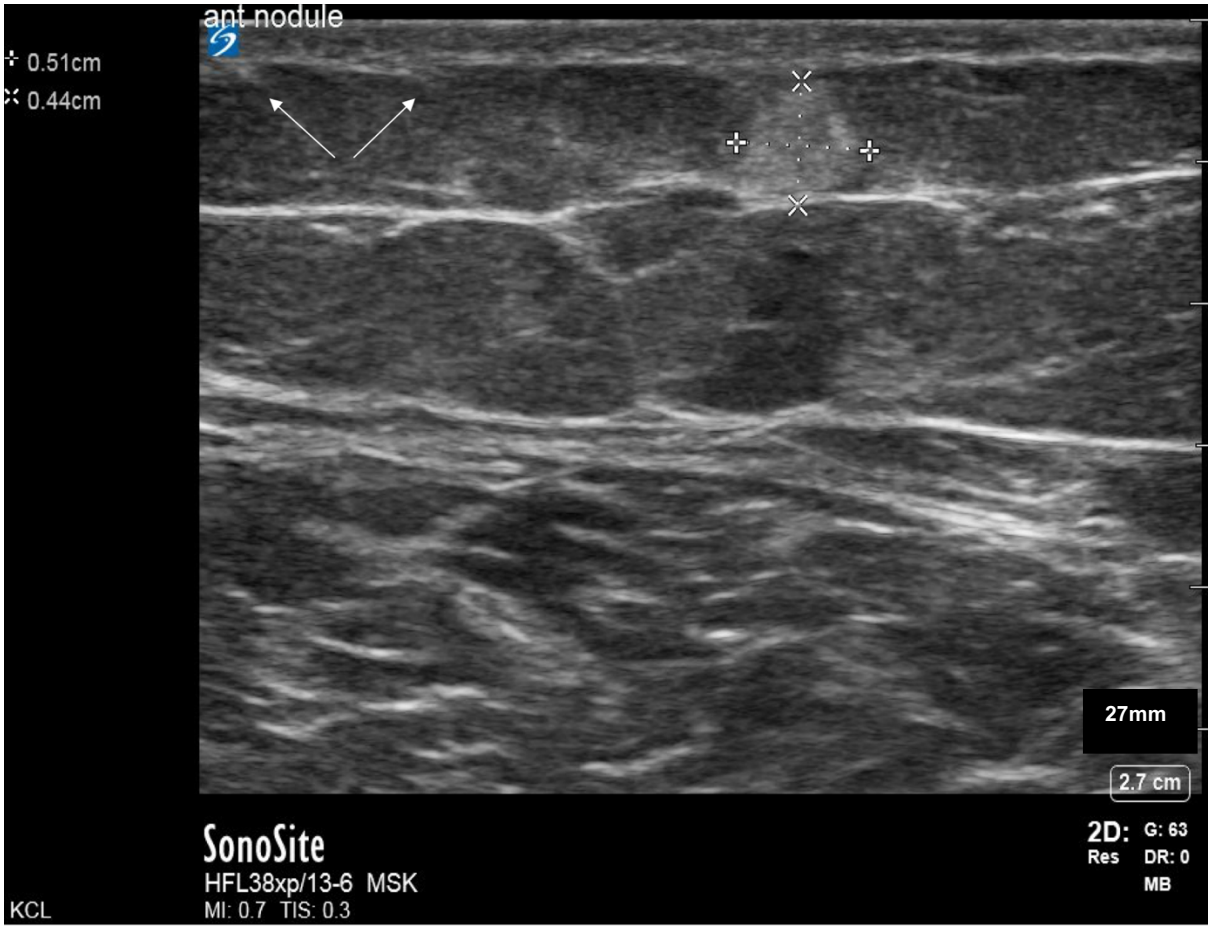


Image 3: Grade 2

This image is from an anterior thigh injection site. The image shows one clear small nodule with a diameter of 5.1mm. The nodule is at a depth of 4mm (this participant was using 4mm needles). The dermal layer is normal in thickness, but with some disruption to the margin on the left side of the image.

Grade 3

Diffuse areas of the injected subcutaneous tissue with nodules of ≥6mm to <8mm (see Image 4).

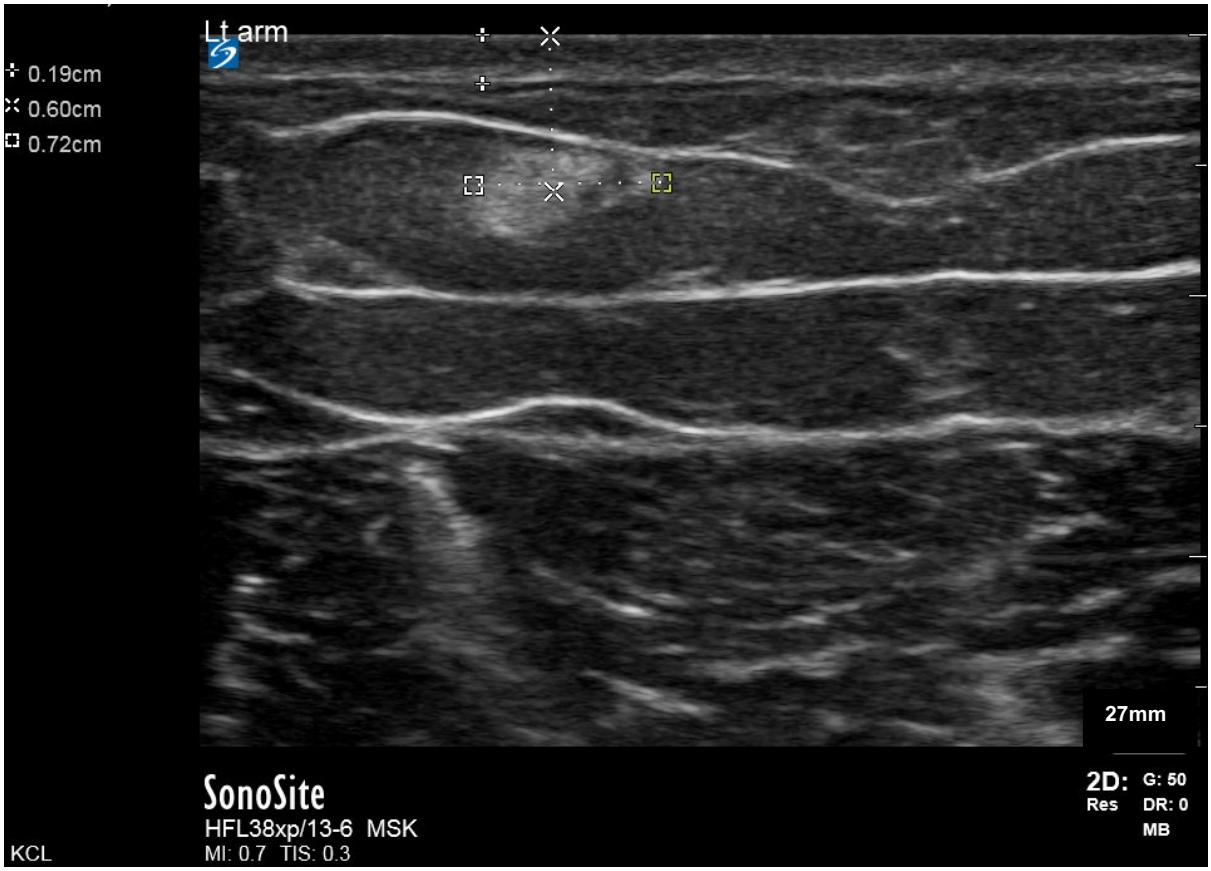


Image 4: Grade 3

This image is from the left triceps. The image shows a dermal thickness of 1.9mm and a clear margin between the dermis and subcutaneous layer. There is a nodule with a diameter of 7.2mm at a depth of 6mm (participant used 5mm needles).

Grade 4

Larger nodules ≥8mm to <10mm in size (see Image 5).

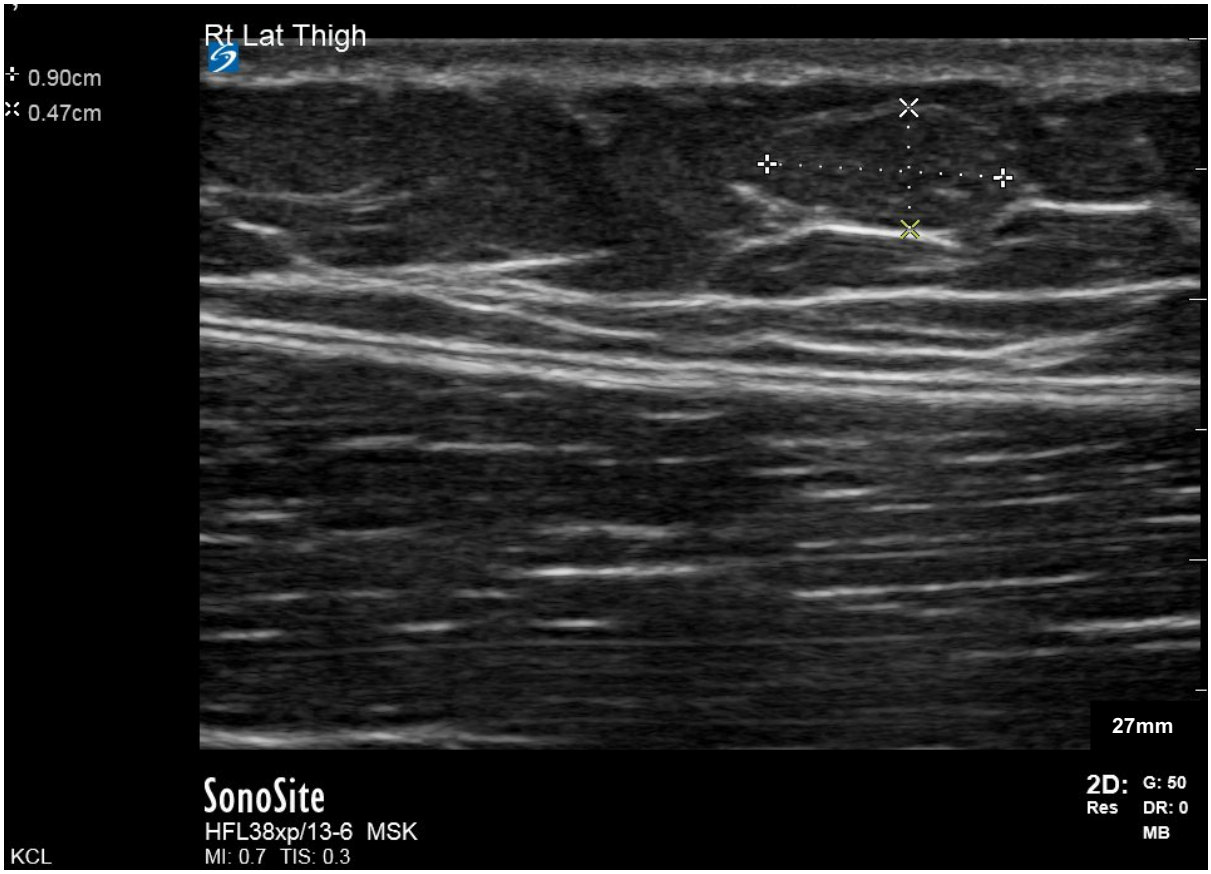


Image 5: Grade 4

This image shows lateral thigh tissue. There is loss of differentiation in the dermal margin, but not significant thickening. There is a large nodule with a width of 9mm and a diffuse area with a small nodule developing to the right of the large nodule.

Grade 5

Diffuse areas of the injected subcutaneous tissue, with nodules ≥10mm (see Image 6).

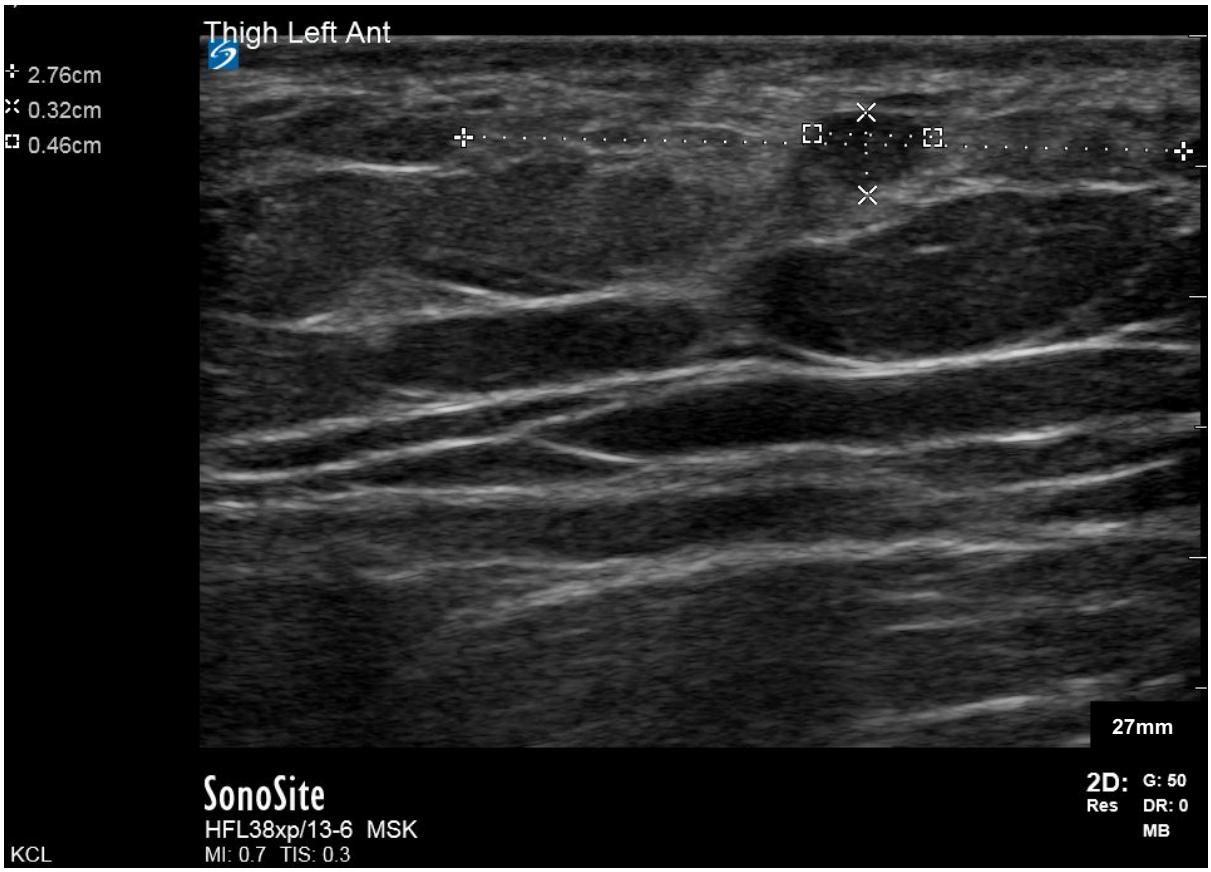


Image 6: Grade 5

This image is of an anterior thigh area. There is almost no delineation between the dermal margin and the subcutaneous tissue. The nodule is greater >10mm across and therefore was graded 5, and in this image there is also a diffuse area which is 27.6mm across. There is also a necrotic patch in the centre of the area, with a diameter of 4.6mm. This participant used 4 mm needles.

In contrast the below image (image 7) is of the same participants left anterior thigh as seen in image 6, but in an area adjacent to the injection site – not used for insulin injections.

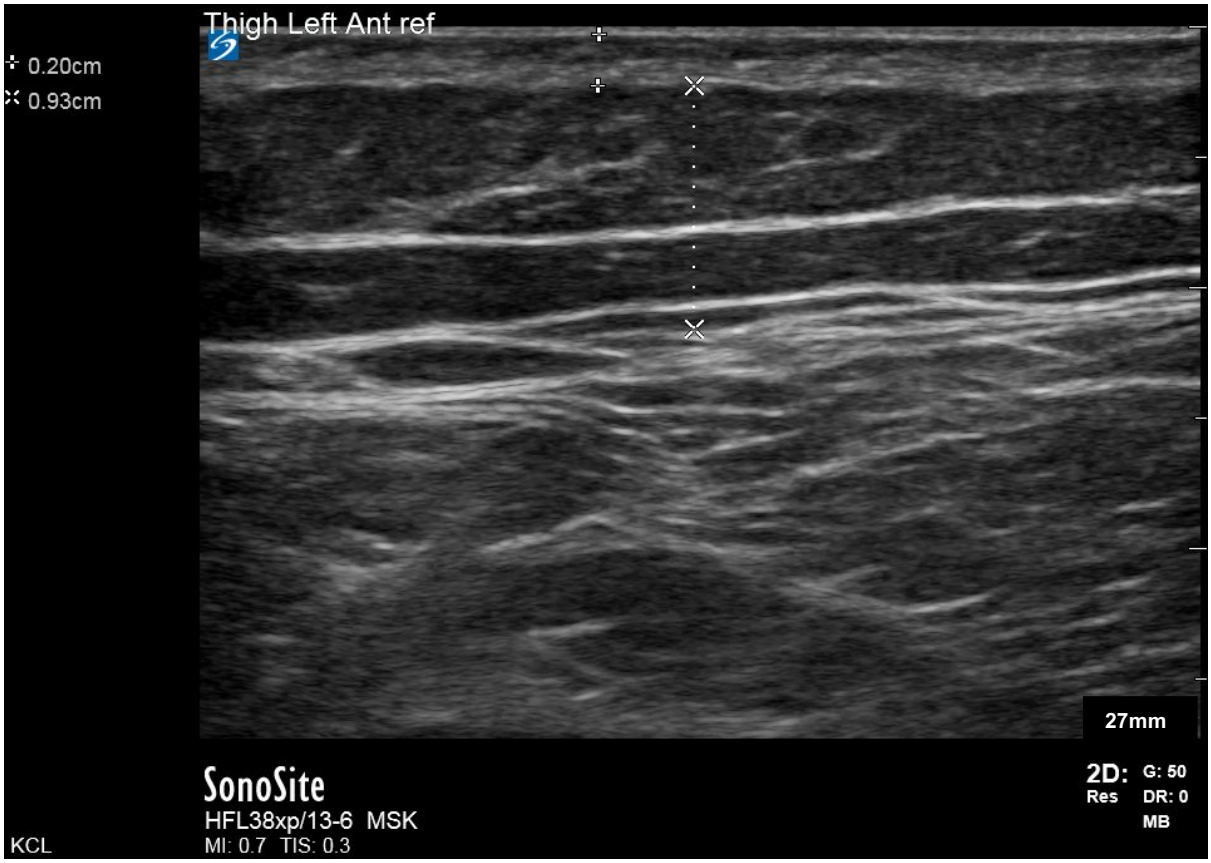


Image 7: Normal reference tissue

Normal reference tissue in the left anterior thigh of the same participant as in image6. The dermal thickness is 2mm and delineation is seen between the dermal layer and subcutaneous tissue, unlike in image 6 where it is difficult to see any dermal differentiation at all and the thickness of the dermal layer is 3mm. The subcutaneous tissue also shows normal reflectivity and no presence of LH.

Additional features of LH were observed in some of the participants US images, illustrating some of the variability in the LH presentation. In US scans of two of the participants, small to medium size nodules (1.8mm to 6.5mm) were identified within the thigh and without evident diffuse tissue (Images 8). During the scanning one of these participants confirmed that this site was rested for the last eight years but had previously been used for ten years. One explanation for this could be that while the diffuse tissue has receded due to the cessation of insulin injections in this area, whereas the nodules are more enduring.

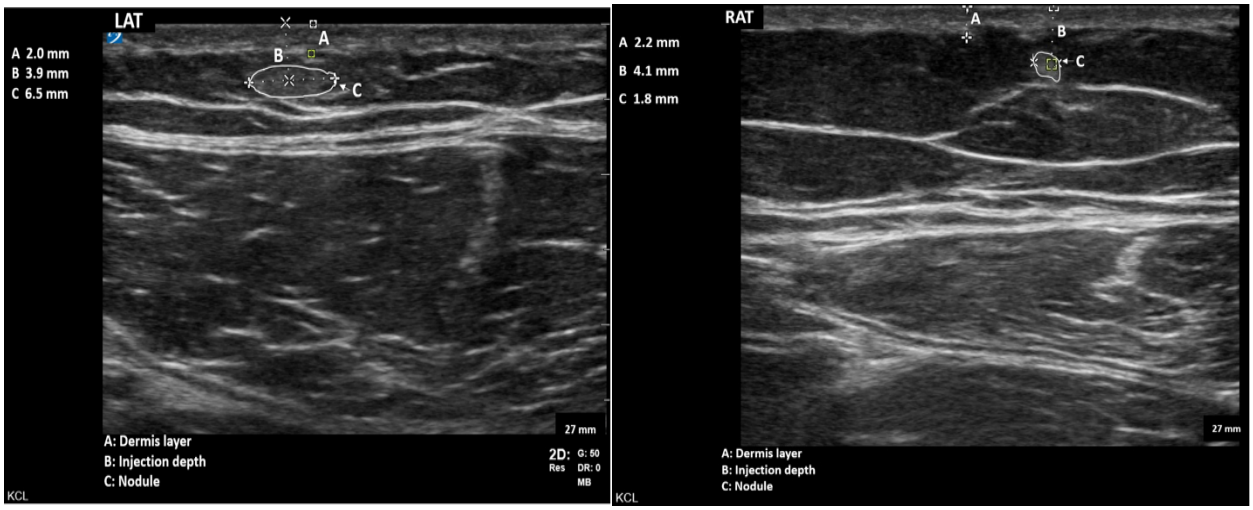


Image 8: Previous injection sites show two nodules form the anterior thighs of two participants. The width sizes of the nodules are 6.5mm and 1.8mm respectively.

As previously indicated in Image 6, there were some hypoechogenic areas that may indicate the presence of necrotic tissue within the nodules. These areas were identified within five participants in six different injection sites (four in the thigh areas and one in each of the lower abdomen and the gluteal region) (see Image 9).

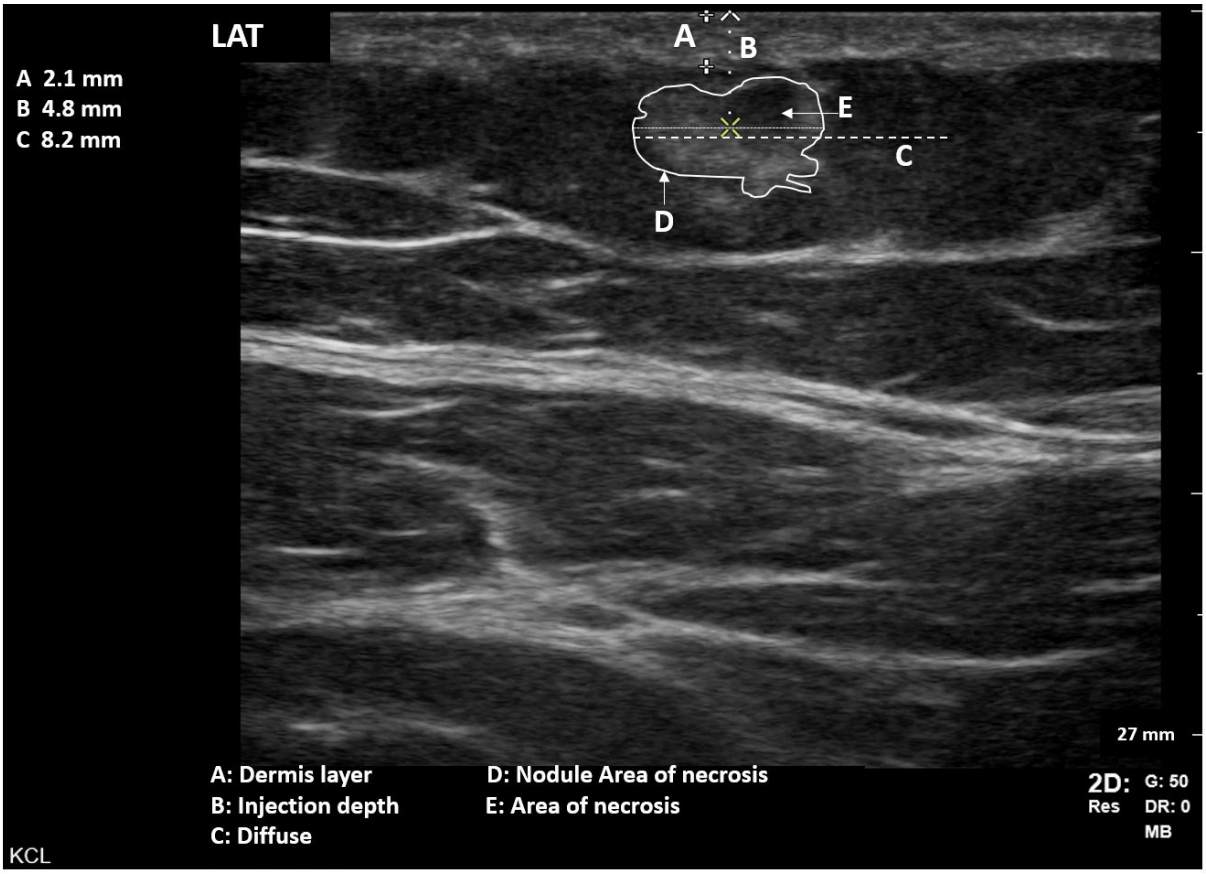


Image 9: Hypoechogenic areas

This image is of a participant's left anterior thigh area; the image shows a dermal thickness of 2.1mm (A) with one large nodule at a depth of 4.8mm to the midpoint (B). This participant was using 6mm needles. The nodules were located within a diffuse area (C) of the injected subcutaneous tissue and had a width of 8.2mm (D). The right-hand part of the LH nodule (E) continues a patch of hypoechogenic.

In some participants the dermal layer exhibited features suggestive of inflammation. In these areas the disruption to the interface between the epidermis and the underlying subcutaneous tissue was gross; this is indicative of inflammation in response to insulin and/or needle exposure. Image 10 shows a large diffuse area, with a width of 30.9mm, exhibiting very little differentiation between the dermis and the subcutaneous tissue (A).



Image 10: Injection site inflammation

This image is of a participant's left flank hip area, the participant was using a 4mm needles.

4.3.2 Anatomical distribution and frequency

LH nodules were detected in 14 of the participants, while one participant had no nodules but did have a large region of diffuse tissue. A total number of 160 nodules were observed, with the highest proportion being identified in the thigh (55%, n=88), followed by the abdomen (34.4%, n=55), triceps area (6.2%, n=10), and gluteal region (4.4%, n=7). The frequency of the LH nodules observed in the thigh area was greatest on the lateral aspect (59%, n=52) with the balance being observed on the anterior aspect of the thigh (41%, n=36). The proportion of nodules at the abdominal site was greatest in the lower abdomen (89%, n= 49), with very few being seen in the upper abdomen (11%, n=6) making this a good site for the LH-free tissue required for Condition 2. A body map of the anatomical distribution and frequency of the nodules is presented in Figure 8. The figure also indicates the largest nodules in each anatomical area.

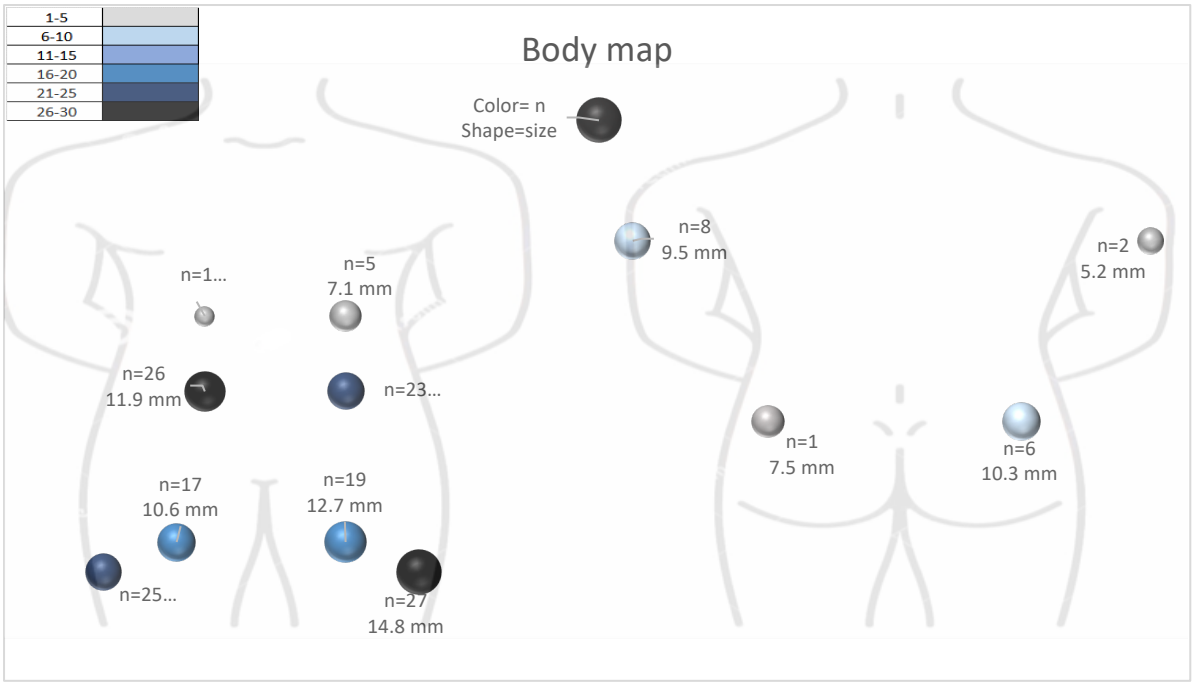


Figure 8: This body map shows the frequency of nodules at each anatomical site by different colours, while the size of the symbol represents the maximum size of nodules observed.

Diffuse areas were noticed in most of the injection sites (n=55), while in four different injection sites the diffuse tissue was presented without nodules formation (see image 2). The highest proportion of diffuse areas was seen in the thigh (53%, n=29), followed by the abdomen (31%, n=17), gluteal region (9%, n=5), and the triceps area (7%, n=4).

A summary of the anatomical distribution and frequency of the nodules and diffuse areas observed is presented in Table 12. The table also describes the observations made of the non-completers, in which the incidence of LH nodules and diffuse areas was lower.

Table 12: Anatomical distribution and frequency of the nodules and diffuse areas based on US scans

(n (%); or median (IQR)						
Characteristic	Participants (n=14)			Non-completers (n=12)		
LH nodules Total observed (n) Median (IQR) number per person	160 10 (4-16)			130 9 (6.3-13.8)		
Anatomical sites	Total	Right	Left	Total	Right	Left
Thigh	88 (55)	42 (48)	46 (52)	33 (25)	15 (45)	18 (55)
Gluteal region	7 (4.4)	6 (86)	1 (14)	41 (32)	21 (51)	20 (49)
Abdomen	55 (34.4)	27 (49)	28 (51)	50 (38)	26 (52)	24 (48)
Triceps	10 (6.2)	2 (20)	8 (80)	6 (5)	3 (50)	3 (50)
Characteristic	Participants (n=15)			Non-completers (n=12)		
Diffuse areas Total observed (n) Median (IQR) number per person	55 4 (2-5)			51 4.5 (2.3-5.8)		
Anatomical Sites	Total	Right	Left	Total	Right	Left
Thigh	29 (53)	13 (45)	16 (55)	16 (31)	7 (44)	9 (56)
Gluteal region	5 (9)	3(60)	2 (40)	12(24)	6 (50)	6 (50)
Abdomen	17 (31)	8 (47)	9 (53)	18 (35)	9 (50)	9 (50)
Triceps	4 (7)	1 (25)	3 (75)	5 (10)	2 (40)	3 (60)

4.3.3 LH nodule size, grade and severity of LH

The number of nodules identified was multiple in 14 of the participants, one participant presented with no nodules but had a large area of diffuse tissue. Of the participants with multiple nodules, two had less than five nodules, and 12 had more than five nodules. The size of nodules ranged between 1.8 and 14.8mm. In terms of average nodule size, the nodules in the thigh were greatest in size, followed by those in the abdomen; this distribution is the same when considering the maximum sized nodule in each area (see Table 13).

Table 13: LH nodule size based on US scans

median (IQR) of all nodules and largest nodule				
Characteristic	Participants (n=14)			
Anatomical sites	All nodules (mm)		Largest nodule (mm)	
	Right	Left	Right	Left
Thigh	5.5 (4.2-6.2)		8.1 (6.4-10.4)	
Anterior thigh	4.8 (2.7-6.5)	5.3 (4.4-6.5)	6.3 (4.3-8)	7.3 (5.2-8.2)
Lateral thigh	5 (4.5-5.9)	5.5 (4.6-8.3)	6.8 (4.6-9.1)	7.6 (5.5-9.8)
Gluteal region	7	7.5	10	7.5
Abdomen	4.2 (3.8-5.3)		6.1 (5.1-7.1)	
Upper abdomen	2.8	5	3	7.1
Lower abdomen	5 (4.5-5)	3.5 (3.4-5.8)	6.1 (5.3-6.4)	4.9 (4.4-7.1)
Triceps	4	4	5.2	7.2

The frequency of the LH grades observed for the largest nodules in each anatomical area is detailed in Table 14. Higher grade LH areas were clustered in the thigh area, followed by the lower abdomen.

Table 14: Grading of largest LH nodules per anatomical area

		n (%)				
Area	Total max graded LH per area	Grade				
		Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Thigh	13	-	3 (23)	1 (8)	6 (46)	3 (23)
Anterior thigh*	7	-	2 (28.6)	2 (28.6)	2 (28.6)	1 (14.2)
Lateral thigh*	10	-	-	4 (40)	4 (40)	2 (20)
Gluteal region	5	2 (40)	-	1(20)	1(20)	1 (20)
Abdomen	10	1 (10)	3 (30)	5 (50)	-	1 (10)
Upper abdomen	2	-	1(50)	1 (50)	-	
Lower abdomen	8	1(12.5)	2(25)	4 (50)	-	1 (12.5)
Triceps	3	-	1 (33.3)	1 (33.3)	1 (33.3)	-
* Number inflation is observed when counting max grade in the anterior and lateral thigh independently.						

The severity scale (severity= ((max grade^2 x nLH)/100), which reflects the size and number of nodules observed shows that 67% (n=10) of participants had scores of >1. Higher scores related to participants with multiple large nodules; one participant (ID GV-14) scored 0 as they had no LH nodules (Table 15).

Table 15: Ranked LH Severity per participant

Participants (n=15)						
Participant ID	Number of diffuse areas	Number of nodules	Range of the nodules sizes (mm)	Max size (mm)	Max grade	Severity
GV-26	5	22	3.2-14.8	14.8	5	5.5
GV-27	6	17	3.5-12.7	12.7	5	4.3
GV-5	6	16	3.5-11.6	11.6	5	4
GV-23	2	12	5.8-11.9	11.9	5	3
GV-28	4	15	3.5-8.1	8.1	4	2.4
GV-18	7	19	3-7.2	7.2	3	1.7
GV-19	4	10	5-9.6	9.6	4	1.6
GV-21	4	10	5-8.2	8.2	4	1.6
GV-24	3	9	1.8-8.2	8.2	4	1.4
GV-10	2	7	5.6-8	8	4	1.1
GV-4	2	3	9-10.3	10.3	5	0.8
GV-7	2	4	2.7-9.2	9.2	4	0.6
GV-11	3	12	3.3-5.2	5.2	2	0.5
GV-12	4	4	2.8-7.5	7.5	3	0.4
GV-14	1	0	-	-	1	0.0

Collectively, these data show that most participants had a high exposure to LH tissue damage in a broad anatomical distribution across injection sites, predominantly in the thigh and lower abdomen.

4.3.4 LH severity and insulin antibodies

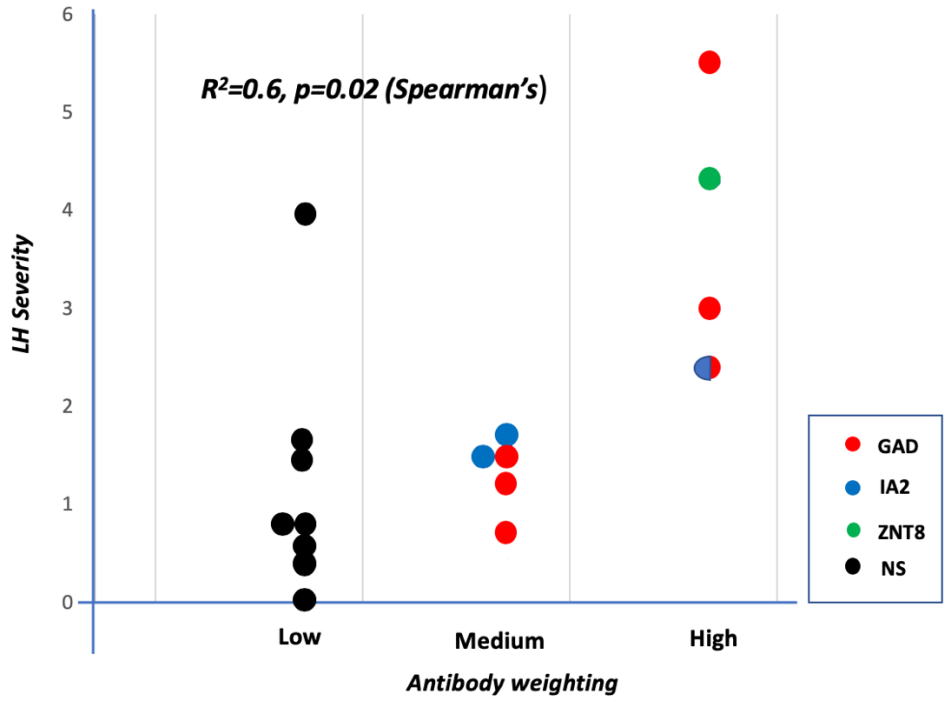
Data were collected on insulin antibodies to consider any potential interplay between insulin antibodies and the observed LH. The antibodies included: insulin auto-antibodies(IAA); islet antigen type 2 (IA2); and glutamic acid decarboxylase (GAD). The insulin auto- antibodies were replaced by zinc transporter 8 (ZnT8) antibodies during the study by the local laboratory. The antibody levels together with LH severity index are presented in table 16, for each participant ranked by LH severity. To provide a summative assessment of the antibody levels, participants were graded as having high, medium or low antibody levels based on the strength of the antibodies detected (high= 1 or more highly elevated antibody; medium= 1 or more moderate antibody; and low minimal or no antibodies).

Table 16: LH severity and insulin antibodies

Participant	LH severity	IAA	IA2	GAD	ZnT8	Antibody grade
GV-26	5.5	-	0.0	>2000	<1	3
GV-27	4.3	-	72.4	2.3	668.5	3
GV-5	4.0	3.4	0.8	9.4	-	1
GV-23	3.0	-	0.0	461.4	<1	3
GV-28	2.4	-	601.4	1401.1	6.9	3
GV-18	1.7	-	78.9	17.5	3.1	2
GV-19	1.6	-	111.4	0.5	<1	2
GV-21	1.6	-	0.0	39.3	<1	2
GV-24	1.4	-	39.5	57.9	<1	2
GV-4	0.8	0.6	0.4	6.8	-	1
GV-10	0.8	1.2	6.2	1.6	-	1
GV-7	0.6	0.2	0.0	106.8	-	2
GV-11	0.5	44.1	0.1	0.7	-	1
GV-12	0.4	16.3	9.19	0.2	-	1
GV-14	0.0	0.4	0.0	0.5	-	1

It was evident from the data that there seemed to be some relationship between the level of antibodies and the severity of LH. There was a positive correlation (Sperarman'd Ranked Coefficient) of R^2 0.6 between the grade of the antibody level and the LH severity index (p=0.02), (see Figure 9). While the limited number of observations mean that interpreting the types of specific antibodies that seem most relevant is very limited, the GAD antibodies seemed to be the most commonly observed.

Figure 9: LH severity and antibody levels



4.4 Insulin injection behaviour

4.4.1 Injection sites

The most frequent areas for injections used by participants were the thighs, followed by the abdomen, and gluteal region, while triceps were seldomly utilised. Table 17 and 18 presents percentages for specific injection areas or combination of areas, with the largest percentage of participants using a combination of abdomen/thigh/gluteal region sites.

Table 17: Most to least frequently used injection areas (alone or combination) by participants at Condition 1

n (%)		
Injection areas	Participants (n=15)	Cases
Abdomen/thigh/Triceps	1 (6.7)	GV-5
Abdomen/thigh/gluteal region	6 (40)	GV-4, GV10, GV-12, GV-19, GV-21 and GV-27
Abdomen/thigh	3 (20)	GV-23, GV-24 and GV-28
Abdomen/thigh/ Triceps/gluteal region	2 (13.3)	GV-18 and GV-26
Thigh/gluteal region	2 (13.3)	GV-7 and GV-14
Abdomen alone	1 (6.7)	GV-11

Table 18: Injection sites used by participants and US-confirmed LH

Case	Participants (n=15)								
	Abdomen		Thigh		Triceps		Gluteal region		US-confirmed LH
	C1	C2	C1	C2	C1	C2	C1	C2	
GV-4	Y(LL)	Y (RL)	Y	-	-	-	Y(R)	Y(L)	3 nodules at right gluteal region and 1 diffuse at left lower abdomen
GV-5	Y(L)	Y(U)	Y	-	Y	-	-	-	7 nodules at lower abdomen, 4 nodules at thigh, and 5 nodules at triceps
GV-7	-	Y(L)	Y	-	-	-	Y	-	4 nodules at thigh
GV-10	Y(L)	Y(U+L)	Y	-	-	-	Y	-	7 nodules at thigh
GV-11	Y(L)	Y (U)	-	-	-	-	-	-	7 nodules at lower abdomen, one diffuse at right thigh and 5 nodules at left thigh
GV-12	Y(U+L)	Y(U+L)	Y	-	-	-	-	-	1 nodule at upper abdomen, 2 nodules at thigh, and 1 nodule at gluteal region
GV-14	-	Y	Y	-	-	-	Y	-	1 diffuse at gluteal region
GV-18	Y(L)	Y(U)	Y	-	Y	-	Y	-	8 nodules at lower abdomen, 10 nodules and 1 diffuse at thigh a, and 1 nodule at triceps
GV-19	Y(L)	-	Y	-	-	-	Y	-	5 nodules at upper abdomen, 5 nodules at thigh and 1 diffuse at right gluteal region
GV-21	Y(L)	Y(U)	Y	-	-	-	Y	-	10 nodules at thigh
GV-23	Y(L)	Y(U)	Y	-	-	-	-	-	9 nodules at lower abdomen and 3 nodules at thigh
GV-24	Y(L)	Y(U+L)	Y	-	-	-	-	-	9 nodules at thigh
GV-26	Y(U+L)	-	Y	Y	Y	-	Y	Y	7 nodules at lower abdomen, 11 nodules at thigh, and 4 nodules at triceps
GV-27	Y(L)	Y(U)	Y		-	-	Y	-	4 nodules at lower abdomen, 10 nodules at thigh, and 3 nodules at gluteal region
GV-28	Y (L)	Y(U)	Y	-	-	-	-	-	7 nodules at lower abdomen and 8 nodules at thigh
Y, Yes; RL, Right lower; L, Lower; U, Upper; C1, Condition 1; C2, Condition 2									

4.4.2 Insulin doses and action

To consider whether participants were taking higher insulin doses to compensate for the potential attenuating effect of their LH, their daily insulin requirement was estimated (0.6 units per kg body weight) and compared with their total daily insulin dose. The median daily insulin requirement calculated at baseline was 43.4 (IQR, 36.3-49.3) units and the median total daily insulin dose used by participants was 43 (IQR, 35.5-55) units. There were, however, variations between participants in the amount of insulin used compared with their estimated requirement as detailed in Table 19.

Table 19: Participants' total daily insulin doses and requirement

Participant ID	EIR (units)	TDI at Condition 1 (units)	DIFF TDI (C1) - EIR (units)
GV-5	35	75	+40
GV-19	43	68	+25
GV-27	33	46	+13
GV-18	51	60	+9
GV-14	43	49	+6
GV-11	52	55	+3
GV-26	40	39	-1
GV-24	36	36	0
GV-4	46	44	-2
GV-7	47	43	-4
GV-12	36	31	-5
GV-23	49	42	-7
GV-21	47	39	-8
GV-28	35	27	-8
GV-10	51	25	-26
TDI, Total daily insulin; EIR, Estimated insulin requirement; DIFF., Difference; C1, Condition 1			

4.5 Change in Time in range and GV

In this section the findings in respect of the changes observed in TIR and GV between Conditions 1 and 2 are reported. The primary outcome of TIR was assessed with the CGM data. The mean average time recorded by the CGM device was 5.7 (±0.7) days and 5.6 (±0.6) at Conditions 1 and 2 respectively.

In relation to the primary outcome TIR (glucose range 4-10 mmol/L), there was a significant difference in the median values between Conditions 1 and 2 which were 46.4% (IQR, 43.2-53.6) and 54.2% (IQR, 46.1-66.7) respectively (p=0.02)- this equates to a medium effect size of 0.6 (Cohen's-d) based on the mean difference. Five participants achieved the primary outcome of a ≥10% improvement in TIR. These observations are summarised along with the other GV indicators in Table 20. No statistically different changes were observed for any measure.

Table 20: Change in Time in range and GV

CGM Measure (Unit)	C1 Median	IQR	C2 Median	IQR	Change C2-C1 Median	P-value
TIR (%)	46.4	43.2-53.6	54.2	46.1-66.7	+7.8	**0.02**
TBR (%)	7.4	3.7-9.6	4.5	3.2-8.3	-2.9	0.5
TAR (%)	44.5	37.1-52.1	39.8	26.3-49.6	-4.7	0.1
SD (mmol/L)	4.2	3.7-4.7	4.2	3.5-4.7	0	0.3
CV (%)	43.2	38.5-47.5	44.2	40.5-48.2	+1	0.8
CONGA-4 (mmol/L)	6.6	6.2-7.6	7	6.5-7.6	+0.4	0.9
MODD (mmol/L)	4.4	3.9-4.8	4.3	3.7-5.1	-0.1	0.7
MAGE (mmol/L)	9.2	8.9-10.6	9.6	7.7-10.5	+0.4	0.5
MAG (mmol/L/hr)	2.3	1.9-2.5	2.2	2.2-2.6	-0.1	0.4
TIR, Time In Range; TBR, Time Below Range; TAR, Time Above Range; SD, Standard Deviation of blood glucose; CV, Coefficient of Variation; CONGA, Continuous Overlapping Net Glycaemic; MODD, Mean of Daily Difference; MAGE, Mean Amplitude of Glycaemic Excursions; MAG, Mean Absolute Glucose						

It was observed that there were variations between individual participants in relation to the change in TIR, after altering their injecting sites between Conditions 1 and 2. A third of the participants showed an improvement in TIR, with the remainder showing little change or a slight reduction, although in one participant the reduction was more marked. Table 21 presents the LH characteristics for each participant ranked in respect of how much improvement was shown in respect of the time spent in range. Throughout this section this ranking is used, to consider the factors that may explain the individual differences in the impact of changing injection sites to avoid LH areas, in relation to GV between participants. The data in Table 21, show no clear pattern in relation to the changes observed in TIR and the distribution, number or severity of LH areas.

Table 21: Cases ranked by improved TIR and LH characteristics

Case	Change TIR (%)	Number of diffuse areas	Number of nodules	Range of the nodules width sizes (mm)	Max Grade	Severity
GV-7	+24.7	2	4	2.7-9.2	4	0.6
GV-28	+20.3	4	15	3.5-8.1	4	2.4
GV-4	+20.2	2	3	9 -10.3	5	0.8
GV-11	+11.9	3	12	3.3-5.2	2	0.5
GV-21	+10	4	10	5-8.2	4	1.6
GV-27	+7.7	6	17	3.5-12.7	5	4.3
GV-5	+6.4	6	16	3.5-11.6	5	4
GV-26	+3.6	5	22	3.2-14.8	5	5.5
GV-12	+2.7	4	4	2.8-7.5	3	0.4
GV-14	+1.2	1	0	-	1	0
GV-23	+0.6	2	12	5.8-11.9	5	3
GV-24	+0.04	3	9	1.8-8.2	4	1.4
GV-10	-0.1	2	7	5.6-8	4	0.8
GV-18	-3.7	7	19	3-7.2	3	1.7
GV-19	-10.3	4	10	5-9.6	4	1.6

4.5.1 Insulin doses and action

In this section the findings concerning changes that were observed in respect of insulin dose and action (number of effective injections) are reported, comparing Conditions 1 and 2 with individual participant data ranked by changes in TIR (most to least improved).

The data in Table 22 show the changes observed in TDI for each participant ranked, in relation to improvements in TIR. The data show that three participants (GV-4,GV-7, GV-28) improved TIR by ≥10% with no changes in their insulin doses, with two others (GV-5 and GV-11) improving with reduced insulin doses (≥ 20 Units of insulin). There were some patterns in the participants showing no improvement in TIR which may explain this observation. In two cases (GV-19 and GV-24) it was observed that they reduced their insulin (mainly quick acting) and then spent less time in hypoglycaemia (Time Below Range), with a corresponding increase in time spent in hyperglycaemia (Time Above Range). In the case of GV-23 the main change was an increase in period spent in the time below range. While this may be explained by the participant increasing their quick acting insulin by 4 units, it could also indicate that by injecting in the non-LH area the insulin exposure was increased (see Figures 10 and 11).

Figure 10: GV-23 Condition 1- CGM trace shows limited impact of QA insulin on blood glucose

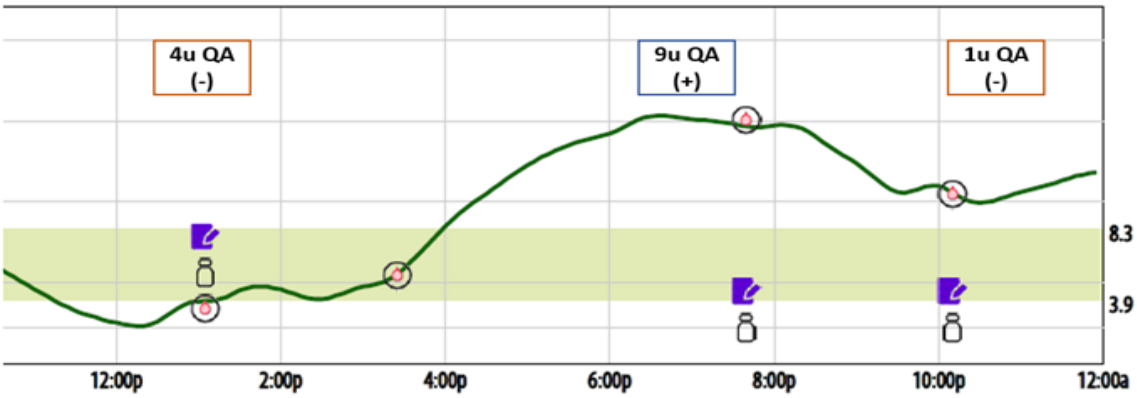


Figure 11: GV-23 Condition 2- CGM trace shows stronger impact of QA insulin on glucose

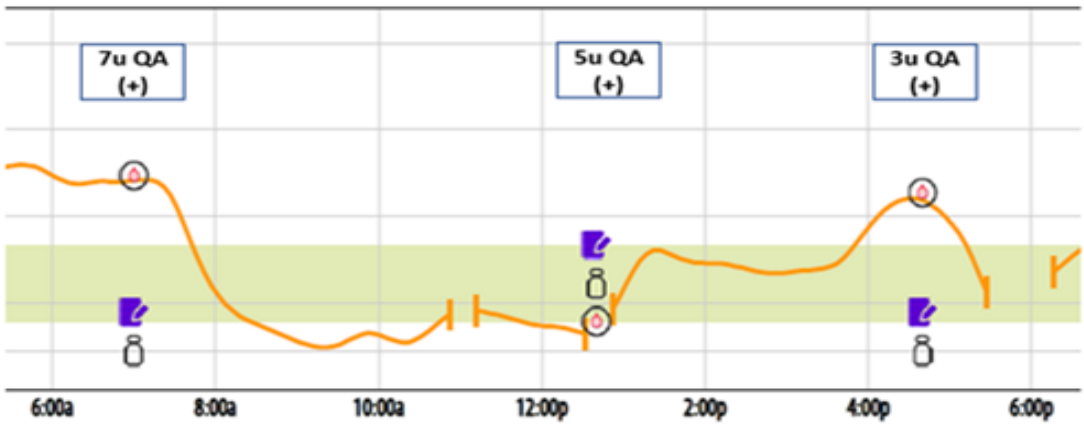


Table 22: Insulin doses

Case	*Participants (n=15)*													
	Condition 1			Advice		Condition 2					Glucose level			
	Ch.TDI (C1) - EIR (units)	TDI at C1 (units)	Basal insulin (% of TDI)	TDI Advice (units)	Basal insulin (% of TDI)	TDI at C2 (units)	Basal insulin (% of TDI)	Ch.TDI (C2-C1) (units)	Ch. Basal (C2-C1) (units)	Ch. Bolus (units)	C2 TIR (%)	Ch. TIR (%)	Ch. TBR (%)	Ch. TAR (%)
GV-7	-4	43	25 (58)	42	20 (48)	44	26 (59)	+1	+1	0	67.9	+24.7	+3.1	-28.6
GV-28	-8	27	15 (56)	27	15 (56)	29	15 (52)	+2	0	2	56	+20.3	-4.3	-16.5
GV-4	-2	44	30 (68)	38	24 (63)	45	32 (71)	+1	+2	-1	78.3	+20.2	+0.2	-19.9
GV-11	+3	55	30 (56)	49	24 (49)	35	24 (69)	-20	-6	-14	68.8	+11.9	-15.3	+3.2
GV-21	-8	39	18 (47)	39	18 (47)	34	18 (53)	-5	0	-5	48.5	+10	-4.3	-5.7
GV-27	+13	46	26 (57)	42	22 (52)	44	24 (55)	-2	-2	2	54.2	+7.7	+5	-12.3
GV-5	+40	75	38 (51)	45	20 (44)	50	20 (40)	-25	-18	-7	44.1	+6.4	-15	+8.6
GV-26	-1	39	24 (62)	35	20 (57)	41	24 (59)	+2	0	+2	54.3	+3.6	-2.6	-1.9
GV-12	-5	31	19 (62)	29	17 (59)	31	19 (61)	0	0	0	49.5	+2.7	+1.3	-4.7
GV-14	+6	49	31 (64)	41	24 (59)	41	24 (59)	-8	-7	-1	46.1	+1.2	+8.7	-9.3
GV-23	-7	42	24 (57)	38	20 (53)	46	24 (52)	+4	0	+4	54.2	+0.6	+5.3	-5.5
GV-24	0	36	17 (48)	33.5	15 (45)	27	16 (59)	-9	-1	-8	66.7	+0.04	-1.4	+1.2
GV-10	-26	25	10 (41)	24.5	10 (41)	28	10 (36)	+3	0	+3	46.6	-0.1	-0.3	+2.2
GV-18	+9	60	40 (67)	56	36 (64)	60	36 (60)	0	-4	+4	40.5	-3.7	+0.3	+2.9
GV-19	+25	68	34 (50)	60	30 (50)	62	40 (65)	-6	+6	-12	34.5	-10.3	-5.7	+15.8
TDI, Total daily insulin; C1, Condition 1; Ch, Change; TIR, Time in range; C2, Condition 2; TBR, Time below range; TAR, Time above range Participants ranked by improvement in time in range														

The number of bolus injections taken by participants at Condition 1 varied from 11 to 26 injections per week (median=17 (IQR, 14-22)) and the percentage of effective injections ranged between 50 and 95% (see Table 23). Effective injections were considered to be those where the insulin effect was clearly evident (considering a peak effect at two hours from injection of a quick acting insulin on the glucose levels in the CGM trace). Non-effective injections relate to those where the insulin had a limited or poorly defined action; injections where the CGM signal was disrupted were not included as it was not possible to fully interpret the effect of the insulin, and doses of ≤1 unit were also excluded. The data show a significant improvement in the median percentage of effective injections from 69% (IQR,62-73) to 86% (IQR,82-93) ($p<0.001$).

Table 23: Effective bolus injections at Condition 1 and 2

Participants (n=15)										
Case	Condition 1 CGM					Condition 2 CGM				
	Number of bolus injections	Effective injection	Non-effective injection	% Effective	Unclear* injection	Number of bolus injection	Effective injection	Non-effective injection	% Effective	Unclear injection*
GV-7	11	8	3	73	1	12	12	0	100	1
GV-28	19	18	1	95	6	22	19	3	86	5
GV-4	22	16	6	73	1	20	17	3	85	3
GV-11	22	18	4	82	-	16	15	1	94	-
GV-21	16	11	5	69	6	22	19	3	86	-
GV-27	21	15	6	71	1	20	20	0	100	2
GV-5	12	8	4	67	-	15	12	3	80	-
GV-26	14	8	6	57	-	15	14	1	93	-
GV-12	20	12	8	60	2	17	14	3	82	1
GV-14	13	7	6	54	4	17	14	3	82	-
GV-10	16	10	6	63	2	14	12	2	86	5
GV-24	26	19	7	73	-	18	14	4	89	-
GV-23	17	11	6	65	1	20	15	5	81	-
GV-18	23	17	6	74	-	18	16	2	89	1
GV-19	16	8	8	50	-	16	14	2	88	-
*Unclear: Single unit of insulin or no data available between bolus injections within one day										

4.6 Glycaemic control

Glycaemic control was assessed using HbA1c and 1,5-Anhydroglucitol (1,5-AG). No difference was observed in HbA1c between Condition 1 (baseline) and Condition 2 (follow-up visit) (median score: 65 (IQR, 55-70) mmol/mol [8.1 (IQR, 7.2-8.6) %] compared with 64 (IQR, 56-72) mmol/mol [8 (IQR, 7.3-8.7) %], respectively; p = 0.4). While seven participants did show some improvement in 1,5-AG, there was no change in the with median values between Conditions 1 and 2, being 8.7 (IQR,7.9-9.5) and 9 (IQR,8.3-10.7) respectively (p = 0.40). Table 24 summarises the individual participant (ranked as previously detailed) level data; three participants showed clinically important change in HbA1c (GV-10, GV-14, GV27). GV-10 demonstrated an increase of 8 mmol/mol [0.8%] in HbA1c, which may be related to potential under insulinisation as the participant's TDI was 26 units lower than their estimated physiological requirement.

Table 24: Glycated haemoglobin and 1,5-Anhydroglucitol

Case	Participants (n=15)					
	HbA1c at C1 mmol/mol (%)	HbA1c at C2 mmol/mol (%)	Ch. HbA1c mmol/mol (%)	1,5-AG at C1 µg/mL	1,5-AG at C2 µg/mL	Ch. 1,5-AG µg/mL
GV-7	64 (8.0)	66 (8.2)	+2 (+0.2)	9.2	8.4	-0.8
GV-28	100 (11.3)	100 (11.3)	0	7.7	7.3	-0.4
GV-4	54 (7.1)	55 (7.2)	+1 (+0.1)	-	13	-
GV-11	46 (6.4)	51 (6.8)	+5 (+0.4)	9.2	8.6	-0.6
GV-21	66 (8.2)	69 (8.5)	+3 (+0.3)	8.5	11.4	+2.9
GV-27	55 (7.2)	63 (7.9)	+8 (+0.7)*	11.8	15.5	+3.7
GV-5	103 (11.6)	102 (11.5)	-1 (-0.1)	6.3	9.7	+3.4
GV-26	63 (7.9)	64 (8.0)	+1 (+0.1)	8.2	9	+0.8
GV-12	67 (8.3)	65 (8.1)	-2 (-0.2)	9.6	8.3	-1.3
GV-14	65 (8.1)	60 (7.6)	-5 (-0.5)*	13.1	6.7	-6.4
GV-23	57 (7.4)	56 (7.3)	-1 (-0.1)	8.8	8.6	-0.2
GV-24	54 (7.1)	54 (7.1)	0	7.9	7.1	-0.8
GV-10	69 (8.5)	78 (9.3)	+9 (+0.8)*	6.4	9.5	+3.1
GV-18	70 (8.6)	67 (8.3)	-3 (-0.3)	9.5	10.7	+1.2
GV-19	85 (9.9)	-	-	8.4	9.6	+1.2

Reference Range for 1,5-AG: Normal= 10 – 31 µg/mL; 1,5-AG Abnormal=< 10 µg/Ml
*Indicates clinically significant change
C1, Condition 1; C2, Condition 2; 1,5-AG,1,5-Anhydroglucitol

4.7 Participant level measures (insulin satisfaction, diabetes distress, quality of life)

This section reports the findings concerning the participant level measures, which included insulin treatment satisfaction, diabetes distress and quality of life.

4.7.1 Treatment satisfaction using ITSQ

There were no differences in the overall or sub-scale scores for the ITSQ treatment satisfaction scale between Conditions 1 and 2, as detailed in Table 25. While there were no statistical differences (this being attributable to insufficient power) the change in lifestyle flexibility score suggests that participants may have found their insulin routines less flexible as they changed their injections to unfamiliar sites.

Table 25: Insulin treatment satisfaction

	median (IQR)		
ITSQ items	Condition 1	Condition 2	P-value
Inconvenience of regimens	65.7 (45.7-74.3)	61.4 (54.3-74.3)	0.9
Lifestyle flexibility	66.7 (47.6-76.2)	59.5 (55.9-67.9)	0.1
Glycaemic control	47.6 (33.3-52.4)	47.6 (46.4-57.1)	0.3
Hypo control	48.6 (28.6-60)	51.4 (33.6-62.9)	0.7
Insulin delivery device satisfaction	64.3 (47.6-78.6)	64.3 (52.9-74.4)	0.8
Total score	55.2 (48.7-66.9)	55.5 (47.4-67.2)	0.9

Table 26 presents the ITSQ data for each of the ranked participants. In relation to the lifestyle flexibility scale, which includes questions concerning exercise and whether participants are confident that they can avoid hypoglycaemia. The responses suggest that changing injection sites impacted on the way participants experienced the effect of insulin, reducing their confidence in considering how much insulin to take. This response was seen both in those who had improved their TIR and in those who had not. Conversely, in relation to the hypoglycaemia control scale some participants showed increased satisfaction.

Table 26: Insulin treatment satisfaction data for each of the ranked participants

Case	Participants (n=15)											
	ITSQ		IR		LF		HC		GC		DS	
	C1	C2 Ch	C1	C2 Ch	C1	C2 Ch	C1	C2 Ch	C1	C2 Ch	C1	C2 Ch
GV-7	66.9	+3.3	74.3	-2.9	76.2	-4.8	68.6	+2.9	33.3	+23.8	71.4	+2.4
GV-28	39.6	+6.5	42.9	+11.4	71.4	-9.5	22.9	+17.1	52.4	+4.8	28.6	+2.4
GV-4	44.8	-6.5	45.7	-5.7	61.9	0	31.4	-5.7	47.6	0	45.2	-14.3
GV-11	58.4	-2.6	65.7	-5.7	66.7	-9.5	54.3	-2.9	42.9	+4.8	59.5	0
GV-21	55.2	-7.8	60	0	47.6	+19.1	57.1	-17.1	33.3	0	64.3	-23.8
GV-27	48.7	+5.2	37.1	+17.1	52.4	0	25.7	+8.6	57.1	-14.3	71.5	+4.8
GV-5	48.7	+11	65.7	-5.7	85.7	-28.6	14.3	+51.4	52.4	+4.8	42.9	+14.3
GV-26	57.8	-2.6	74.3	0	38.1	-19.1	48.6	+14.3	23.8	0	78.6	-11.9
GV-12	53.9	+12.3	37.1	+31.4	76.2	-9.5	45.7	+5.7	61.9	+9.5	59.5	+14.3
GV-14	68.8	-3.9	77.1	-14.3	80.9	-4.8	60	0	38.1	+9.5	78.6	-4.8
GV-23	64.9	-17.5	68.6	-20	66.7	-23.8	48.6	-17.1	61.9	-9.5	76.2	-16.7
GV-24	68.2	+1.9	77.1	+5.7	57.1	0	62.9	0	47.6	0	80.9	+2.4
GV-10	74.7	-2.6	85.7	-5.7	85.7	-14.3	60	+2.9	47.6	0	85.7	0
GV-18	49.4	+5.2	65.7	+8.6	42.9	+14.3	40	-14.3	42.9	+9.5	50	+11.9
GV-19	38.9	-	74.3	-	9.5	-	28.6	-	9.5	-	47.6	-

ITSQ, Insulin treatment satisfaction; IR, Inconvenience of regimens; LF, Lifestyle flexibility; HC, Hypoglycaemia control; GC, Glycaemic control; DS, Insulin delivery device satisfaction; C1, Condition 1; C2 Ch, Change between Condition 2 and Condition 1 (C2-C1).

No significant changes were observed in the DDS score between Conditions 1 and 2 ($p = 0.8$) as summarised in Table 27.

Table 27: Diabetes distress scale

	median (IQR)		
DDS items	Condition 1	Condition 2	P-value
Emotional burden	2.2 (1.4-2.8)	2.1 (1.4-2.7)	0.7
Physician-related distress	1.8 (1.5-2.3)	1.9 (1.5-2.6)	0.9
Regimen-related distress	2.0 (1.6-2.8)	2.2 (1.6-2.9)	0.9
Interpersonal distress	2.3 (1.7-3)	2.5 (1.3-3.4)	0.5
Total score	2.1 (1.5-2.7)	2.1 (1.6-2.5)	0.8

The majority of participants had moderate or severe distress, as shown in Table 28.

Table 28: Diabetes distress score

	n (%)	
TOTAL DDS	Participants (n=14) Condition 1	Participants (n=14) Condition 2
Little or no distress (<2.0)	5 (35.7)	4 (28.6)
Moderate distress (2.0–2.9)	8 (57.1)	9 (64.3)
High distress (≥3.0)	1 (7.1)	1 (7.1)

In relation to the EQ-5D-5L quality of life measure, a median visual analogue scale score of 70% (IQR, 65-80) was observed, ranging from 30% to 100% for the participants at Condition 1, with 40% (n=6) of the participants rating their health >80% on the EQ-VAS (100 = the best health you can imagine). The median index-based value was 0.88 (IQR,0.8-1.0), ranging from 0.5 to 1.0. After changing the injection sites (Condition 2), the median visual analogue scale score was increased to 80% (IQR, 69.5-96.3), ranging from 60% to 100%, with 57% (n=8) of the participants rated their health >80%. The median (IQR) index-based value was 0.95 (0.9-1.0), ranging from 0.7 to 1.0, with no difference between the median of the index value at Condition 1 and Condition 2 ($p = 0.2$).

4.8 LH characterisation study

In this section the data from the participants in the LH characterisation study are presented. The participants in this study were those who declined involvement in the GV study but were willing to have an US scan of their injection sites. These participants are defined as the LH group in the study. To enhance the scale of the analysis the baseline data from the GV participants, including those who did not complete the GV study, were included in the analysis (n=27), as the data in relation to the LH areas and characteristics were common. These participants are defined as the GV group. The analysis addressed the following areas:

- The prevalence of LH (number, type, size and anatomical distribution);
- A comparison of LH detection using US and via digital palpation.

4.8.1 Sample characteristics of LH group

Study participants (GV group and LH group) were similar in terms of age, duration of diabetes, ethnicity, education level, BMI and HbA1c. On average, participants were 40.6 (±14.2) years old, with a mean duration of T1DM of 18.3 (±10.9) years. Most of the participants were of White ethnicity (82.4%, n=61) and had a degree or equivalent educational qualification (67.6%, n=51). The mean body mass index (BMI) was 25.5 (±4.2) Kg/m^2, and the mean baseline HbA1c value was 68 (14.5±) mmol/mol [8.4 (±1.3) %]. English was the first language for 62 (83.7%) people and for 12 (16.2%) it was a second language. There were slightly more male participants 59.5% (n=44). One third of the participants (35.14%, n=26) reported diabetes complications, which included retinopathy (n=19), neuropathy (n=4), foot complications (n=4), nephropathy (n=2), and cardiovascular complications (n=1). A summary of the demographic characteristics of the 74 participants and each study groups is presented in Table 29.

Table 29: Demographic characteristics

	(n (%); or mean ±SD and range)		
Characteristic	Total (n=74)	LH group (n=47)	GV group (n=27)
Age in years			
Mean ±SD	40.6 ±14.2	40 ±13.4	39.9 ±15.9
Range (years)	20-71	25-71	20-71
<30	21 (28.4)	12 (25.5)	9 (33.3)
30-50	35 (47.3)	23 (48.9)	12 (44.4)
>50	18 (24.3)	12 (25.5)	6 (22.2)
Gender			
Male	44 (59.5)	31 (66)	13 (48.1)
Female	30 (40.5)	16 (34)	14 (51.9)
Ethnicity			
White	61 (82.4)	39 (83)	22 (81.5)
Black	4 (5.4)	1 (2.1)	3 (11.1)
Asian	3 (4.1)	2 (4.3)	1 (3.7)
Mixed	6 (8.1)	5 (10.6)	1 (3.7)
BMI n (%)			
Mean ±SD	25.5±4.2	25.3±3.6	25.8±5.0
Range	18.2-38.6	18.2-37.1	19.3-38.6
<20	5 (6.8)	4 (8.5)	1 (3.7)
20-24.9	30 (40.5)	16 (34)	14 (51.9)
25-29.9	30 (40.5)	22 (46.8)	8 (29.6)
30>	7 (9.5)	3 (6.4)	4 (14.8)
Unrecorded	2 (2.7)	2 (4.3)	-
T1DM Duration (years)			
Mean ±SD	18.3 ±10.9	17.9 ±11.5	19 ±9.9
Range (years)	3-44	3-44	3-40
Year groups			
1-4	4 (5.4)	3 (6.4)	1 (3.7)
5-9	13 (17.6)	12 (25.5)	1 (3.7)
10-14	16 (21.6)	7 (14.9)	9 (33.3)
15-19	10 (13.5)	6 (12.8)	4 (14.8)
20-24	13 (17.6)	7 (14.9)	6 (22.2)
25>	18 (24.3)	12 (25.5)	6 (22.2)

	(n (%); or mean ±SD and range)		
Characteristic	Total (n=74)	LH group (n=47)	GV group (n=27)
HbA1c			
Mean at baseline ±SD mmol/mol	68 (±14.5)	68 (±15)	67 (±13.8)
Range mmol/mol	44-110	44-110	46-103
Mean at baseline ±SD %	8.4±1.3	8.4±1.4	8.3±1.3
Range %	6.2-12.2	6.2-12.2	6.4-11.6
42-52 mmol/mol [6.0-6.9 %]	5 (6.8)	4 (8.5)	1 (3.7)
53-63 mmol/mol [7.0-7.9 %]	27 (36.5)	17 (36.2)	10 (37)
64-74 mmol/mol [8.0-8.9 %]	24 (32.4)	12 (25.5)	12 (44.4)
75-85 mmol/mol [9.0-9.9 %]	9 (12.2)	8 (17)	1 (3.7)
≥ 86 mmol/mol [≥10 %]	9 (12.2)	6 (12.8)	3 (11.1)
Education level			
Secondary school level	23 (31.1)	14 (29.8)	9 (33.3)
University level or above	51 (67.6)	32 (68.1)	18 (66.7)
Unrecorded	1 (1.4)	1 (2.1)	
English			
as first language	62 (83.7)	36 (76.6)	26 (96.3)
as second language	12 (16.2)	11 (23.4)	1 (3.7)
Dominant hand			
Right-handed	64 (86.5)	39 (83)	25 (92.6)
Left-handed	6 (8.1)	5 (10.6)	1 (3.7)
Both	1 (1.4)	1 (2.1)	-
Unknown	3 (4.1)	2 (4.3)	1 (3.7)

N, Number; SD, Standard deviation; BMI, Body mass index; %, Percentage; T1DM, Type 1 diabetes mellitus; HbA1c, Glycated haemoglobin

No differences were found between those who participated in the GV and LH characterisation study in respect of insulin types, total daily insulin at baseline and needle length. Basal-bolus therapy was used by all participants for bolus insulin; 75.7% (n=56) were taking insulin aspart, and 20.3% (n=15) used insulin lispro. Basal insulins were glargine (36.5%, n=27), and detemir (58.1%, n=43). The mean total daily dose of insulin at baseline was 49.79 units (±18.13). The needle sizes used by the participants varied; 39% (n=29) used 4-mm needles, 32% (n=24) used 5-mm needles, 23% (n=17) used 6-mm needles, and one reported using 8-mm needles. Three quarters of participants (n=54) reported testing their blood glucose four or more times per day. The majority of the participants (59%, n=44) had attended a structured education programme, including dose adjustment and carbohydrate counting, and 78% (n=58) observed carbohydrate counting; out of 58 participants 18 (31%) had not attended a structured programme (Table 30).

Table 30: Insulin treatment characteristics

	(n (%); or mean ±SD and range)		
Characteristic	Participants (n=74)	LH group (n=47)	GV group (n=27)
Insulin requirement*			
Mean ±SD	46.3 ±8.5	46.8 ±7.7	45.5 ±9.8
Baseline total insulin dose	49.8 ±18.1	51.1 ±17.1	47.5 ±19.9
Baseline total basal Insulin	26.5 ±10.9	26.7 ±11.2	25.9 ±10.9
Baseline total bolus insulin	23.8 ±9.3	25.0 ±8.5	21.5 ±10.3
Long-acting insulin n (%)			
Glargine	27 (36.5)	18 (38.3)	9 (33.3)
Detemir	43 (58.1)	27 (57.4)	16 (59.3)
Degludec	2 (2.7)	1 (2.1)	1 (3.7)
Isophane Insulin	2 (2.7)	1 (2.1)	1 (3.7)
Quick-acting insulin n (%)			
Aspart	56 (75.7)	38 (80.9)	18 (66.7)
Lispro	15 (20.3)	7 (14.9)	8 (29.6)
Glulisine	1 (1.3)	-	1 (3.7)
Unrecorded	2 (2.7)	2 (4.2)	
Needle length n (%)			
4 mm	29 (39.1)	19 (40.4)	10 (37)
5mm	24 (32.4)	16 (34)	8 (29.6)
6mm	17 (23)	10 (21.3)	7 (25.9)
8mm	3 (4.1)	1 (2.1)	2 (7.4)
12.5mm	1 (1.4)	1 (2.1)	-
Blood glucose monitoring test/day n (%)			
<4	19 (25.7)	18 (38.3)	1 (3.7)
4	20 (27)	10 (21.3)	10 (37)
>4	34 (45.9)	19 (40.4)	15 (55.6)
Unrecorded/missing	1 (1.4)	-	1 (3.7)
Mean number of tests ±SD	3.9±1.5	3.6±1.6	4.5±0.9
Attended structured education n (%)	44 (59)	27 (57)	17 (63)
Carbohydrate counting n (%)	58 (78)	39 (83)	19 (70)
*= 0.6 units per Kg body weight			

4.8.2 Hypoglycaemia awareness and frequency

The awareness of hypoglycaemia is summarised in Table 31. Seventy-three participants completed the Gold score question, and ten of them were noted to have impaired awareness (Gold score >4), and while 41% (n=31) of the participants had previously experienced a hypoglycaemic episode requiring third party assistance, with 27%(n=20) experiencing such an episode in the past 12 months.

Table 31: Awareness of hypoglycaemia

	n (%)		
Characteristic	Participants (n=74)	LH group (n=47)	GV group (n=27)
Hypoglycaemia awareness (Gold score)			
1-2 (Aware)	54 (73)	34 (72.3)	20 (74.1)
3	9 (12.2)	6 (12.8)	3 (11.1)
4-7(Unaware)	10 (13.5)	6 (12.8)	4 (14.8)
Unrecorded	1 (1.4)	1 (2.1)	
Hypoglycaemia assistance	31 (41.9)	19 (40)	12 (44.4)
Frequency of severe hypoglycaemia during last year			
None	53 (71.6)	32 (68.1)	21 (77.9)
1	13 (17.6)	10 (21.2)	3 (11.1)
2	3 (4.1)	2 (4.3)	1 (3.7)
3	-	-	-
4	-	-	-
>4	4 (5.4)	2 (4.3)	2 (7.4)
Unrecorded	1 (1.4)	1 (2.1)	-

4.8.3 LH characteristics

A total of 740 nodules were observed in the participants. The highest proportion was identified in the abdomen (43.2%, n= 320); followed by thigh (36.2%, n=271); gluteal region (17%, n=122); and triceps (3.6%, n=27) areas. Diffuse areas were noticed in most of the injection sites (n=304). A summary of the frequency of the nodules and diffuse areas observed is presented in Table 32 and Figure 12.

Table 32: Anatomical distribution and frequency of the nodules and diffuse areas based on US scans

(n (%); or Mean ±SD			
Characteristic	Total (n=71)	LH group (n=45)	GV group (n=26)
LH nodules Total observed (n) Mean ±SD Range	 740 10.4 ±6.2 1-29	 450 10.0 ±6.2 1-28	 290 11.2 ±6.1 3-29
Anatomical sites	Total		
Thigh*	271 (36.2)	150 (33.3)	121 (41.7)
Gluteal region	122 (17)	74 (16.4)	48 (16.6)
Abdomen	320 (43.2)	215 (47.8)	105 (36.2)
Triceps	27 (3.6)	11 (2.4)	16 (5.5)
Characteristic	Total (n=74)	LH group (n=47)	GV group (n=27)
Diffuse areas Total observed (n) Mean ±SD Range	 304 4.1 ±1.8 1-10	 198 4.2 ±1.8 2-10	 106 3.9 ±1.7 1-7
Anatomical sites	Total		
Thigh	115 (37.8)	70 (35.4)	45 (42.5)
Gluteal region	54 (17.8)	37 (18.7)	17 (16)
Abdomen*	113 (37.2)	78 (39.4)	35 (33)
Triceps	22 (7.2)	13 (6.5)	9 (8.5)
*Thigh area includes right and left lateral and anterior thigh * Abdomen area includes right and left upper and lower abdomen			

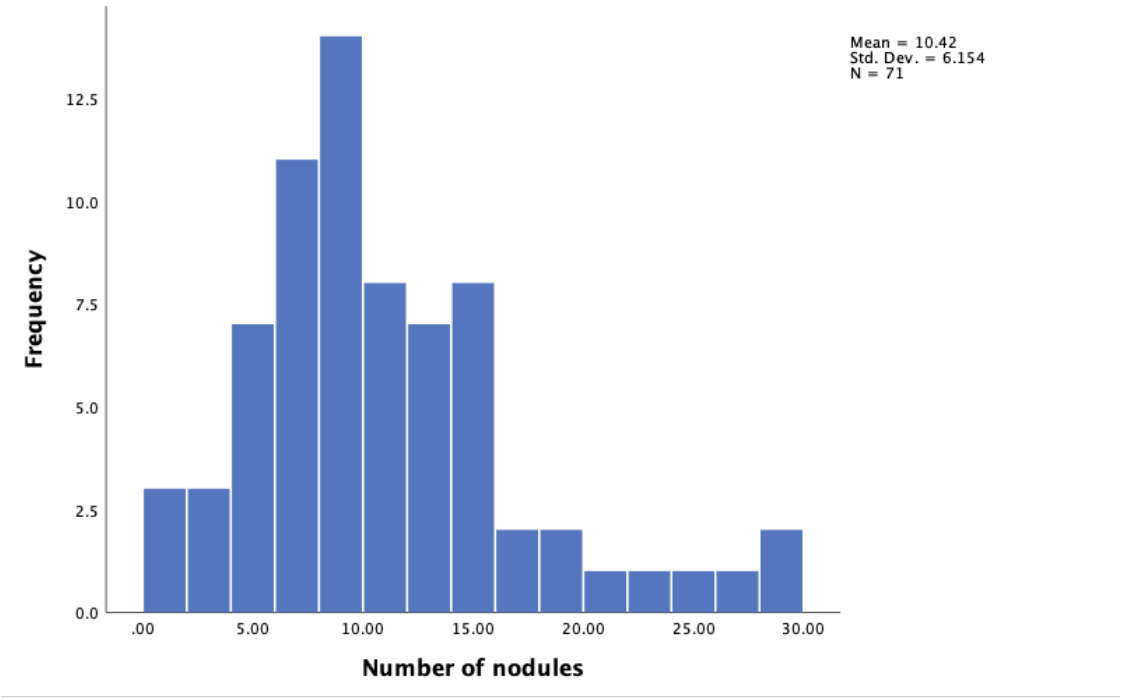


Figure 12: Number and frequency of the nodules

The majority of the participants (n=71) had multiple nodules, with three participants presenting with one large diffuse area without nodules. Of the participants with multiple nodules, 15% (n=11) had less than five nodules, 43% (n=32) had between 5 and 10 nodules, 23% (n=17) had between 11 and 15 nodules and 15% (n=11) had more than 15 nodules. The size of the nodules ranged between 1.8 and 40mm. In terms of average nodule size, the nodules in the thigh were largest followed by those in abdomen, this distribution is the same when considering the maximum sized nodule in each area (see Table 33). Table 34 shows the LH nodule size based on US for the LH characterisation group.

Table 33: LH nodule size based on US scans

Mean ±SD of all nodules and largest nodule				
Characteristic	Participants (n=71)			
Anatomical Sites	All nodules (mm)		Largest nodule (mm)	
	Right	Left	Right	Left
Thigh	6.6±2.9		10.6±9.3	
Anterior thigh	5.9±2.6	6.6±3.4	7.3±2.8	8.2±3.7
Lateral thigh	6.3±3.3	6.1±2.4	8.6±5.2	7.3±3.2
Gluteal region	5.9±2.1	5.2±1.4	8.3±6.9	6.7±2.5
Abdomen	6.1±3.9		8.7±5.1	
Upper abdomen	5.8±2.8	6.1±1.0	6.6±3.5	8.8±1.4
Lower abdomen	6.2±4.5	6.1±4.1	7.6±4.7	8.1±5.2
Triceps	4.9±1.1	8.6±8.3	5.6±1.1	9.8±7.7

Table 34: LH nodule size based on US for the LH characterisation group

Mean ±SD of all nodules and largest nodule				
Characteristic	LH group (n=45)			
Anatomical Sites	All nodules (mm)		Largest nodule (mm)	
	Right	Left	Right	Left
Thigh	7.5±3.5		10.2±5.4	
Anterior thigh	6.9±2.8	8.6±4.9	8.1±2.9	10.7±4.5
Lateral thigh	7.2±3.8	6.7±2.4	9.8±6.0	7.7±2.9
Gluteal region	6.5±2.0	5.5±1.2	9.4±8.1	7.3±2.5
Abdomen	6.8±4.6		9.7±5.9	
Upper abdomen	6.5±2.7	6.0	7.6±3.3	9.9±0.5
Lower abdomen	6.9±5.3	6.9±4.9	8.2±5.5	9.2±6.0
Triceps	5.9±0.4	2.3	6.4±0.9	2.3

The frequency of the LH grades observed for the largest nodules in each anatomical area is detailed in Table 35. Higher grade LH areas were found in the abdomen area, followed by the thigh. In the GV group the thigh was the area most affected by LH (see table 14 page 135).

Table 35: Grading of largest LH nodules per anatomical area in 74 participants

		n (%)				
Area	Total max graded LH per area	Grade				
		Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Thigh	55	8 (14.5)	8 (14.5)	14 (25)	12 (22)	13 (24)
Anterior thigh*	31	5 (16)	6 (19)	8 (26)	8 (26)	4 (13)
Lateral thigh*	40	5 (12.5)	10 (25)	8 (20)	7 (17.5)	10 (25)
Gluteal region	33	6 (18.2)	6 (18.2)	8 (24.2)	5 (15.2)	8 (24.2)
Abdomen**	58	5 (9)	13 (22)	20 (34)	9 (16)	11 (19)
Upper abdomen	10	2 (20)	2 (20)	2 (20)	2 (20)	2 (20)
Lower abdomen	55	4 (7)	14 (26)	20 (36)	7 (13)	10 (18)
Triceps	11	5 (46)	2 (18)	2 (18)	1 (9)	1 (9)
* Number inflation is observed when counting max grade in the anterior and lateral thigh independently. ** Number deflation is observed when counting max grade in the upper and lower abdomen as one.						

Table 36: Grading of largest LH nodules per anatomical area in LH group

		n (%)				
Area	Total max graded LH per area	Grade				
		Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Thigh	35	7 (20)	5 (14)	8 (23)	5 (14)	10 (29)
Anterior thigh*	17	4 (23)	3 (18)	2 (12)	5 (29)	3 (18)
Lateral thigh*	26	5 (19)	4 (15)	6 (23)	3 (12)	8 (31)
Gluteal region	22	4 (18)	4 (18)	4 (18)	3 (14)	7 (32)
Abdomen**	39	4 (10)	8 (20)	10 (26)	7 (18)	10 (26)
Upper abdomen	7	2 (29)	1 (14)	1 (14)	1 (14)	2 (29)
Lower abdomen	38	3 (8)	10 (26)	10 (26)	6 (16)	9 (24)
Triceps	6	4 (66)	-	1 (17)	-	1 (17)
* Number inflation is observed when counting max grade in the anterior and lateral thigh independently. ** Number deflation is observed when counting max grade in the upper and lower abdomen as one.						

Table 37. details the severity index for LH, with the LH group having a higher mean index compared with the GV group. The severity distribution is shown in a histogram (Figure 13). The data show that while the majority of participants have a severity level <2.0, a significant proposition of participants with large multiple nodules score above 4.0.

Table 37: Total severity of LH nodule

Mean ±SD (Range)		
Participants (n=74)	LH group (n=47)	GV group (n=27)
1.8±1.5 (0-6.3)	1.8± 1.6 (0-6.3)	1.6±1.4 (0-5.5)

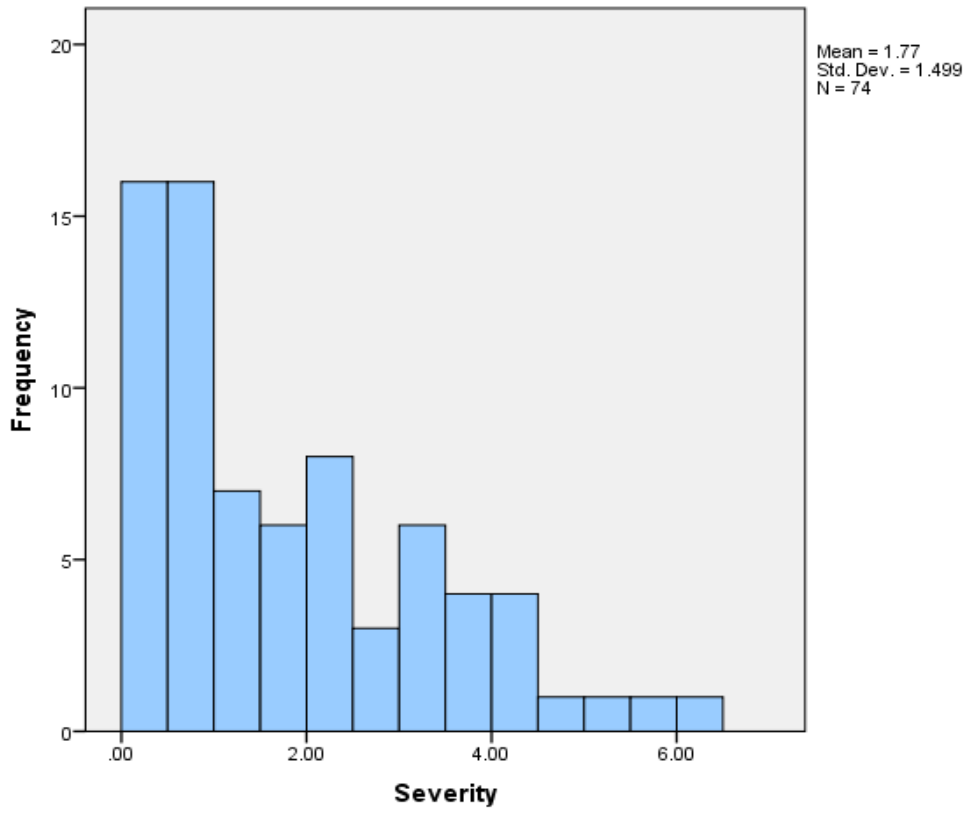


Figure 13: Severity and frequency of the nodules

The scans identified 42 necrotic patches in nodules, this would suggest a crude prevalence for necrotic nodules of about 6%, and that around 30% of the participants had nodules with necrotic features on US scans (Table 38).

Table 38: Anatomical distribution and frequency of necrotic patches

Participants (n=22) Total necrotic patches observed (n=42)			
Anatomical sites	Total	Right	Left
Thigh	13	5	8
Anterior thigh	7	2	5
Lateral thigh	6	3	3
Gluteal region	11	7	4
Abdomen	18	7	11
Upper abdomen	1	1	-
Lower abdomen	17	6	11
Triceps	-	-	-

Table 39 shows that the necrotic tissue was more common in higher grade (i.e. larger) LH nodules, with most being observed in the lower abdomen.

Table 39: Location of necrotic patches by LH grade per anatomical area

Participants (n=22)					
Anatomical sites	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Anterior thigh	1	-	1	2	3
Lateral thigh	2	2	-	1	1
Gluteal region	-	-	6	1	4
Upper abdomen	-	-	-	-	1
Lower abdomen	-	2	3	3	9

4.8.4 Change in glycated haemoglobin (HbA1c) and TDI dose

Follow-up data for HbA1c and TDI doses were available for 54 (73%) and 48 (64%) participants respectively (Table 40 and 41). No difference was observed in HbA1c between Condition 1 and Condition 2, with means of 67mmol/mol (8.3%) and 68 mmol/mol (8.4)% (p = 0.09).

Table 40: Change in glycated haemoglobin between Condition 1 and Condition 2

Mean ±SD mmol/mol (%)		
Participants (n=54)	LH group (n=31)	GV group (n=23)
1.1±5.5 (0.1±0.5) p=0.09	1.1±6.6 (0.1±0.6) p=0.3	1.1±4.4 (0.1±0.4) p=0.9

There was a modest reduction in the mean total daily insulin dose of the participants who completed the follow-up (n=48) between Conditions 1 and 2, of 2.9±8.1 (P= 0.01); this is detailed in Table 41.

Table 41: Total daily insulin between Condition 1 and Condition 2

Mean ±SD (Unit)					
Participants (n=48)		LH group (n=27)		GV group (n=21)	
C1	C2	C1	C2	C1	C2
47.7±16.6	44.7±15.0.7	50.0± 17.7	47.2±16.1	44.8±14.9	41.6±13.4
C1, Condition 1; C2, Condition 2.					

It was not possible to estimate GV or effectiveness of insulin response as these data were not collected, and while there were some data from blood glucose monitoring tests; many in the LH group tested <4 times per day. Hence, it was difficult to estimate any effects of identifying the LH areas and advising them to avoid them in this sample. Of the participants who were testing ≥4 times per day (n=34) the mean change was not significant with a reduction in SD of -0.4 mmol/L.

4.8.5 Education attendance and left/right handedness

Previous attendance at a structured education programme was considered in relation to the prevalence of LH, comparing those who had and had not attended. No statistically significant difference was observed between those who had attended (n=42) and do those who have not attended (n=29) education in terms of the number of LH nodules observed, with means of 11.5 (±6.9) and 8.8 (±4.3) for those who had attended structured education and those who had not attended respectively (p=0.8).

In terms of left/right handedness, 64 (86%) participants were right handed with distribution of the LH regions in these participants, being: skewed to the right in 32 participants (50%); skewed to the left in 23 participants (36%); with remainder showing an even distribution (n=9, 14%). Of the six participants who were left-handed, four had more LH on the left side and two participants had more LH on the right side of their injection areas. One participant who was ambidextrous (using both the right and left hands for injections) had more LH areas on the right.

4.8.6 Digital palpation compared with ultrasound

As all the participants had their LH assessed by digital palpation blind to the US scans, it was possible to compare how consistent palpation following a clinical protocol was compared with the US observations. A total of 528 areas where examined using both palpation and US. Table 42 identifies the number of agreements and disagreements between US and palpation.

Table 42: Palpation detected LH compared to US

Nodules US	Palpation agree	Palpation disagree
Observed	147	149
Not observed	226	6

The data show that palpation had lower sensitivity in detecting LH compared with US. The number of areas in which LH was detected by US was double that of palpation. Overall concordance between palpation and US was 70.6%, with a Cohen's kappa of 0.44 indicating moderate agreement.

4.8.7 Insulin treatment satisfaction, diabetes distress and QoL

In this section the data on insulin treatment satisfaction and diabetes distress are presented, comparing responses before and after participants were advised to change their injection sites to avoid the identified LH areas.

No differences were observed in the total ITSQ scores or in any of the sub-scales between Conditions 1 and 2 ($p = 0.5$) (see Table 43). Likewise, no differences were observed in the DDS scores between Conditions 1 and 2 ($p = 0.6$) (see Table 44).

In terms QoL at Condition 1 the mean (SD) visual analogue scale score of EQ5-5D-5L was 71.5% (±17.8) and the mean (SD) index-based value was 0.9 (±0.2). In Condition 2 the mean (SD) visual analogue scale score was increased slightly to 76.2% (±14.7) ($p=0.01$); while the index-based value remained unchanged at 0.9 (±0.2) ($p =0.3$).

Table 43: Insulin treatment satisfaction

Mean±SD									
ITSQ items	Participants (n=60)			LH group (n=37)			GV group (n=23)		
	C1	C2	P	C1	C2	P	C1	C2	P
IR	58.8±17.2	59.2±16.5	0.8	58.1±17.3	58.8±17.5	0.7	61.0±17.2	58.7±15.2	0.5
LF	61.7±16.3	60.9±15.8	0.7	62.5±14.9	63.7±15.5	0.6	60.4±18.7	56.3±15.4	0.2
GC	42.7±12.9	46.9±11.8	0.6	40.9±11.8	45.9±11.4	0.8	45.2±14.2	48.3±12.4	0.4
HC	43.7±18.9	44.9±20.4	0.5	43.8±18.8	44.9±22.0	0.5	43.6±19.5	44.9±17.9	0.7
DS	39.5±17.4	39.4±17.3	0.9	58.1±18.5	58.4±18.2	0.9	64.3±3.1	64.2±15.6	0.9
Total score	54.3±12.6	55.1±13.1	0.5	53.2±12.8	54.8±14.2	0.2	55.9±12.5	55.4±11.6	0.8
ITSQ, Insulin treatment satisfaction; IR, Inconvenience of regimens; LF, Lifestyle flexibility; HC, Hypoglycaemia control; GC, Glycaemic control; DS, Insulin delivery device satisfaction; 1, Condition 1; 2, Condition 2.									

Table 44: Diabetes distress

Mean±SD									
DDS items	Participants (n=60)			LH group (n=37)			GV group (n=23)		
	C1	C2	P	C1	C2	P	C1	C2	P
EB	2.3±1.1	2.3±1.0	0.9	2.4±1.2	2.4±1.2	0.6	2.2±0.7	2.3±0.8	0.5
PD	2.1±1.0	2.2±0.9	0.2	2.2±1.2	2.2±1.1	0.7	1.9±0.7	2.1±0.8	0.2
RD	2.4±1.0	2.3±0.9	0.9	2.5±1.2	2.4±1.1	0.6	2.2±0.8	2.3±0.8	0.5
ID	2.4±1.0	2.4±1.2	0.5	2.4±1.3	2.4±1.2	0.9	2.3±0.8	2.4±1.1	0.3
Total score	2.3±0.9	2.3±0.9	0.6	2.4±1.1	2.3±1.1	0.8	2.1±0.6	2.3±0.8	0.3
DDS, Diabetes distress scale; EB, Emotional burden; PD, Physician-related distress; RD, Regimen-related distress; ID, Interpersonal distress; C1, Condition 1; C2, Condition 2.									

4.9 Qualitative data on participant experiences

Structured exit interview data (Appendix 12) were collected from 62 (84%) participants from both the GV and LH groups. The duration of the interviews ranged from 20 to 45 minutes. The interviewers asked specific closed questions regarding injection behaviours, and participants were asked to comment on how they found changing their injection sites. The data on changing sites is presented in Tables 45, 46 and 47, indicating the responses to each of the questions with illustrative participant comments for each question. The responses suggested that participants had mixed views about changing sites, with the majority reporting no difficulty, although approximately one third of participants did find it challenging, and preferred their previous sites or found new sites painful. Hence, changing sites may be more challenging for some people with diabetes, and they may require additional support in observing site changes. However, the majority of respondents did feel that changing sites had positive impact on their insulin or glucose levels.

Table 45: Changing injection sites

Question	Response n (%)			Comments
	Yes	No	No response	
Difficulty in changing sites	18(29)	40 (65)	4 (6)	• *“Found it difficult to remember”* • *“Took a couple of days to remember”* • *“Easy to follow and change the site but it was harder to do it in public places”*
Desire to use the old injection sites	23 (37)	27 (44)	12 (19)	• *“Injected once or twice in old site"* • *"Occasionally, just due to habit"* • *"Wanted to go back for convenience and to reduce stigma of people noticing me taking injections"* • *"Wanted to go back every single time, but didn't"*
Pain at the new injection sites	22 (35.5)	33 (53.2)	7 (11.3)	• *"More tender in certain spots"* • *" found the old site - legs - more painful"* • *"No difference in pain, bruising in the first week but old site used to bleed more"* • *[New injection site] was painful for two days but then settled"*
Improvement of glucose level	33 (53)	18 (29)	11 (18)	• *"More stable"* • *"Just the same"* • *"Results better"*
Perception the effectiveness of insulin	35 (56.4)	12 (19.4)	15 (24.2)	• *"A measurable difference"* • *"With regard to using the old site it was "a bit of luck" whether the insulin worked; with new site feels "efficiency is much better"*

Participants were also asked to give their views on different methods of managing injection sites to lower the risk of LH. They were asked to consider rotating anatomical sites (upper abdomen; lower abdomen; lateral thigh) every three months or seasonally to rest areas for longer. Participants did not consider either option as appealing (see Table 46)

Table 46: Injection site rotation

Question	Response n (%)			Comments
	Yes	No	No response	
Injecting into one site for three months	55 (89)	4 (6)	3 (5)	*"It would be impracticable to rotate as would not be able to take the trousers down in public space. it would be good to inject early morning and evening in four sites (upper R/L thigh and R/L buttock) and rotating those every month and then rotate injections around four sites in abdomen (upper L&R, lower L&R) during the daytime when in public"*
Seasonal method	45 (73)	10 (16)	7 (11)	• *"Very pleased by using the seasons"* • *"It is too long to inject into just one site"* • *"Would not feel comfortable doing this"*

In terms of receiving advice on injection sites or whether they were reviewed (see Table 47), participants reported mixed experiences. While half reported having been given advice regarding injection sites, three-quarters had not been given clear instructions as to how to do this and less than half had their sites inspected in the last two years. These data suggest that the process of site management may be somewhat ad hoc, and does not seem to be a priority for clinicians.

Table 47: Instruction and examination of injection site management

Question	Response, n (%)			Comments
	Yes	No	No response	
Give advice regarding injection sites	33 (53)	16 (26)	13 (21)	• *“Lots of information at the beginning of the diagnosis but nothing since”* • *“Told to not do it in the same place, keep moving around sites, but vague information."* • *"Keep moving the sites" - nothing specific"*
Prior instruction	5 (8)	44 (71)	13 (21)	• *"Was given forms but didn't read them so doesn't know"* • *"Just told to avoid going into the same area, all news to me when you guys approached me."* • *Remembers having his abdomen divided into quadrants when he was a child but not since then.*
Previous examination (past two years)	26 (42)	27 (44)	9 (14)	• *“Maybe 5 times in 20 years"* • *Asked "any complaints?" but not actually looked at or touched* • *"Examined in the last two years - only if issues"* • *“Only once in clinic prior to referral to the study - usually no examination"*

4.10 Feasibility observations

4.10.1 Injection sites procedure

All participants in the GV and LH characterisation studies had their injection sites scanned and examined by US and digital palpation as per protocol (SOP). The duration of the examination session varied from 10 to 20 minutes, while the US scans ranged from 60 to 90 minutes. No technical issues were observed in following the SOP.

4.10.2 Recruitment

From September 2017 to February 2018, a total of 226 people with T1DM were screened from diabetes out-patient clinics at GSTFT, of whom 95 (42%) met the eligibility criteria and 27 (28%) participated in the GV study. In LH characterisation study the recruitment rate was slightly higher; out of 131 who met the eligibility criteria, 47 (36%) participated in the study (Figure 7 section 4.1.1).

4.10.3 Retention rate

The retention rate in the GV study was 89 %. Three out of 27 participants left the study (two did not complete the Condition 2 and one withdrew). In the LH characterisation group 81% (n=38) of the participants completed the study and 19% (n=9) of the participants did not complete Condition 2.

4.10.4 CGM insertion

CGM sensors were fitted to all participants (n=27) enrolled on the GV study in Conditions 1 and 2. Sensor failure or insufficient CGM data occurred in six observations, three were in Condition 1 and three in Condition 2.

4.10.5 Insulin dose stabilisation

All participants in both the GV and LH characterisation arms of the study received insulin advice as per protocol after having US screening of their injection sites. Unfortunately, the compliance with the insulin advice was not recorded. However, in the exit interviews 19 (31%) of participants reported taking less insulin than before changing the injection sites, with two participants reporting taking more insulin than before, and 29 (47%) did not notice any change in their insulin dose. Twelve participants provided no data on their insulin doses.

In the GV participants, TDI dose data were available from the participants' CGM diaries, allowing a comparison between Conditions 1 and 2. At follow-up (Condition 2 last visit) 40%(n=6) of the participants reduced their insulin by >4 units, 53% (n=8) had not changed their dose and the one increased their doses by >4units. Some participants (20%, n=3) were not advised to reduce their dose as it was consistent with their estimated insulin requirement.

4.10.6 Adverse events

No severe hypoglycaemic events (those that would require third party assistance for recovery), or other adverse events were reported during the study.

4.11 Chapter summary

This chapter has presented the study findings from the GV and LH characterisation studies. The findings from both analyses indicate that LH is a prevalent clinical effect of insulin exposure. The data also suggest that LH is heterogenous in its morphology, with diffuse areas and nodules being the most common observation. The size, number and distribution of LH areas observed in participants varied depending on their injecting habits. The US scan data highlight some novel observations associated with LH areas, including dermal disruption which is potentially indicative of inflammation, and areas that could be necrotic tissue. In terms of the impact of LH on insulin and glycaemic control the data showed a somewhat complex picture. In relation to TIR and GV, there was a mixed picture, with approximately one third of participants exhibiting improvement in time spent in range (4-10mmol/L), while the remainder showed either no improvement, or in a few cases a decrease. The exit interviews reveal that many participants found changing injection sites to be challenging, but most also reported a beneficial impact of the change on their glucose levels and responsiveness to insulin. The interviews also suggest that injection site assessment and management advice is currently limited. The findings are discussed in more detail in the next chapter, with considerations given to their clinical interpretation and the limitations of the study.

Chapter 5. Discussion

An exploratory case-crossover study was presented in this thesis, the study aimed to test the association between US characterised LH lesions and glucose levels (TIR and GV) in people with T1DM. The GV study included data from 27 participants, with completed follow-up data on 15 participants. Data were also presented from an observational study of 47 participants who had their injection sites examined in order to assess whether US scanning of injection sites could be used as a tool to detect, characterise and grade LH tissue.

In this chapter, study findings are discussed in relation to the research aim and objectives with reference to the wider literature on the impact of LH on TIR and GV, the chapter is organised as follows:

- LH characterisation and detection
- Time in range and GV
- Insulin doses and adjustments
- Insulin injecting behaviours
- The use of ultrasound in LH assessment
- Process evaluation findings
- Insulin antibodies and LH
- Insulin satisfaction, diabetes distress and quality of life

The chapter also addresses the study strengths and limitations; and Implications for future research and clinical practice.

5.1 LH characterisation and detection

The study has provided some novel insights into the clinical features of LH in insulin exposed tissue as observed with US scan. The study has shown that LH is heterogeneous in nature in terms of the characteristics and size of the affected tissue observed. The study has also revealed some previously un-noted features of LH related tissue damage. The scanned injection areas mostly revealed areas of increased echogenicity or reflectivity of the dermal tissue compared to sites not exposed to insulin injections. In some areas the delineation between the subcutaneous and dermal layer disappeared and increased in thickness, suggestive of inflammation that may be related to increased insulin exposure. This finding was congruent with those of Perciun (2010), who undertook US scans of 40 insulin treated participants and reported complex multilayer changes including possible inflammatory reactions at injection sites. Some evidence of inflammatory tissue damage was also reported in one of the studies examining the histology of LH samples, with increased fibroblasts being present (Wallymahmed et al. 2004). This finding is important as it extends the classic interpretation of LH as being nodular in form and suggests that tissue damage is more generalised than has been previously considered or as described in current LH guidelines (FIT 2016). While it is not possible to say whether these changes have the same effect on glucose levels when injecting insulin as LH nodules, what can be observed is that nodules rarely occur independently of wider tissue change. Indeed, the comparison between US and digital palpation assessment of LH observed in the study suggest that what is physically palpated and identified as LH is often shown under US to be predominantly diffuse (dense) tissue.

In the study participants a wide range of LH presentations (ranging from single nodules to multiple nodules and diffuse areas) were observed. The anatomical distribution of the LH mirrored the injection site preferences and were concentrated in the thigh and lower-abdominal areas. These observations were again largely congruent with the previous US studies of LH (Kapeluto et al. 2018, Bertuzzi et al. 2017, Perciun et al. 2014, Perciun 2010). Whether the number of presenting nodules or tissue changes inflates the risk of an impaired insulin response is unclear. In the GV arm of the study,

the case (GV-4) that showed an improvement in TIR by 20% had only one large nodule. While the magnitude of effect observed in this case may be explained by the fact that the participant had multiple alternative injections areas without LH to use, it also illustrates the potential effect of one LH site on insulin absorption, which in this case which varied by a third in the LH and non-LH conditions.

Areas of reduced echogenicity were also observed in some nodules, suggesting no or low blood flow indicating the possibility of necrotic tissue, as has been reported in previous studies (Perciun 2010). Perciun (2010) observed hypoechoic irregular shaped lesions attributed to necrosis in 7.5% (n=3) of participants, although they also suggested they could be areas of haematoma. The data from this study, however, suggest that these areas of potential necrotic tissue in LH tissue could be more common, as they were observed in around a third of the participants in both the GV and LH characterisation participants. The likelihood, that these observations are areas of necrotic tissue, is supported by one of the two previous studies that have examined LH tissue, were necrotic tissue was found in the sampled tissue (Wallymahmed et al. 2004). However, what is not known is whether this necrotic tissue plays a role in insulin absorption or glucose regulation or indeed whether they convey any other health hazards. Hence, the presence of necrosis and inflammation warrants further investigation to confirm the US based observations, via either tissue biopsy or screening for inflammatory markers and cytokines that are associated with necrotic adipocytes and may mediate insulin signalling (Stafeev et al. 2017). Another observation that may indicate evidence of inflammatory processes or potentially trauma related to needle insertion, was dermal disruption. These were seen as areas where the margin between the dermis and the underlying subcutaneous tissue is either ill-defined or in some cases absent. Perciun et al. (2010) also referred to this dermal disruption and identified as one of their subtypes of 'subcutaneous lesions', defining it as *'diffusely inhomogeneous hyperechoic thickened subcutis with an poorly defined boundary between dermis/subcutis'* (p.105).

This study also confirms the high prevalence of LH in people with T1DM, with a total of 740 LH nodules observed in the sample. Therefore, given that most participants reported that they had never previously had a thorough clinical examination of the LH areas suggests that this problem is largely undetected. This observation is congruent with the survey undertaken by Frid et al. (2016b) of which reported that 38.9% (n=4864) of the participants had never been examined for LH. This variation suggests that diabetes health professionals are either unaware of the significance of LH or are unsure as to the appropriate methods for detecting it (Gentile et al. 2016a). While the palpation procedure adopted for this study was based on the latest clinical guidelines and tested method for assessing LH (FITTER 2016, Frid et al. 2016a, Gentile et al. 2016a), the clinical examination was significantly inferior to US as suggested by previous studies (Kapeluto et al. 2018, Volkova et al. 2015). The agreement observed between the US and the clinical examination was moderate (κ=0.44) which is very similar to the findings of Kapeluto et al. (2018) study, who in a study of 103 participants (8% had T1DM) also reported moderate agreement (κ=0.50). Therefore, finding better ways for detecting LH are important, while raising awareness of LH and promoting assessment guidelines are important, first steps it is important to develop more robust data on the clinical impact of LH on glucose regulation to support more extensive consideration of LH in people with T1DM. This will require larger studies examining the multi-modal effects of LH on diabetes management and insulin action. Technology may also enhance and objective LH detection and assessment. Portable US technology is now being developed which has the same accuracy as larger scanning machines. Hence with training, member of the diabetes team may be able to undertake LH scanning with US either routinely or in cases where insulin absorption problems are suspected.

An additional output from the study was the introduction of a novel LH grading model. While it was not possible to assess or validate the grading system against an external clinical metric such as GV, it was possible to apply the grading consistently in assessing the LH areas identified. The study also provided additional features to consider in grading such as the presence of diffuse tissue. Hence, the study has

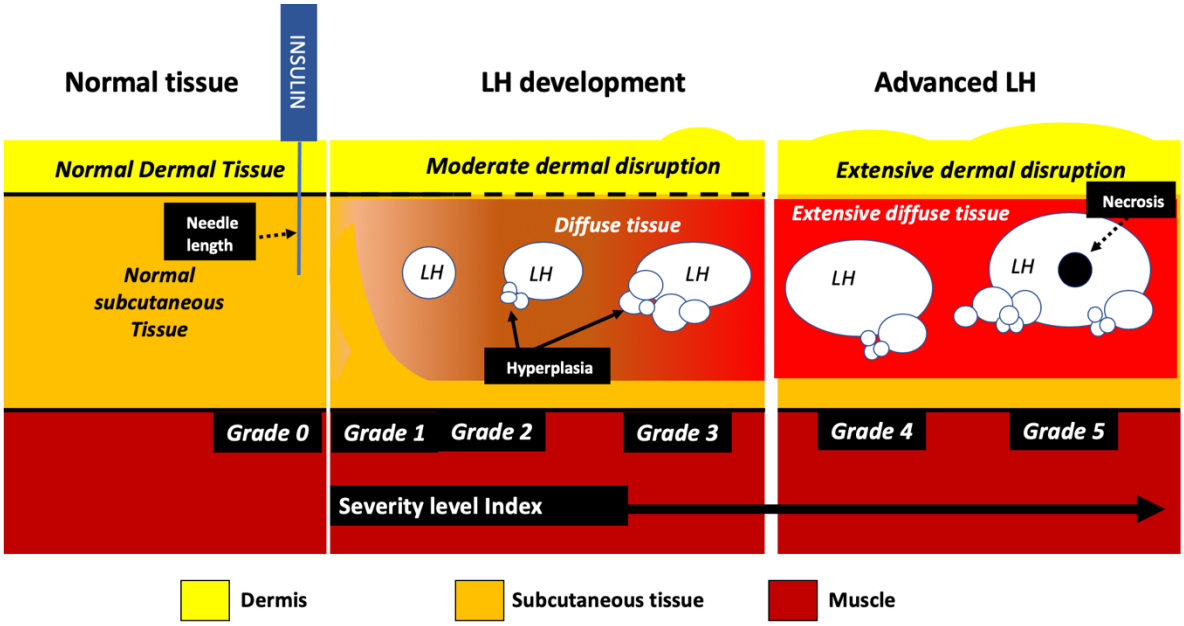
introduced a grading model that has more detailed features compared to some of the earlier grading systems based on visualisation or measurement alone (Conwell et al. 2008, Kordonouri et al. 2002, Hauner et al. 1996). While the grading system shares some similarities with the US-based grading system of Perciun (2010) who identified five grades of LH, it is perhaps easier to interpret as it integrates the diffuse tissue with nodule size and the presence of necrotic tissue. It would now be important to possibly compare the two grading systems in terms of clinical application and to consider whether the grade indicates a risk for altered insulin action. Indeed, it may be the grade of the LH rather the number of LH areas that conveys that risk as evidence in the participant with one large grade nodule who significantly improved his TIR by avoiding that area.

Another new metric introduced into the analysis was a LH severity index (severity= ((max grade^2 x nLH)/100), based on size and number of nodules. However, again this index showed no pattern in respect of the GV findings suggesting that frequency of nodules may not be so important. Therefore, larger nodules which are used more frequently may be more important than smaller more distributed areas of LH. One association that was observed was between the level of severity and insulin antibodies, which may be an additional area to explore in respect of the value of the severity index (see discussion section 5.7).

Putting the collective outputs from the characterisation finding of the study together, including the grading criteria it is possible to identify a hypothetical model of the pathological features of LH. While it is not possible from a cross-sectional observational study to speculate on the progression of LH, it is possible to illustrate the pattern of observations from single to multiple nodules and the presence of disrupted and necrotic tissue. This model is presented figuratively below (see Figure 14) and integrates the observations made in this study with those reported in previous studies (such as hyperplasia). The model illustrates the grading and severity index used in the study and shows some of the other novel observations made in the study such as dermal disruption. It is hoped that this model will provide a useful reference

for future studies and provide a tool for clinically characterising LH in people with diabetes.

Figure 14: A Model of LH Morphology and Grading



Finally, there was one observation in the study that may relay something of the enduring nature of LH. In the US scanning of one participant had a defined nodule in an area which had not been used for injecting insulin for eight years. The image showed a clearly identified nodule but with slightly less dense tissue than was observed in active areas (see Image 8 page 129). Hence, it is possible that the effect of LH may be enduring, although this could also be an anomaly as no other such observations were made. Therefore, whether LH lesions recover when insulin exposure and injections cease remains unknown. Conducting a study to consider recovery may be challenging, as it could require a prolonged period of observation to establish recovery. There would also be a number of factors to consider, such as: the size of the affected area and the duration of insulin exposure; and most importantly what would determine recovery, would that be the point at which insulin adsorption in the area returns to normal.

5.2 Time in range and GV

The findings of the case-crossover study have provided some important new insights into the relationship between LH and glucose regulation. While the effects observed in the TIR findings were varied, the data showed that in about a third of cases there was an improvement in TIR ≥10% in the observation period, suggesting some potential benefit in changing sites. The improvements observed in TIR seemed to occur independently from changes in insulin doses, with the participants who improved their TIR either reducing their insulin dose or maintaining it at the Condition 1 level. This is the first study to report such findings. While previous studies have shown the effects of LH on insulin absorption and glucose levels, these studies used insulin clamps and were conducted in highly controlled conditions (Famulla et al. 2016, Hovelmann et al. 2015). In this study the effects observed were in the real-world context and are ,therefore, important as they show that there may be potential clinical benefits in preventing and managing LH in people with T1DM.

However, the study has also identified that the relationship between LH and insulin action and glucose regulation is quite complex in the clinical setting. In relation to the GV measures and glycaemic control no differences were observed in between Condition 1 and Condition 2. The GV measures showed no pattern of association with the changes seen in TIR, with the exception of two participants (GV-5 and GV-11) who showed the largest reductions in their glucose CV on CGM, at 13 and 14% which corresponded with a reduction in their time below range of 15%. While it is also important to recognise that the lack of impact observed on the GV measures could be related to the small sample size and potentially to the insulin transition process as discussed below, it may also be that studying GV in this context is too heavily confounded (see study limitations section 5.9).

In terms of glycaemic control, no significant differences were observed in HbA1c or 1,5-AG. While the HbA1c result was may be related to the short time interval between measures (6 weeks) and that the participants did not have very high HbA1c's at baseline, with a median HbA1c of 65 mmol/mol [8.1 %]; the 1,5-AG test, which is more

responsive to short-term changes in glucose exposure and is particularly sensitive post-prandial glucose levels, might have shown more sensitivity to the changes in the glucose levels observed. However, again there was no consistent pattern of change in either HbA1c or 1,5-AG. The 1,5-AG test which is particularly sensitive to post-prandial hyperglycaemia (Dungan et al. 2006), did not show a consistent response in the participants who had the largest reductions in time above range which is somewhat unexpected. Hence, the value of this measure in assessing glucose exposures in relation to studies of LH avoidance may need further consideration.

Therefore, while the results show an indication of clinical benefit in relation to glucose regulation in avoiding LH effected areas, the data suggest that the association is likely to be heavily confounded by extraneous factors. There are multiple factors at play in glucose regulation (diet, activity, stress etc.) and these may well have had an impacted on the study findings particularly given the small sample of participants and the short period of glucose monitoring (see study limitations). A particularly important mediating factor in respect of the study may have been the process of adjusting insulin doses for Condition 2. The intention was to reset insulin levels to a baseline level based on the participant's body weight (0.6 units per Kg) to reduce the risk of hypoglycaemia and then to stabilise doses following each participants usual approach to insulin dose adjustment based on blood glucose testing in a six week wash-out phase prior to a follow-up CGM. The aim was to do this without changing the participants insulin regimen, the type of insulin and how they estimated their insulin doses. The difficulty was that not all the participants observed this advice and maintained their current dose (see section 5.4). Furthermore, participants may have benefited from more support when they switched away from their LH areas as exemplified in one participant (GV-14) with no change in TIR. This participant reduced their time above range by 9.3% but had a corresponding increase in time below range of 8.7% despite reducing the insulin dose by 8 units. This could indicate that the participant was receiving more active insulin doses following site changes. The learning point perhaps was that while the participant had a reduction of 8 units in the TDI from 49 units to 41 units, 7 of those units were background insulin, when a proportional reduction in the quick acting may

have been more appropriate. Hence, had the post transition insulin reductions been better considered they would have improved their TIR significantly, Identifying, a model for transitioning insulin doses to avoid hypoglycaemia when transitioning away from LH affected areas should be an important consideration for future clinical studies.

One additional observation that may indicate a beneficial effect of changing sites was observed in the increased number of bolus injections with evident effect on glucose levels. While there are many factors that could have mediated these observations in a small sample of participants the changes were generally consistent across the sample, suggesting some improvement in the action of quick acting insulin when moving to LH free sites.

Therefore, despite its limitations, the GV study has provided some important insights into the relationship between GV and LH than has been provided by the previous studies (Strollo et al. 2016, Blanco et al. 2013, Ibarra & Gallego 1998). As outlined in chapter 2, these studies used very crude estimates of GV (using mainly the SD of self-monitored blood glucose tests) and non-standardised methods for detecting LH. These studies were further limited by using non-matched controls comparing GV in people with and without LH which does not allow for any estimation of the association between LH and GV independent of a large number of confounding variables (age, duration of diabetes, gender, education, self-management behaviour etc.). In this case-cross-over study it would seem that the association between LH and GV is likely to be heterogenous in nature in people with T1DM. This would suggest the need for a more pragmatic approach, in which people with diabetes can (once their LH areas have been identified) be supported in trying new sites with more individualised in titrating insulin doses following the change (as considered further in the next section).

5.3 Insulin doses and adjustments

The study has identified some important learnings for future studies in respect of insulin doses in the transition away from LH affected injection sites. In studying the impact of LH on GV in a clinical context (rather than using insulin clamping models) it is clear that there is a need to actively manage the insulin adjustment process when transitioning away from LH areas. In this study participants were reset to a physiological estimate based on 0.6 units per KG (with 50% being allocated to basal) to avoid hypoglycaemia, although not all observed this advice. Avoiding potential hypoglycaemia was deemed of high importance in the study, as the clinical supervisors for the project had previously observed this problem and the risk was also suggested in previous studies. The Blanco et al. (2013) study, for example, reported a strong association between LH and the incidence of frequent unexplained hypoglycaemia (39%, n=108) in those with LH compared to (6%, n=9) in those without LH, which they explained by exposure to insulin in areas with and without LH. An advantage of this approach was thought to be that would individualise reductions, whereas other studies have adopted a 20% overall reduction to TDI (Campinos et al. 2017).

The overall picture observed in the GV study in respect of changes in insulin doses was inclusive and inconsistent. Overall, considering participants in both GV and LH characterisation arms of the study, there was a modest reduction in insulin doses with a drop in TDI of around 3.0 units. This reduction corresponds with a 2.0 unit (95%CI 1.4-2.5 units) drop in insulin reported in another multi-centre study of 346 participants assessing the impact of LH advice on injecting behaviours, although they include people with both T1DM and T2DM and did not specify the proportion of each (Grassi et al. 2014). In a similar intervention study (RCT) of LH education and changes to needle length with 123 participants (50% with T1DM), a drop in TDI of 5 units over six months was observed in the intervention group, although the controls also had a reduction of 3 units (Campinos et al. 2017). Hence, the average impact of LH avoidance on insulin requirements seems to be quite modest.

However, when looking at patterns of changes in insulin dose and impact on TIR in the GV study the picture becomes more complex. The data showed that while some participants had quite significant reductions in insulin doses, with corresponding reductions in time above range or increased hypoglycaemia; others who did not change doses also showed a reduction in their time above range, suggesting some stability in their insulin requirements following transition away from LH areas. As highlighted in the previous section, one participant increased her time below range despite reducing her TDI dose. Therefore, it is important to identify more effective processes for managing insulin doses when avoiding LH areas, to compensate for this variability. In this study the process was perhaps too passive, as it followed the participants usual adjustment practices to stabilise the participants doses rather than more active support, this was done to avoid confounding. It may also be that the physiological estimate used in resting insulin levels for the wash-out phase was too conservative in some participants. In addition, it might be important to give greater attention to bolus insulin doses as there were seen to be more active when moving away from LH areas. Therefore, it may have been better to have been more active in supporting participants in assessing and reviewing insulin ratios as well as basal insulin doses in a more managed way; while acknowledging that this could conflate the impact of the LH on glucose regulation, as providing insulin adjustment support may do this independently to the avoidance of LH areas. Establishing how best to manage this transition is important clinically, as current LH guidelines only advise the need to reduce insulin when moving away from an LH area without specifying in detail how this should be achieved other than increasing blood glucose monitoring (FIT 2016). One possible study design, that might compensate for the confounding effect of providing insulin advice, would be to: compare LH assessment/advice plus insulin support; with insulin support without LH advice to see if the former showed superiority over insulin advice in isolation, in people with identified LH (although blinding might be problematic in such a study as it would be necessary to screen both intervention and control subjects for LH). Despite these methodological challenges, identifying a strategy for this transition is of high importance, to ameliorate the risk or problematic

or severe hypoglycaemia, while also optimising insulin doses in order that the benefits of avoiding LH tissue can be fully and safely assessed.

5.4 Insulin injecting behaviours

A key factor in understanding LH is the behavioural habits associated with insulin injecting. Most people with T1DM not using CSII, will need to inject insulin at least 4-5 times daily. They will need to inject in the context of their lives fitting it around activities and their work. Given the somewhat limited range of easily accessible places for injecting insulin, it is highly likely that people with T1DM will have favourite injection sites. In addition, these habits will become habituated over a significant period of time and may be difficult to change. Previous studies have shown that many people with diabetes do not rotate between injections sites (Gentile et al. 2016b, De Coninck et al. 2010, Patton et al. 2010) despite education of the importance of rotation. In the qualitative interviews of all the participants across the GV and LH characterisation arms of the study (n= 62), around a third of participants said that they found changing sites difficult. With remembering to use their new sites and problems in using them in public places, being the most common explanations. The majority of participants also said they had not been given detailed education on changing injection sites and only half had their sites checked in the last two years. The impression would seem to be that whatever advice people with diabetes are given on site rotation the advice provided does not translate into their behaviours in many. This suggests that people with diabetes are either not inclined or able to follow advice or the advice provided is not clearly received or reinforced by health professionals. It is likely, the psychological, cognitive and social processes play an important role in regulating injecting behaviours in people with diabetes contributing to the development of LH. To date, the data on these processes are limited and need further exploration to inform the education and support provided to people with T1DM. One study of people with and without LH (n=215 people with T1DM), they reported higher levels of depression and lower levels of treatment satisfaction, although there were no differences in motivation or diabetes distress (Hernar et al. 2017). Therefore, if people with diabetes are going to be better

support in attending to site rotation to avoid LH, a better understanding of the behavioural regulators in respect to injections is required.

An anecdotal observation made during the conduct of the study was that participants valued seeing the LH areas on the US, suggesting that this visualisation may be useful in incentivising behaviour change. Although, this did not make the adjustment to new injection areas, as detailed above, any easier for some participants. Future studies of LH should consider these behavioural factors and adopt more robust procedures to monitor compliance with injection site changes. This may involve the development of behavioural support systems such as prompts, reminders and to-up education by clinicians.

There have been some studies that have considered combinations of needle length changes and supportive education that have shown some success in helping participants to adjust insulin behaviours (although most are sponsored by needle length manufactures). The Grassi et al. (2014) study, which involved palpation of LH and one-to-one education from a nurse, showed a 25% increase in the number of participants who rated injection technique as being very important with an increase from 40% (n=138) at baseline to 65% (n=224) three months post-intervention. They did not, however, assess or report on whether this translated into behaviour change. In the Campinos et al. (2017) study, which involved education from a nurse (avoid LH areas, leaving 1 cm between injections, resting sites for 2-4 weeks and injection grids), two-thirds of participants met their criteria of satisfactory injection behaviour (avoiding LH, switching to a 4mm needle, and changing needles after each injection) at the 6 month follow-up. Again, they provided no data on fidelity to the advice as the outcome was based on patient self-report. In the study of Smith et al. (2017), 75 participants (20 with T1DM) were exposed to injection site training based on the FIT guidance and again change to 4mm needles (the study was sponsored by a needle manufacturer), showed some impact on LH indirectly with a 65% increase in site rotation in accord with the FIT guidelines based on self-report (3-6 month follow-up). An interesting finding in this study was that they report a reduction in the amount of LH found on

palpation at follow-up with a reported reduction in the size of the LH of 14% and 8% in the abdomen and thigh areas respectively. This observation was not objectively verified, and it is not consistent with the findings of this study where evidence of historical LH was found for a number of years after they had rested an injection sites. Therefore, a potentially important question to consider is what happens to a hypertrophic nodule when insulin exposure ceases and whether there is resolution or recovery in the area in respect of insulin absorption.

This study also undertook detailed mapping of the LH areas to consider the anatomical distribution of the lesions. The data from the whole study sample, showed the highest concentration was in the abdomen followed by the thigh, gluteal area and triceps. While the overall distribution was general to both the left and right side of the body, in right-handed participants the distribution of LH was skewed to the right side of the body by a margin of 14%. There were only six left-handed participants, four of whom had a bias of LH on the left-side. While it is not possible to unequivocally claim that hand dominance influences the distribution all LH lesions, it is likely that people who are right-handed tend to have a preference for injecting on their right side. If the distribution of insulin injections are important in LH development, then finding methods to help people with diabetes extend their sites more evenly (left/right and anatomically) may be useful. Although, the practicalities of such an intervention would need to be considered with people who have diabetes to ensure that such advise was feasible for them and easy to maintain.

The findings of this study suggest that these behavioural factors are of major importance in the development of LH, and any intervention to prevent or minimise the clinical impact of LH should consider these carefully. Simply advising people with diabetes about the need to rotate may not be adequate. One potential way this could be addressed would be to engage people with diabetes and diabetes health professionals in a co-design process to consider optimal strategies for: education;

overcoming psychological and social barriers; and to activate and reinforce behaviours in people with diabetes.

5.5 The use of ultrasound in LH assessment

Another important contribution made by this study has been in developing a protocol for the use of US in assessing and detecting LH, which could be utilised clinically or in future studies. As outlined in the discussion on the characterisation of LH the study has revealed important insights into LH. While this study was not designed to test or consider the clinical application of using US for LH detection, it has shown that with access to the appropriate equipment and with training diabetes professionals could undertake such assessments reliably. The Standard Operating Procedure (SOP) for the scanning, developed with an international expert in sonography with expertise in training health professionals, could provide a basis for the clinical use of US in LH detection and management. The SOP provides a framework, detailing: equipment specifications and settings; procedure for scanning anatomical sites; classifications for determining LH lesions; and a process for measuring, recording and reporting LH (Appendix 13.1). It was also used to show participants which areas were free of LH so they could inject in those areas. There has only been one other study group who have reported a protocol for assessing LH. Perciun and Mihu (2014) presented a protocol for US assessment of LH, which included some similar features to the SOP used in this study. This included, mapping and characterising lesions as nodular and diffuse, and providing children and their parents with a map of unaffected areas for future injections. However, their protocol was designed for use in a paediatric context, and they observed high degree of variability relatable to the age and development of the child.

The learning from this study suggests that the SOP, together with a training package could be tested clinically by training diabetes health professionals. While the practicalities of doing this would challenging particularly in accessing scanning equipment, there are now clinically equivalent portable handheld US scanning devices

that could be used in any diabetes clinic. However, it is important to emphasise that the primary objective for future research is still to provide more evidence on the clinical hazards of LH (hyper and hypoglycaemia) and the benefits of a more systematic approach to its assessment and clinical management, to justify the costs of introducing such a procedure. As part of the cost benefit analysis for assessing clinical effect consideration could also be given to whether preventing and avoiding LH, will reduce the amount of insulin used by participants, In this study, the overall reduction in insulin in terms of TDI was 6%, in the participants who provided the data (n=48). Although, it is important to note that estimate was only at six weeks follow-up and participants were advised to reduce doses following the US assessment. Therefore, future studies need to consider the impact of LH avoidance on insulin doses over a longer period of time. A further consideration would be the need to develop robust systems to support and reinforce injection behaviours that would prevent the development of further lesions and avoid existing LH areas, as discussed in the previous section.

5.6 Process evaluation findings

One of the objectives of this study was to evaluate and consider the study design in terms of recruitment and retention of participants and their experiences in the study. As outlined in Chapter 3 processes evaluations are important in exploratory phase studies as they can help inform the design of future larger studies.

In terms of recruitment and retention to the GV arm of the study, around 42% of screened participants were eligible (SD of mean glucose $\geq$ 4mmol/L) for the study, and a third of these agreed to participate in the study. While this represents a reasonable level of recruitment for a relatively involved clinical study, the study also struggled to meet its recruitment target. The reasons for this were not fully identified, although it is likely that the frequency of visits involved in the study may have been seen as challenging for a population of working adults; particularly when people with T1DM also have to attend frequent outpatient appointments. In hindsight, greater attention should have been paid to this through a wider discussion of recruitment procedures

with a larger PPI group. In addition, it would have been useful to have conducted either a survey or short interviews with people who decided not to participate in the study.

In terms of potential bias to the study, while bias in a small exploratory study is somewhat inevitable, it would have been useful to look in more detail at those who declined participation to establishing factors that may impact on study findings. Some indication of these factors was gained from the retention data. Participants who exited the GV study without completing (40% of those recruited), tended to be male by a margin of two to one (male to female). Non-completers were also likely to be older with longer diabetes duration. Hence, prior to another trial study it would be important to model and optimise strategies for both recruitment and retention to reduce this potential bias. A further consideration would be whether participants should be financially compensated for their participation.

Another important learning for the study was the way participants were recruited to the study. Referring clinicians were aware of the purpose of the study but were advised not to reveal this to the participants prior to recruitment so we could observe their glucose regulation following their usual insulin management behaviours. However, some participants revealed they had been given recent LH advice by their clinician. While this was partially compensated for by instructing participants to take their insulin as normal in Condition 1, this advice may have either consciously or unconsciously moderated their behaviour. It is quite challenging to consider how this potential contamination might be overcome in future studies exploring the association between LH and GV, although one possibility would be to recruit a random sample of people with diabetes with a high SD from clinic registries and directly invite them to participate. The assumption based on known prevalence of LH would be that at least 40-60% of people with diabetes would have significant LH. Participants would then be monitored with CGM and then assessed for LH, this would potentially provide GV data on people with and potentially without LH for comparison. Although this study showed that in a sample of participants with a SD of mean glucose ≥4mmol/L, all bar 3 people had evident LH with mean of 10 nodules, limiting the range of potential comparisons.

Bearing in mind that there are many other factors particularly the number and timing of blood tests performed that determine SD; and there may also be bias in the sample as those with an elevated glucose SD with LH may have been more likely to participate in the study. Hence, further consideration needs to be given to the utility of SD in mean glucose as a possible indicator for LH.

Other process related findings included: a small number of CGM sensor failures, which occurred in six observations, three were in Condition 1 and three in Condition 2 (this failure level could be modelled in sample size estimations for future studies involving CGM); and no significant adverse events were recorded, which was important given the potential risk of hypoglycaemia.

Finally, the exit interviews gave some useful insights in respect of the process evaluation, in addition to the points already discussed in relation to insulin injecting behaviours. The participants provided comments on site rotation and some of the perceived benefits of changing their injection sites. While most participants reported that the adhered to their new sites for the study, they also reported that observing and potentially sustaining rotation can be challenging. Hence, it would seem to be a priority that future studies consider more optimal strategies to support people with site rotation.

5.7 Insulin antibodies and LH

A novel and very tentative observation made in the study (given the small sample size), was potential association between LH severity and insulin antibodies. While previous studies have shown that insulin antibodies are associated with lipoatrophy (Raile et al. 2001, Reeves et al. 1980), only one previous study in children with diabetes has found an association between insulin antibodies and LH (Raile et al. 2001). In this study participants with strong GAD antibodies had the most severe LH based on size and number of LH nodules, followed by those with IA2 antibodies. However, in the small sample observed it was not possible adjust for the multiple

confounding variables which may also mediate the development of LH for example duration of insulin exposure. It is likely therefore that if antibodies do play a role in the development of LH that contribution is likely to be minimal. Therefore, further study, in a larger sample of people with T1DM, is required to consider whether there is an interaction between insulin antibodies and the development/severity of LH. Such studies could also consider whether the association is related to an antibody mediated inflammatory reaction to subcutaneous insulin exposure or insulin resistance resulting in higher insulin requirements. Although, in relation to the latter point, the participants in this study with high antibody levels did not have high insulin requirements, with the exception of one participant (GV-27) who had high ZnT8 antibodies and a TDI dose 13 units above the physiological estimation (0.6 units per kilogramme).

5.8 Insulin satisfaction, diabetes distress and quality of life

In relation to insulin satisfaction, diabetes distress and quality-of-life, the results showed little impact on these measures, with the exception of a slight improvement in the visual analogue scale of EQ-5D-5L. This was largely related to the size of the sample which precluded any estimation of change in the GV study arm, although the observations were same when the data from participants of the LH characterisation study was pooled. There have been very few previous studies looking at the association between LH and these measures. In a cross sectional study of participants (n=215) with T1DM, Hernar et al. (2017) group participants by LH severity (based on the number of LH nodules) and compared their diabetes distress (DDS) and insulin satisfaction scores (ITSQ). They found no differences in either measure between people: with no LH (n=95); one nodule(n=86); or two of more nodules (n=25). The median values they reported for the ITSQ and DDS in those with two or more nodules were 61.4 and 2.4 respectively, this compares to median values at baseline of 57.4 (ITQS_total) and 2.0 (DDS_total) in this study (combining the GV and LH arms of the study). A correlation was performed to explore further whether the number of LH lesions or the severity index of LH associated with the EQ-5D-5L, ITSQ or DDS scores and no significant correlations were observed.

One secondary observation in relation to the survey instruments was that they had good completion rates, which would support their use in larger future studies, although it may be necessary to consider some more sensitive measures such as patient centred individualised outcome measures that could incorporate specific patient selected outcomes (Paterson 1996). In addition, it may be useful to develop some questions specific to LH, including injection behaviours and activation in managing injection sites. Such measures would be useful in establishing the impact of site management education.

5.9 Strength and limitations of the study

In conducting a study, it is important to recognise its limitations. In the context of this study which was exploratory in nature this is particularly important so as to inform future studies as well was identifying potential bias in the study observations. This section considers some of the main strength and potential limitations of the study.

An important limitation was recruitment and retention, only 10% of participants screened for eligibility (n=226) were recruited to the GV study, as the majority were ineligible due to inadequate testing. While 34 participants were recruited as suggested by the power calculation, only 27 of these entered Condition 1 and full data following Condition 2 was only available on 15 participants. Therefore, the study was ultimately underpowered. The fact that so many participants had to be assessed for eligibility meant that there was insufficient time to recruit further. Nevertheless, the study has provided some important insights and has shown that more consideration needs to be given to elements of the research process in determining the impact of LH of GV as outlined in the previous sections. This limitation also suggests that in advance of a future study the recruitment protocol may need further intensification particularly in identifying eligible participants and in making the study more attractive to people with diabetes. It may be that with the increasing use of Flash Glucose Monitoring, the blood testing limitation may be overcome.

As has been already been discussed another limitation of the study was the wash-out phase in which insulin levels were adjusted to prevent hypoglycaemia. While this was an important consideration for participant safety, the study has shown that actioning this and re-adjusting insulin doses may need more to consider individual factors such as BMI and patient preferences, participants may need more active support with this without altering the insulin delivery model or type of insulin. An alternative approach would be to consider whether an overt clinical intervention involving LH assessment, and insulin injection site education led to improved glucose regulation and improved glycaemia in a RCT; with the control and intervention groups both being given some education on managing insulin doses.

A further limitation of the study was the short follow-up duration of six weeks. While this suited the assessment of short-term effects such as TIR and GV, it limited others such as glycaemic control as measured by Hb1Ac, although 1,5AG was used to compensate for this as a short term glycaemic marker, a longer follow-up period may provide greater insights into the impact of LH sites on metabolic control and also establish whether participants can sustain the changes. Again, this may be suited to a clinical intervention study with adequate follow-up (6-12 months).

Perhaps the most important limitations of this study are in respect of measurement, particularly in relation to GV. Considering GV in a real world setting with people with diabetes, particularly with relatively short periods or observation, can be influenced by multiple extraneous factors. Dietary intake, carbohydrates, fats and proteins all impact on glucose levels. While people with diabetes generally only use insulin to compensate for carbohydrates, fat and protein based foods also elevated glucose levels. Mixed meals and the type of carbohydrates consumed can alter glucose responses. Other factors that can impact on glucose levels and variability include: activity and exercise levels (anaerobic and aerobic); stress mental or physical due to illness; alcohol consumption; menstruation; and many more. Hence, attempting to measure GV in two six day windows may not be adequate to assess the impact of LH on GV. The intention of the study design was to use the participants as their own controls, by keeping the

insulin model consistent and sticking with their normal routines. As has been highlighted the former was problematic, as is the latter without prescribing a fixed diet and routine. Although we asked participants to record their activities and did consider evidence of insulin action by looking at the glucose response to bolus injections in Conditions 1 and 2, this may not have been enough to compensate for these extraneous factors. A potential solution to this problem would be to use other glucose sensing technologies that provide data over longer time periods. In a study considering the validity and reliability of GV measures using CGM, it was identified that a minimum 12 day period was required to ensure an acceptable level of data variability (Neylon et al. 2014). Therefore, the study had insufficient glucose data for a reliable analysis and this may explain the case to case variation observed. There are now a number of CGM and flash glucose monitoring systems available and these could be used to deliver longer-duration data, although not all have extractable glucose data and only a few are available blinded. This study set out with intention of providing some insights into the clinical relevance of LH by considering is impact on glucose regulation. While, the findings of this study are limited as identified above, the learning generated by this study will hopefully inform future studies. Identifying the clinical impact of LH remains an important objective, without this understanding gaging the risk to people with diabetes is problematic and risks either over or under considering the problem of LH clinically. It is also important to estimate the hazard to justify expanding or intensifying LH screening and the use of more expensive time consuming activities such as using portable US.

There may also have been a contamination issue, with some participants having been informed by clinical staff to change injection sites prior to the CGM assessment of Condition 1 (as relayed in section 5.6). This may have meant that the opportunity to observe the full LH effect on TIR and GV in all participants was reduced. Hence, in future studies it would be important to screen people with diabetes to ascertain if they had changed their injections in anyway in the last month prior to starting the study, or whether they had received recent advice as recorded in their medical record.

Equipment availability was a significant limiting factor, particularly in respect of recruitment. There was only one US machine allocated for the study and it was only available one day per week. This limited the number of recruitments as it excluded people who may have a preference for other days and it also complicated scheduling participants' clinical visits which affected retention. The inflexible timing contributed to significant drop out of participants as the study progressed. Going forward the use of portable US equipment with a designated scanner for studies should be considered. These technologies are becoming more affordable and available, with the equivalent scanning power and functionality of the larger machines.

The fact that the study was quite involved in terms of participant commitment may have biased recruitment and reduced the potential to observe the interaction between LH and GV in a larger pool of people with diabetes. Participants in the study had to test and report their blood glucose levels regularly and completing their CGM diary. The study also involved multiple clinic visits which might be difficult for some, and perhaps the £50 voucher was not adequate compensation for their time. In addition, some of participants showed limited compliance in avoiding the LH sites, potentially reducing the estimation of the LH effect on GV. In addition, six participants from the GV study (approximately 20%) had no CGM data in Condition 1 or 2. Generally, in a parallel-group study, this can lead to an imbalance between groups. However, as the participants in this study acted as their own controls this was less of a problem, although it did reduce the power of the study significantly. For future studies some form of electronic data recording may make this easier for participants making fidelity monitoring more accurate.

Despite these limitations the study has yielded some important insights shedding light on the complex relationship between LH and the action of insulin on glucose levels. The study has also exposed some of the many challenges in conducting studies to consider the clinical effect of LH on people with diabetes. It is hoped that these insights will be useful in future studies as outlined in the following section.

5.10 Implications for future research and clinical practice

The research has identified a number of new insights into LH and its potential clinical relevance. The study has also shown that LH is a prevalent and under addressed clinical issue. In order to advance further the study of LH to generate evidence to support clinical guidelines further research is required as follows:

- Studies to assess the clinical impact of LH on diabetes outcomes are required these should include both acute and long-term outcomes. Studying the impact of LH on GV is also useful in determining the clinical effects of LH, from the experience of this study such studies need: carefully designed recruitment protocols to encourage greater participation this would be enhanced with more PPI involvement; if blinding is included in the design then robust procedures for concealment of study intention need to be developed and referring clinicians need to adhere to the procedure; methods for the maintenance of usual injecting behaviours for baseline observations need to be developed and for monitoring adherence; more individualised insulin adjustment protocols with more active support during the wash-out phase should be considered; and longer periods of glucose monitoring (optimally 12 days) or use alternative monitoring systems such as flash-glucose monitoring.
- Studies looking at insulin doses would be useful. Such studies could look at TDI doses before and after LH assessment and consider other outcomes such as glycaemic control and incident hypoglycaemia.

- More studies on individual behaviours in respect of injection site practices and preferences are needed, these could be qualitative or observational studies. Such data will be useful in designing individual injection behaviour change interventions, as it is clear from the literature review and from this study that attending to injection sites can be challenging for people with T1DM.

- Robust assessment of different methods of LH detection should be undertaken. Studies are needed to: establish how frequently LH screening should be

performed and whether screening should consider all people with diabetes or only those exhibiting potential insulin absorption issues; the application of new technologies in routine clinical assessment such as portable US technology should also be considered, including cost-benefit analysis; and further validation work is required to identify an optimal grading model in terms of clinical utility.

- More studies on the natural history of LH may be useful, considering how quickly lesions develop and when or if affected tissues return to normal and recover equivalence with normal tissue in terms of insulin absorption.

- Studies examining LH tissue samples might be useful as there have been very few studies examining the histological characteristics of LH. Such studies could consider provide more insight into the necrotic of inflamed tissues observed on US. As part of these studies or in parallel it may be worth measuring inflammatory cytokines which could impact on insulin signalling or other metabolic pathways.

- Studies of different site rotation methods together with technologies to support people with diabetes in managing their insulin sites to reduce the risk of LH are important, as the ultimate mediator for the development of LH is individual behaviour. There are currently a number of mobile applications and adapted insulin pen technologies in development which need evaluating in respect of this. A key factor in sustaining the rotation will be that the method is achievable and that the people with diabetes can recognise the benefits of it. As with all studies requiring people to change a frequent behaviours this may be best achieved through a co-design process.

- Studies considering the interface between people with diabetes and professional perspectives on managing LH may help inform interventions that will engage both groups. Again co-design studies that consider the nature of a problem form people with diabetes and professional perspectives may yield some novel approaches.

- Finally, an area that is currently significantly understudied is the interaction of LH and insulin action in CSII users. As the number of people with diabetes using CSII increases this may be an important consideration as insulin exposure in one site can be quite extended with set changes only occurring every third day.

In terms of clinical implications, this study provides some useful new learning and confirms many established truths about LH. The study reinforces the fact that LH is a very common clinical finding in people with T1DM, and that it is often under considered by health professionals. While the participants may have had some education on LH and site management it is clear that the education provided does not necessarily resonate with them. Hence, it is important that diabetes health professionals address LH and reinforce messages about site management with people with diabetes frequently. Some of the other specific clinical considerations, include:

- Physical or visual examination of LH may significantly underestimate the presence of LH lesions even when current best practice is observed. This suggests that new approaches are required and if cost-effective US could play a role in LH detection.
- Changing injection sites can significantly alter insulin requirement in some people with diabetes and insulin adjustments are important when sites are being changed. It is an important area for future development that standard methods or considerations are used to support this.
- The classical view, held by many health professionals, of LH as a defined fatty lump needs to be reconsidered. This study allied with others shows that LH is heterogeneous in nature with changes to the skin and surrounding subcutaneous tissues as well as the hypertrophic cells.

5.11 Conclusion

This exploratory study has generated some important and useful insights into LH in relation to its characteristics (presentation, grade, prevalence) and its impact on insulin action. While the number of participants studies was small, it was possible to observe some impact on insulin response in relation to LH when participants changed their sites. Hence, the study has exposed that the relationship between LH insulin action and glucose regulation is complex and difficult to measure in a real-world setting. The impression given by the observations gleaned in the studies suggests that LH management strategies will need to carefully consider insulin management in the transitioning injection sites away from LH affected areas. Overall the study has identified a number of important questions that need to be addressed in future studies to identify the evidence needed to prevent and manage LH more effectively. The study has also provided some useful insights into how future studies may be more optimally designed.

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Appendices

Appendix 1 Systematic Review Search Terms

- Appendix 1.1 Web of Science
- Appendix 1.2 MEDLINE
- Appendix 1.3 CINAHL
- Appendix 1.4 Embase

Appendix 2 Data extraction tool

Appendix 3 Quality appraisal tool

- Appendix 3.1 Quality appraisal scores

Appendix 4 Study Identification Card

Appendix 5 Participant Information Sheets

- Appendix 5.1 Glucose Variability Study- Condition 1
- Appendix 5.2 The Effect of Changing Injection Site- Condition 2
- Appendix 5.3 Lipohypertrophy Characterisation Study

Appendix 6 Study Consent Forms

- Appendix 6.1 Glucose Variability Study- Condition 1
- Appendix 6.2 The Effect of Changing Injection Site- Condition 2
- Appendix 6.3 Lipohypertrophy Characterisation Study

Appendix 7 Schedule of Procedures

Appendix 8 Study Questionnaire

Appendix 9 Clinical Data Forms

- Appendix 9.1 Digital Palpation
- Appendix 9.2 Ultrasound Examination

Appendix 10 Continuous Glucose Monitor Diary

Appendix 11 Injection Site and Dose Calculation Form

Appendix 12 Exit Interview Guide

Appendix 13 Standard Operator Procedures

- Appendix 13.1 Standard Operator Procedures 1- Physical examination and ultrasound scan technique
- Appendix 13.2 Standard Operator Procedures 2- *Insertion Technique for the IPro2 Continuous Glucose Monitor*
- Appendix 13.3 Standard Operator Procedures 3- *Identification of lipohypertrophy free injection sites and safety issues regarding use of these new injection sites*

Appendix 14 Ethical Approval

Appendix 15 Publications

Appendix 1 Systematic Review Search Terms

Appendix 1.1 Web of Science

TOPIC: (Diabetes Mellitus OR Diabetes mellitus type 1 OR insulin dependent diabetes mellitus OR Insulin-dependent diabetes patients OR Insulin-dependent diabetic patients OR Type 1 diabetes OR Type 1 diabetic OR T1D OR T1DM OR DM type 1 OR IDDM OR Juvenile diabetes OR Diabetes mellitus type 2 OR Type 2 diabetes OR Type 2 diabetic OR T2D OR T2DM OR DM type 2 OR diabetic patient OR Diabetic patients OR Diabetes OR Diabetic OR Insulin treated patients)

AND

TOPIC: (Lipohypertrophy OR lypohypertrophy OR Diabetic lipohypertrophy OR Diabetes lipohypertrophy OR lipohypertrophic OR Lipohypertrophied OR lipohypertrophies OR Insulin dystrophy OR dystrophy OR Dystrophies OR Subcutaneous Dystrophy OR Subcutaneous dystrophies OR Subcutaneous tissue dystrophies OR lipodystrophy OR Lipodystrophies OR Lipodystrophic OR hypertrophy OR Fat hypertrophy)

NOT

TOPIC: (CARDIOVASCULAR DISEASE OR HEART DISEASE OR HYPERTENSIVE OR HYPERTENSION OR MYOCARDIAL DISEASE)

NOT

TOPIC: (GROWTH HORMONE)

NOT

TOPIC: (LIVER DISEASE OR HEPATIC DISEASE) NOT TOPIC: (AIDS)

NOT

TOPIC: (KIDNEY DISEASE OR RENAL DISEASE OR DIALYSIS)

NOT

TOPIC: (PREGNANCY OR GESTATIONAL DIABETES)

NOT

TOPIC: (VITAMIN D)

Refined by: [excluding] RESEARCH AREAS: (BIOCHEMISTRY MOLECULAR BIOLOGY OR HISTORY OR RESPIRATORY SYSTEM OR GENETICS HEREDITY OR MEDICAL INFORMATICS OR UROLOGY NEPHROLOGY OR RHEUMATOLOGY OR HISTORY PHILOSOPHY OF SCIENCE OR PUBLIC ENVIRONMENTAL OCCUPATIONAL HEALTH OR CARDIOVASCULAR SYSTEM CARDIOLOGY OR ENVIRONMENTAL SCIENCES ECOLOGY OR BUSINESS ECONOMICS OR TRANSPLANTATION OR NEUROSCIENCES NEUROLOGY OR MATHEMATICAL COMPUTATIONAL BIOLOGY OR IMMUNOLOGY OR PARASITOLOGY OR PLANT SCIENCES OR ENGINEERING OR ZOOLOGY OR VETERINARY SCIENCES OR NUTRITION DIETETICS OR DENTISTRY ORAL SURGERY MEDICINE OR GASTROENTEROLOGY HEPATOLOGY OR OTORHINOLARYNGOLOGY OR INFECTIOUS DISEASES OR SPORT SCIENCES OR OBSTETRICS GYNECOLOGY OR OPHTHALMOLOGY OR TOXICOLOGY OR SURGERY OR MICROSCOPY OR WOMEN APOS S STUDIES OR URBAN STUDIES OR FOOD SCIENCE TECHNOLOGY OR TELECOMMUNICATIONS OR ANESTHESIOLOGY OR FILM RADIO TELEVISION OR HEMATOLOGY OR REHABILITATION OR EVOLUTIONARY BIOLOGY OR ONCOLOGY OR EMERGENCY MEDICINE OR AGRICULTURE OR CULTURAL STUDIES OR ORTHOPEDICS OR COMMUNICATION OR VIROLOGY OR REPRODUCTIVE BIOLOGY)

Timespan=All years

Search language=Auto

Appendix 1.2 MEDLINE

Ovid® Wolters Kluwer

My Account | Support & Training | Help | Feedback | Logged in as rabab hashem at kings college | Logoff

Search | Journals | Books | My Workspace | Multimedia

▼ Search History (73) View Saved

# ▲	Searches	Results	Type	Actions	Annotations
1	exp Diabetes Mellitus/	397938	Advanced	Display Results | More	
2	Diabetes Mellitus.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	401998	Advanced	Display Results | More	
3	exp Diabetes Mellitus, Type 1/	72374	Advanced	Display Results | More	
4	Diabetes mellitus type 1.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	72476	Advanced	Display Results | More	
5	insulin dependent diabetes mellitus.mp.	15560	Advanced	Display Results | More	
6	Insulin-dependent diabetes patients.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	45	Advanced	Display Results | More	
7	insulin-dependent diabetic patients.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	2426	Advanced	Display Results | More	
8	Type 1 diabetes.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	36181	Advanced	Display Results | More	
9	Type 1 diabetic.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	5661	Advanced	Display Results | More	
10	T1D.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	5492	Advanced	Display Results | More	
11	T1DM.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	3949	Advanced	Display Results | More	
12	DM type 1.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	175	Advanced	Display Results | More	
13	IDDM.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	6817	Advanced	Display Results | More	
14	Juvenile diabetes.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	1364	Advanced	Display Results | More	
15	exp Diabetes Mellitus, Type 2/	120910	Advanced	Display Results | More	
16	Diabetes mellitus type 2.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	122047	Advanced	Display Results | More	
17	Type 2 diabetes.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	111744	Advanced	Display Results | More	
18	Type 2 diabetic.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	15710	Advanced	Display Results | More	
19	T2D.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	7823	Advanced	Display Results | More	
20	T2DM.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	16842	Advanced	Display Results | More	

Contract

21	DM type 2.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	248	Advanced	Display Results | More	
22	diabetic patients.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	55807	Advanced	Display Results | More	
23	Diabetes.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	568726	Advanced	Display Results | More	
24	Diabetic.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	244507	Advanced	Display Results | More	
25	Diabetes*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	568888	Advanced	Display Results | More	
26	Diabetic*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	261782	Advanced	Display Results | More	
27	Diabetie$.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	31	Advanced	Display Results | More	
28	Insulin treated patients.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	746	Advanced	Display Results | More	
29	Insulin-treated patient$.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	759	Advanced	Display Results | More	
30	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 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29	639683	Advanced	Display Results | More	
31	lipohypertrophy.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	308	Advanced	Display Results | More	
32	Diabetic lipohypertrophy.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	4	Advanced	Display Results | More	
33	Diabetes lipohypertrophy.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	1	Advanced	Display Results | More	
34	lipohypertrophic.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	23	Advanced	Display Results | More	
35	Lipohypertrophied.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	3	Advanced	Display Results | More	
36	lipohypertrophies.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	6	Advanced	Display Results | More	
37	Insulin dystrophy.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	1	Advanced	Display Results | More	
38	dystrophy.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	47761	Advanced	Display Results | More	
39	Dystrophies.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	22475	Advanced	Display Results | More	
40	Subcutaneous dystrophies.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	1	Advanced	Display Results | More	
41	exp Lipodystrophy/	4674	Advanced	Display Results | More	

☐	42	lipodystrophy.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	6324	Advanced	Display Results | More	🗨
☐	43	Lipodystrophies.mp.	293	Advanced	Display Results | More	🗨
☐	44	Lipodystrophic.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	383	Advanced	Display Results | More	🗨
☐	45	exp Hypertrophy/	72589	Advanced	Display Results | More	🗨
☐	46	hypertrophy.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	97335	Advanced	Display Results | More	🗨
☐	47	Fat hypertrophy.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	52	Advanced	Display Results | More	🗨
☐	48	31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45 or 46 or 47	182007	Advanced	Display Results | More	🗨
☐	49	30 and 48	9121	Advanced	Display Results | More	🗨
☐	50	exp Cardiovascular Diseases/	2255481	Advanced	Display Results | More	🗨
☐	51	exp Hypertension/ or hypertension.mp.	458453	Advanced	Display Results | More	🗨
☐	52	50 or 51	2401852	Advanced	Display Results | More	🗨
☐	53	49 not 52	4543	Advanced	Display Results | More	🗨
☐	54	aids.mp. or exp Acquired Immunodeficiency Syndrome/	209793	Advanced	Display Results | More	🗨
☐	55	53 not 54	4479	Advanced	Display Results | More	🗨
☐	56	liver disease.mp. or exp Liver Diseases/	544763	Advanced	Display Results | More	🗨
☐	57	hepatic.mp.	293920	Advanced	Display Results | More	🗨
☐	58	56 or 57	709841	Advanced	Display Results | More	🗨
☐	59	55 not 58	3914	Advanced	Display Results | More	🗨
☐	60	exp Kidney Diseases/ or exp Kidney/ or renal disease.mp.	731524	Advanced	Display Results | More	🗨
☐	61	59 not 60	2803	Advanced	Display Results | More	🗨
☐	62	exp Vitamin D Deficiency/ or exp Vitamin D/ or vitamin D.mp.	87237	Advanced	Display Results | More	🗨
☐	63	61 not 62	2791	Advanced	Display Results | More	🗨
☐	64	growth hormone.mp. or exp Growth Hormone/	72102	Advanced	Display Results | More	🗨
☐	65	63 not 64	2756	Advanced	Display Results | More	🗨
☐	66	exp Pregnancy/ or pregnancy.mp.	930311	Advanced	Display Results | More	🗨
☐	67	gestational diabetes.mp. or exp Diabetes, Gestational/	16151	Advanced	Display Results | More	🗨
☐	68	66 or 67	931663	Advanced	Display Results | More	🗨
☐	69	65 not 68	2602	Advanced	Display Results | More	🗨
☐	70	limit 69 to humans	1599	Advanced	Display Results | More	🗨
☐	71	limit 70 to (humans and yr="2015 -Current")	293	Advanced	Display Results | More	🗨
☐	72	limit 70 to yr="2016 -Current"	229	Advanced	Display Results | More	🗨
☐	73	limit 72 to yr="2018 -Current"	66	Advanced	Display Results | More	🗨

Save Remove Combine with: AND OR

Appendix 1.3 CINAHL

#	Query	Limiters/Expanders	Last Run Via	Results
S57	S55 NOT S56	Limiters - Published Date: 20180901-20190331 Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	46
S56	"growth hormone" OR (MH "Vitamin D+") OR "vitamin d" OR (MM "Vitamin D Deficiency+")	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	24,987
S55	S53 NOT S54	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	791
S54	"growth hormone"	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	3,879
S53	S49 NOT S52	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	799
S52	S50 OR S51	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	197,547
S51	(MM "Pregnancy in Diabetes+") OR (MM "Diabetes Mellitus, Gestational") OR "gestational diabetes"	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	7,720
S50	(MM "Pregnancy+") OR	Expanders - Apply	Interface - EBSCOhost	196,417

	"pregnancy"	equivalent subjects Search modes - Boolean/Phrase	Research Databases Search Screen - Advanced Search Database - CINAHL	
S49	S47 NOT S48	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	822
S48	"aids" OR (MM "Acquired Immunodeficiency Syndrome")	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	57,574
S47	S45 NOT S46	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	833
S46	"kidney disease" OR (MM "Kidney Diseases+")	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	61,220
S45	S43 NOT S44	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	971
S44	(MM "Liver Diseases+") OR "liver disease"	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	48,641
S43	S41 NOT S42	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	993
S42	(MM "Heart Diseases+") OR "cardiac disease"	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	188,436

S41	S28 AND S40	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	1,398
S40	S29 OR S30 OR S31 OR S32 OR S33 OR S34 OR S35 OR S36 OR S37 OR S38 OR S39	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	20,734
S39	"Fat hypertrophy"	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	6
S38	"hypertrophy"	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	13,895
S37	(MM "Hypertrophy+")	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	1,286
S36	"Lipodystrophic"	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	34
S35	"Lipodystrophies"	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	23
S34	"lipodystrophy"	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	996
S33	(MM "Lipodystrophy+")	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	546

S32	"Dystrophies"	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	582
S31	"dystrophy"	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	5,380
S30	"lipohypertrophic"	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	6
S29	"lipohypertrophy"	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	84
S28	S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7 OR S8 OR S9 OR S10 OR S11 OR S12 OR S13 OR S14 OR S15 OR S16 OR S17 OR S18 OR S19 OR S20 OR S21 OR S22 OR S23 OR S24 OR S25 OR S26 OR S27	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	191,474
S27	"Insulin treated patients"	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	197
S26	"Diabetic"	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	57,222
S25	"Diabetes"	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	173,381

S24	"diabetic patients"	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	18,634
S23	(MM "Diabetic Patients") OR "diabetic patient"	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	5,664
S22	(MM "Diabetic Patients")	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	4,848
S21	"DM type 2"	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	37
S20	"T2DM"	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	4,376
S19	"T2D"	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	2,116
S18	"Type 2 diabetic"	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	3,624
S17	"Type 2 diabetes"	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	52,761
S16	"Diabetes mellitus type 2"	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	50,406

S15	(MM "Diabetes Mellitus, Type 2")	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	39,114
S14	"Juvenile diabetes"	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	103
S13	"IDDM"	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	16,365
S12	"DM type 1"	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	26
S11	"T1DM"	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	16,560
S10	"T1D"	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	1,603
S9	"Type 1 diabetic"	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	1,253
S8	"Type 1 diabetes"	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	51,509
S7	"Insulin-dependent diabetic patients"	Expanders - Apply equivalent subjects Search modes - Boolean/Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced	105

Appendix 1.4 Embase

Ovid® Wolters Kluwer

My Account | Support & Training | Help | Feedback | Logged in as rabab hashem at kings college | Logoff

Search | Journals | Books | My Workspace | Multimedia

▼ **Search History** (73) View Saved

# ▲	Searches	Results	Type	Actions	Annotations
1	exp Diabetes Mellitus/	912760	Advanced	Display Results | More	
2	Diabetes Mellitus.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	884761	Advanced	Display Results | More	
3	exp Diabetes Mellitus, Type 1/	107343	Advanced	Display Results | More	
4	Diabetes mellitus type 1.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	2929	Advanced	Display Results | More	
5	insulin dependent diabetes mellitus.mp.	313636	Advanced	Display Results | More	
6	Insulin-dependent diabetes patients.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	63	Advanced	Display Results | More	
7	insulin-dependent diabetic patients.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	2690	Advanced	Display Results | More	
8	Type 1 diabetes.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	57644	Advanced	Display Results | More	
9	Type 1 diabetic.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	8059	Advanced	Display Results | More	
10	T1D.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	11547	Advanced	Display Results | More	
11	T1DM.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	8055	Advanced	Display Results | More	
12	DM type 1.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	371	Advanced	Display Results | More	
13	IDDM.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	8000	Advanced	Display Results | More	
14	Juvenile diabetes.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	3949	Advanced	Display Results | More	
15	exp Diabetes Mellitus, Type 2/	227267	Advanced	Display Results | More	
16	Diabetes mellitus type 2.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	8633	Advanced	Display Results | More	
17	Type 2 diabetes.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	173346	Advanced	Display Results | More	
18	Type 2 diabetic.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	23679	Advanced	Display Results | More	
19	T2D.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	15704	Advanced	Display Results | More	
20	T2DM.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	31242	Advanced	Display Results | More	
21	DM type 2.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	584	Advanced	Display Results | More	
22	diabetic patients.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	84794	Advanced	Display Results | More	
23	Diabetes.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	999257	Advanced	Display Results | More	
24	Diabetic.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	383445	Advanced	Display Results | More	
25	Diabetes*.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading	999588	Advanced	Display Results | More	

Contract

		word, candidate term word]				
☐	26	Diabetic*.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	405577	Advanced	Display Results | More	
☐	27	Diabetie$.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	92	Advanced	Display Results | More	
☐	28	Insulin treated patients.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	1180	Advanced	Display Results | More	
☐	29	Insulin-treated patient$.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	1197	Advanced	Display Results | More	
☐	30	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 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29	1086198	Advanced	Display Results | More	
☐	31	lipohypertrophy.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	996	Advanced	Display Results | More	
☐	32	Diabetic lipohypertrophy.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	5	Advanced	Display Results | More	
☐	33	Diabetes lipohypertrophy.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	1	Advanced	Display Results | More	
☐	34	lipohypertrophic.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	28	Advanced	Display Results | More	
☐	35	Lipohypertrophied.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	5	Advanced	Display Results | More	
☐	36	lipohypertrophies.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	13	Advanced	Display Results | More	
☐	37	Insulin dystrophy.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	1	Advanced	Display Results | More	
☐	38	dystrophy.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	81707	Advanced	Display Results | More	
☐	39	Dystrophies.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	9201	Advanced	Display Results | More	
☐	40	Subcutaneous dystrophies.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	1	Advanced	Display Results | More	
☐	41	exp Lipodystrophy/	11813	Advanced	Display Results | More	
☐	42	lipodystrophy.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	12957	Advanced	Display Results | More	
☐	43	Lipodystrophies.mp.	420	Advanced	Display Results | More	
☐	44	Lipodystrophic.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	524	Advanced	Display Results | More	
☐	45	exp Hypertrophy/	307932	Advanced	Display Results | More	
☐	46	hypertrophy.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	197379	Advanced	Display Results | More	
☐	47	Fat hypertrophy.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]	86	Advanced	Display Results | More	
☐	48	31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45 or 46 or 47	438531	Advanced	Display Results | More	
☐	49	30 and 48	26098	Advanced	Display Results | More	
☐	50	exp Cardiovascular Diseases/	4110340	Advanced	Display Results | More	
☐	51	exp Hypertension/ or hypertension.mp.	923480	Advanced	Display Results | More	
☐	52	50 or 51	4175742	Advanced	Display Results | More	
☐	53	49 not 52	9191	Advanced	Display Results | More	
☐	54	aids.mp. or exp Acquired Immunodeficiency Syndrome/	231247	Advanced	Display Results | More	
☐	55	53 not 54	9069	Advanced	Display Results | More	
☐	56	liver disease.mp. or exp Liver Diseases/	1000335	Advanced	Display Results | More	
☐	57	hepatic.mp.	419458	Advanced	Display Results | More	

☐	58	56 or 57	1190861	Advanced	Display Results | More	🗨
☐	59	55 not 58	8098	Advanced	Display Results | More	🗨
☐	60	exp Kidney Diseases/ or exp Kidney/ or renal disease.mp.	1243843	Advanced	Display Results | More	🗨
☐	61	59 not 60	6120	Advanced	Display Results | More	🗨
☐	62	exp Vitamin D Deficiency/ or exp Vitamin D/ or vitamin D.mp.	154533	Advanced	Display Results | More	🗨
☐	63	61 not 62	6030	Advanced	Display Results | More	🗨
☐	64	growth hormone.mp. or exp Growth Hormone/	104633	Advanced	Display Results | More	🗨
☐	65	63 not 64	5921	Advanced	Display Results | More	🗨
☐	66	exp Pregnancy/ or pregnancy.mp.	942762	Advanced	Display Results | More	🗨
☐	67	gestational diabetes.mp. or exp Diabetes, Gestational/	35318	Advanced	Display Results | More	🗨
☐	68	66 or 67	945214	Advanced	Display Results | More	🗨
☐	69	65 not 68	5601	Advanced	Display Results | More	🗨
☐	70	limit 69 to humans	4138	Advanced	Display Results | More	🗨
☐	71	limit 70 to (humans and yr="2015 -Current")	1177	Advanced	Display Results | More	🗨
☐	72	limit 70 to yr="2016 -Current"	897	Advanced	Display Results | More	🗨
☐	73	limit 70 to yr="2018 -Current"	339	Advanced	Display Results | More	🗨

Save Remove Combine with: AND OR

Appendix 2 Data extraction tool

Data extraction tool - LH and glycaemic control (HbA1c) and /or GV studies

Extraction items	Details
Citation	
Aims and objectives or research question	
Eligibility criteria of the participants • Inclusion • Exclusion	
Participants characteristics	
Study design	
Description of the method (detection of LH)	
Results (HbA1c, glucose variability and prevalence of LH)	

Appendix 3 Quality appraisal tool (NIH 2014)

Criteria	Yes	No	Other (CD, NR, NA)*
1. Was the research question or objective in this paper clearly stated?			
2. Was the study population clearly specified and defined?			
3. Was the participation rate of eligible persons at least 50%?			
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?			
5. Was a sample size justification, power description, or variance and effect estimates provided?			
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?			
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?			
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?			
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?			
10. Was the exposure(s) assessed more than once over time?			
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?			
12. Were the outcome assessors blinded to the exposure status of participants?			
13. Was loss to follow-up after baseline 20% or less?			
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?			

Appendix 3.1 Quality appraisal scores

Items	Deeb et al. (2019)	Pozzuoli et al. (2018)	Ji et al. (2017)	Strollo et al (2016)	Blanco et al (2013)	Hajheydari et al (2011)	Ibarra & Gallego (1998)	Hauner et al. (1996)	McNally et al. (1988)
1	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+
3	CD	+	+	+	+	CD	CD	CD	CD
4	+	+	+	+	+	+	-	-	+
5	-	+	+	-	-	+	-	-	-
6	+	+	+	+	-	+	+	+	+
7	+	+	+	+	-	+	+	+	+
8	+	+	+	+	NA	+	+	+	NA
9	+	+	+	+	+	+	+	+	+
10	-	-	-	-	-	-	-	+	-
11	+	+	+	+	+	-	-	+	-
12	NA	NA	NA	NA	+	NA	NA	NA	NA
13	NA	NA	+	NA	NA	+	NA	NA	+
14	-	+	+	+	-	+	+	+	-
Total	8 out of 14	11 out of 14	12 out of 14	10 out of 14	7 out of 14	10 out of 14	7 out of 14	9 out of 14	7 out of 14
Yes, +; No, -; Cannot determine, CD; Not applicable, NA; Not reported, NR									

Appendix 4 Study Identification Card

The Titanic Studies

We would like to recruit people you think may have LH e.g. people with any of the following: erratic control, sudden unexplained hypo, running high to avoid sudden lows. With or without palpable areas or lumps, and meet the following criteria:

Testing regularly ≥ 4 = possible CGM study – (**pink** info sheet) (n34)	**Testing < 4** times a day = US study – (**yellow** info sheet) (n70)

Age> 20 years
Diagnosed T1DM and using insulin for >3 years
Taking multiple daily injections ≥4 per/day
Using the same insulin type and delivery method for the past 6 months
Able to speak and read English

Contact details:

Henri(etta) Mulnier and Rabab Hashem
[redacted]

Appendix 5 Participant Information Sheets

Appendix 5.1 Glucose Variability Study- Condition 1

Appendix 5.2 The Effect of Changing Injection Site- Condition 2

Appendix 5.3 Lipohypertrophy Characterisation Study

Appendix 5.1 Glucose Variability Study- Condition 1

Glucose Variability Study

Invitation to take part in a research study

We would like to invite you to take part in a research study looking at the variations in blood glucose (sugar) that can occur during the day. This information sheet will explain why this study is being done and what we would like you to do if you wish to take part. We will be happy to answer any questions you may have. Taking part is completely voluntary and you can decide to withdraw at any time. This study is being conducted as part of a PhD study in collaboration with Guy's and St Thomas' NHS Foundation Trust and King's College London.

Purpose of the study

We want to understand more about why blood glucose levels can vary day-to-day. The study would measure your glucose variability, which means the changes (swings) that take place in the amount of glucose in your blood over a given period of time. To do this we will use a special sensor called a Continuous Glucose Monitor (CGM) for six days. The sensor will record your glucose automatically and painlessly the whole time you are wearing it. You will not be able to see the results, but when we download the readings at the end of the week it will provide us and you with:

- An assessment of how much variation there is in your blood glucose
- An understanding of how food, physical activity, and your insulin influence your blood glucose level.

Why have I been invited?

You are being invited to take part in this study because:

- You have had type 1 diabetes for more than 3 years
- You have had some significant swings in blood glucose level in the past four weeks
- You take four or more insulin injections per-day
- You test your glucose at least four times a day

Taking part in the study

It is up to you to if you would like to take part in the study or not. If you do decide to take part you are free to withdraw from the study at any time without giving a reason. This decision will not affect the care you receive at the hospital in any way.

What will happen to me if I take part?

Following attendance at clinic today with your permission a diabetes specialist nurse will contact you by phone within the next few days. They will be able to answer any questions you may have and arrange an appointment with you at the clinical research centre at St Thomas' Hospital to explain the study further and ask you for your consent to take part.

If you agree to take part, we will arrange for the sensor to be put on. The sensor and recording device is small - the size of a £2 coin. It has a tiny plastic tube that lies just under the skin to record your glucose continuously. It is secured to your tummy area with tape and the whole thing is covered with a waterproof adhesive film, which means you can bath or shower, and continue activities as normal.

You will still need to measure your blood glucose with a regular glucose meter at least four times a day. You will also need to keep a diary recording your blood glucose levels, your insulin, and what you eat, so we can check and interpret the readings from

the sensor. We will ask your permission to access some of your medical records such as your current medication, recent blood test results, your blood pressure and weight.

We would also like to ask you to complete a few brief questionnaires that assess how people with diabetes feel about their diabetes and its treatment. There are no right or wrong answers to any of these and you can choose to answer all or none of the questions.

Benefits of taking part

The results of the glucose monitoring will be shared with you and discussed in detail, so that we can look at the variability, what might be causing it, and possible ways to reduce this.

Risks of taking part

While the sensor is painless to wear, there may be some mild discomfort when it is inserted. There is a possible risk of skin irritation, inflammation, infection and bleeding at the sensor insertion site, although this is unusual. Please let the researcher know if you have any skin allergies before they insert the sensor. If you have any problems while wearing the sensor it can easily be removed without any assistance or medical help.

What if there is a problem?

If you have any concerns during the study you can contact the research team on the telephone numbers you will be provided with. If you have concerns regarding the study or you are unhappy in anyway and wish to complain formally, you can do this by contacting the Patients Advice and Liaison Service (PALS): phone: 020 7188 8801 email: pals@gstt.nhs.uk. The PALS team is based in the main entrance on the ground floor at St Thomas' Hospital and on the ground floor at Guy's Hospital in the Tower Wing.

In the event that something does go wrong and you are harmed during the research you may have grounds for legal action for compensation against Guy's and St Thomas' NHS Foundation Trust and/or King's College London but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you (*If appropriate*).

Will my taking part in the study be kept confidential?

All data will be kept confidential and used anonymously. Information will be stored securely and only the research team will have access to it.

Involvement of the general practitioner/family doctor

Your GP will be informed, with your consent, that you are taking part in the study.

Participation in future research

The researchers will request permission to contact you again for future investigation. This is an optional request and if you do not wish to give consent to be contacted for future investigations, it will not impact your participation in this study nor will it impact your clinical care.

Further information and contact details

Thank you for reading this participant information sheet. If you have any questions, or would like to find out more about our studies please contact the study diabetes specialist nurse who will be happy to discuss this study with you:

Contact name and address inserted here
Tel:(contact details inserted here)
E mail:

Appendix 5.2 The Effect of Changing Injection Site- Condition 2

UlTrasound classIficaTion and grAdiNg of lIpohypertrophy and its ImpaCt on glucose variability in type 1 diabetes (the TITANIC studies): the effect of changing injection site

Invitation to take part in a research study

We would like to invite you to take part in a research study looking at the relationship between fatty lumps that can occur under the skin when injecting insulin at the same area, and their effect on insulin and blood glucose (sugar) levels. These fatty lumps are known as Lipohypertrophy and are sometimes referred to as 'lipos'. This information sheet will explain why this study is being done and what we would like you to do if you wish to take part. Taking part is completely voluntary. This study is being conducted as Doctorate study in collaboration with Guy's and St Thomas' NHS Foundation Trust and King's College London.

Purpose of the study

The lipohypertrophy lumps develop because insulin can cause local fat cells to enlarge. They can sometimes be seen as large raised areas on the skin, felt as harder patches or small lumps under the skin. These small lumps can be difficult to feel, so you may not realise they are there. We think the lumps can affect how the insulin works causing the glucose level to vary unexpectedly. We want to understand what this changed tissue looks like using ultrasound and to describe it. We also want to measure the lumps to see if we can establish a way of grading the lumps and if some lumps or patches might affect glucose variability more than others.

Why have I been invited?

You are being invited because you have already done the Glucose variability study and you may well have lumps, so we would really like to look at your injection sites in detail.

Do I have to take part?

It is up to you to if you would like to take part in the study. If you do decide to take part you are free to withdraw from the study at any time without giving a reason. This decision will not affect the care you receive at the hospital in any way.

What will happen to me if I take part?

The researcher will explain to you the purpose of the study and answer any questions you may have. If you are interested in taking part, you will be asked to sign a new consent form. We will then examine your insulin injection sites using ultrasound. Before we do the scan, we will feel for any lumps in your injection sites, which is the current way of assessing for lipos in clinical practice. The ultrasound is a harmless and painless procedure, which uses high frequency sound waves to produce images. An external probe is held on the surface of the skin and images are recorded. The scan takes around 30 minutes to complete. We would like to scan your arms, abdomen, buttocks and thighs. We will then show you were your lumps are and identify some new areas where you can inject avoiding any lumps. When we have done this with people with diabetes in the past we have found that they need less insulin than when they were using their 'old sites', so the study diabetes nurse will discuss this with you and agree a reduced insulin dose with you. During the next six weeks from your scan you will be supported by the

study DSN, to help ensure that your glucose does not go too high or too low in response to the new calculated dose. We will not change the type of your insulin or any other aspects of your treatment.

We would like to take a blood sample to check your glycated haemoglobin (the test that tells us what your glucose has been like in recent weeks) and another test that can show us glucose variability. This blood samples will be collected by a needle into a vein in your arm and only needs 2 teaspoons (10ml) of blood collected in 2 or 3 vials.

After five weeks of injecting into the new area we will ask you to return so that the continuous glucose monitor (CGM) sensor can be fitted for another weeks recording. As with the previous monitoring you will still need to measure your blood glucose with

a regular glucose meter and keep a diary recording your blood glucose levels, your insulin and what you eat, so we can check and interpret the readings from the sensor. When you return to have the sensor taken off and downloaded, the diabetes nurse will look at the data with you and discuss any differences from your first CGM recording. They will also ask you about your experiences of injecting into the different area (lipo free sites) during a short interview and to complete some of the questionnaires you completed at the beginning of the study. We would also like to repeat the blood test at this final visit.

What will I have to do?

Avoid injecting into your old lipohypertrophy areas

Continue to monitor your glucose in your normal way

Five weeks after the scan you would wear a CGM sensor for six days and keep a monitoring diary including activity and food intake

Return for the final visit to have the sensor downloaded, complete post study questionnaires and have a blood sample taken

What will happen to my blood sample?

Blood samples collected for the purposes of the research will be used immediately and at the end of the study the samples will be disposed of in accordance with HTA policies.

Will I be paid for my involvement?

To say thank you for taking part in the study, we will give you a £50.00 gift voucher

Benefits of taking part

This study may not directly benefit you, but it does give you an opportunity to have your injection sites examined and scanned by ultrasound. The information you get from participating in the study will may help raise your awareness around lipos. It also gives you the chance to tell people about your views and experience of injecting

insulin. In the future your participation may help us develop better ways of supporting people with diabetes to regulate their glucose levels.

Risks of taking part

There may be a small chance that your glucose may rise or fall when you change to the new injection sites and we have adjusted the dose of your insulin. You will be given advice on what to do if this happens and a contact number for the study diabetes nurse. There are no known risks from the type of ultrasound scan you are having.

What if there is a problem?

If you have any concerns during the study you can contact the research team on the telephone number provided and they will do their best to answer your questions or manage your concerns. If you have concerns regarding the study or you are unhappy in anyway and wish to complain formally, you can do this by contacting the Patients Advice and Liaison Service (PALS): phone: 020 7188 8801, email: pals@gstt.nhs.uk. The PALS team is based in the main entrance on the ground floor at St Thomas' Hospital and on the ground floor at Guy's Hospital in the Tower Wing.

In the event that something does go wrong and you are harmed during the research you may have grounds for legal action for compensation against Guy's and St Thomas' NHS Foundation Trust and/or King's College London but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you (*If appropriate*).

Will my taking part in the study be kept confidential?

Yes, you will only be identified by an anonymous code and your name will not be included in any reports of the study. All information will be stored securely and only the research team will have access to it and your personal details.

What will happen to the results of the research study?

Part of the research will be written up as a thesis for a Doctorate at King's College London. On successful submission, the thesis will available in the University archives. The results of the TITANIC studies will be disseminated locally, nationally and

internationally in meetings for healthcare professionals and via diabetes related support groups. Papers will be published in health journals associated with diabetes and newsletters for people with diabetes.

Participation in future research

The researchers will request permission to contact you again for future investigation. This is an optional request and if you do not wish to give consent to be contacted for future investigations, it will not impact your participation in this study nor will it impact your clinical care.

Further information and contact details

Thank you for reading this participant information sheet. If you have any questions, or would like to find out more about our studies please contact the study diabetes specialist nurse who will be happy to discuss this study with you:

Contact name and address inserted here
Tel:(contact details inserted here)
E mail:

Appendix 5.3 Lipohypertrophy Characterisation Study

UlTrasound classIficaTion and GrAdiNg of lIpohypertrophy and its ImpaCt on glucose variability in type 1 diabetes (the TITANIC studies): characterisation of lipohypertrophy.

Invitation to take part in a research study

We would like to invite you to take part in a research study to understand more about the fatty lumps that can occur under the skin when you inject insulin, and how they affect your blood glucose (sugar) levels. These fatty lumps are known as lipohypertrophy and are sometimes referred to as ‘lipos’. This information leaflet explains why this study is being done and what we would like you to do if you wish to take part. We will be happy to answer any questions you may have. Taking part is completely voluntary and you can decide to withdraw at any time. This study is being conducted in collaboration with Guy's and St Thomas' NHS Foundation Trust and King’s College London.

Purpose of the study

The lipohypertrophy lumps develop because insulin can cause local fat cells to enlarge. They can sometimes be seen as large raised areas on the skin, felt as harder patches or small lumps under the skin. These small lumps can be difficult to feel, so you may not realise they are there. We think the lumps can affect how the insulin works causing the glucose level to vary unexpectedly. We want to understand what this changed tissue looks like using ultrasound. We also want to measure the lumps to establish a way of grading the lumps and see if some lumps or patches might affect glucose control more than others.

Why have I been invited?

You are being invited to take part in this study because you have reported glucose variability and you may have lipohypertrophy.

Taking part in the study

It is up to you to if you would like to take part in the study. If you do decide to take part you are free to withdraw from the study at any time without giving a reason. This decision will not affect the care you receive at the hospital in any way.

What will happen to me if I take part?

With your agreement, a study nurse will contact you by telephone and arrange to meet with you to discuss the study. If you agree to take part, you will be asked to sign a consent form. We will ask your permission to access some of your medical records such as your current medication and recent blood tests and arrange an appointment to examine your injection sites with ultrasound and by touch. When you come for this appointment and before we do the scan, we will feel for any lumps in your injection sites, which is the current way of assessing for lipos in clinical practice. The ultrasound is a harmless and painless procedure, which uses high frequency sound waves to produce images. An external scanner (probe) is held on the surface of the skin and images are recorded. The scan takes around 30 minutes to complete. We would like to scan your arms, abdomen, buttocks and thighs. We will show you were your lumps are and identify some new areas where you can inject avoiding any lumps. When we have done this with people with diabetes in the past, they often find that they need less insulin than when they were using their old sites, so the diabetes nurse will discuss this with you and agree a reduced insulin dose with you. We would also like to ask you to complete a few brief questionnaires that assess how people with diabetes feel about their diabetes and its treatment. There are no right or wrong answers to any of these and you can choose to answer all or none of the questions. We will take an additional blood test (about 5ml or one teaspoonful of extra blood) to check what your glucose levels have been in recent weeks – your glycated haemoglobin. Finally, we would like you to return to the clinic for a very short appointment six weeks after the scan to check your glycated haemoglobin again and to complete some of the questionnaires again. If you need ongoing review by your diabetes team after taking part, we will arrange this follow-up for you.

What will happen to my blood sample?

Blood samples collected for the purposes of the research will be used immediately and at the end of the study the samples will be disposed of in accordance with Human Tissue Authority (HTA) policies.

Benefits of taking part

This study may not directly benefit you, but it does give you an opportunity to have your injection sites examined and scanned by ultrasound. This information may help you to reduce the variations in blood glucose you experience.

Risks of taking part

There may be a small chance that your glucose may rise or fall when you change to the new injection sites and we have adjusted the dose of your insulin. You will be given advice on what to do if this happens and a contact number for the study diabetes nurse. There are no known risks from the type of ultrasound scan you are having.

What if there is a problem?

If you have any concerns during the study you can contact the research team on the telephone numbers provided. If you have concerns regarding the study or are unhappy in anyway, you can do this by contacting the Patients Advice and Liaison Service (PALS): phone: 020 7188 8801, email: pals@gstt.nhs.uk. The PALS team is based in the main entrance on the ground floor at St Thomas' Hospital and on the ground floor at Guy's Hospital in the Tower Wing.

In the event that something does go wrong and you are harmed during the research you may have grounds for legal action for compensation against Guy's and St Thomas' NHS Foundation Trust and/or King's College London but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you (*If appropriate*).

Will my taking part in the study be kept confidential?

All data will be kept confidential and used anonymously. Information will be stored securely and only the research team will have access to it.

Involvement of the general practitioner/family doctor

Your GP will be informed, with your consent, that you are taking part in the study.

What will happen to the results of the research study?

The results of the TITANIC studies will be disseminated locally, nationally and internationally in meetings for healthcare professionals and via diabetes related support groups. Papers will be published in health journals associated with diabetes and newsletters for people with diabetes.

Participation in future research

The researchers will request permission to contact you again for future investigation. This is an optional request and if you do not wish to give consent to be contacted for future investigations, it will not impact your participation in this study nor will it impact your clinical care.

Further information and contact details

Thank you for reading this participant information leaflet. If you have any questions, or would like to find out more about our studies please contact the study diabetes specialist nurse who will be happy to discuss this study with you:

Contact name and address inserted here
Tel:(contact details inserted here)
E mail:

Appendix 6 Study Consent Forms

Appendix 6.1 Glucose Variability Study- Condition 1

Appendix 6.2 The Effect of Changing Injection Site- Condition 2

Appendix 6.3 Lipohypertrophy Characterisation Study

Appendix 6.1 Glucose Variability Study- Condition 1

PARTICIPANT CONSENT FORM

Please tick the boxes below

		Yes	No
1.	I confirm that I have read and understand the information sheet (GV Study-PI1 Version.3/date….) for the above study and have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐	☐
2.	I understand that my participation is strictly voluntary and that I may withdraw at any time without the need to justify my decision and that this will not affect my medical care in any way.	☐	☐
3.	I understand that all information obtained during this study will be kept strictly confidential and that any results will not be linked to my personal details.	☐	☐
4.	I understand that relevant sections of my medical notes may be looked at by individuals from King's College London, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	☐	☐
5.	In the event of an abnormality being discovered as a result of the scan; I agree that I should be informed of the abnormality, that a relevant medical practitioner may be contacted and that I may be referred if necessary to the appropriate clinician.	☐	☐
6.	I agree to participate in this study.	☐	☐
7.	I agree to my GP being informed of my participation in the study	☐	☐
8.	I am happy to be contacted in the future regarding follow-on relevant ethically approved research studies.	☐	☐
9.	I would like to receive a summary of the findings from the study when it is completed.	☐	☐

_____	_____	_____
Name of Participant	*Date*	*Signature*
_____	_____	_____
Name of Researcher	*Date*	*Signature*

Appendix 6.2 The Effect of Changing Injection Site- Condition 2

PARTICIPANT CONSENT FORM

Please tick the boxes below

	Yes	No
1. I confirm that I have read and understand the information sheet (GV Study--PI2sVersion.3/date….) for the above study and have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐	☐
2. I understand that my participation is strictly voluntary and that I may withdraw at any time without the need to justify my decision and that this will not affect my medical care in any way.	☐	☐
3. I understand that all information obtained during this study will be kept strictly confidential and that any results will not be linked to my personal details.	☐	☐
4. I understand that relevant sections of my medical notes may be looked at by individuals from King's College London, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	☐	☐
5. In the event of an abnormality being discovered as a result of the scan; I agree that I should be informed of the abnormality, that a relevant medical practitioner may be contacted and that I may be referred if necessary to the appropriate clinician.	☐	☐
6. I agree to participate in this study.	☐	☐
7. I agree to my GP being informed of my participation in the study	☐	☐
8. I am happy to be contacted in the future regarding follow-on relevant ethically approved research studies.	☐	☐
9. I would like to receive a summary of the findings from the study when it is completed.	☐	☐

_____	_____	_____
Name of Participant	*Date*	*Signature*

_____	_____	_____
Name of Researcher	*Date*	*Signature*

Appendix 6.3 Lipohypertrophy Characterisation Study

PARTICIPANT CONSENT FORM

Please tick the boxes below

	Yes	No
1. I confirm that I have read and understand the information sheet (USScanStudyPI2b Version.3/date….) for the above study and have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐	☐
2. I understand that my participation is strictly voluntary and that I may withdraw at any time without the need to justify my decision and that this will not affect my medical care in any way.	☐	☐
3. I understand that all information obtained during this study will be kept strictly confidential and that any results will not be linked to my personal details.	☐	☐
4. I understand that relevant sections of my medical notes may be looked at by individuals from King's College London, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	☐	☐
5. In the event of an abnormality being discovered as a result of the scan; I agree that I should be informed of the abnormality, that a relevant medical practitioner may be contacted and that I may be referred if necessary to the appropriate clinician.	☐	☐
6. I agree to participate in this study.	☐	☐
7. I agree to my GP being informed of my participation in the study	☐	☐
8. I am happy to be contacted in the future regarding follow-on relevant ethically approved research studies.	☐	☐
9. I would like to receive a summary of the findings from the study when it is completed.	☐	☐

_____	_____	_____
Name of Participant	*Date*	*Signature*
_____	_____	_____
Name of Researcher	*Date*	*Signature*

Appendix 7 Schedule of Procedures

Appendix 7.1 Case-crossover Study

Case-crossover study	**OPA**	**Screen visit** **Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**
Participant information sheet- Glucose variability study	X	X			
Inclusion and exclusion check	X	X			
People with diabetes who meet the study inclusion criteria					
Informed consent		X	X		
Demographics		X			
DDS, ITSQ, Gold score, quality of life questionnaires		X			X
Blood sample			X		X
CGM fitted		X		X	
Participant information sheet - GV			X		
Physical examination (digital palpation)			X		
Ultrasound scan			X		
Body map (Injection sites)			X		
CGM diary		X		X	
Insulin stabilisation, advice about injection site and agree which new injection areas to use			X		
Tel contact			Telephone support from the diabetes specialist nurse to ensure participant safety		
CGM removed			X		X
Interview					X

Appendix 7.2 Lipohypertrophy Characterisation Study

Lipohypertrophy characterisation Study	**OPA**	**Screen visit** **Visit 1**	**Visit 2**
Participant information sheet – LH characterisation study	X	X	
Inclusion and exclusion check	X	X	
People with diabetes who do not meet the study inclusion criteria			
Informed consent		X	
Demographics		X	
DDS, ITSQ, Gold score, quality of life questionnaires		X	X
Blood sample		X	X
Ultrasound scan		X	
Physical examination (digital palpation)		X	
Body map (Injection sites)		X	X
Insulin stabilisation, advice about injection site and agree which new injection areas to use		X	
Tel contact		Telephone support from the diabetes specialist nurse to ensure participant safety	
Interview			X

Appendix 8 Study Questionnaire

Dear Participant,

We would be very grateful if you could take some time to complete the below information about you and your diabetes, and questionnaires about: insulin, hypoglycaemia, and your health in general.

We really value your time in completing this and encourage you to express your answers freely whether they are positive or negative. The questionnaire will be kept anonymous and your responses will in no way prejudice the care you receive.

Please try and answer all the questions if you can. However, if you think a question is inappropriate or does not apply to you then you may choose not to give an answer.

Thank you very much, we really appreciate your help.

☐ Please tick here if you do not wish to complete this questionnaire

CODE: __ __ __ __

Section 1. In this section, we will ask you some questions about you and your diabetes	
1. Date of birth: __ __ __ __ __	
2. Are you?	☐ Male or ☐ Female
3. How would you describe your ethnic origin? (check one box)	☐ White ☐ Black ☐ Asian ☐ Chinese ☐ Mixed ☐ Other _____ *please specify*
4. Is English your first language?	☐ Yes ☐ No
5. What is the highest level of education you have completed? (check one box)	☐ Some secondary school ☐ Secondary school graduate ☐ Some college ☐ University graduate ☐ Some postgraduate education ☐ Post graduate degree ☐ Advanced Graduate work or Ph.D. ☐ Not Sure
6. What is your current weight?	__ __ __ kg
7. What is your height?	__ __ __ cm
8. Which insulins do you use?	Long acting (basal insulin) name: _____ Do you take this once daily ☐ or twice ☐: What dose do you take Once daily dose: _____ **or** Twice daily doses: _____/_____ Quick acting (bolus) insulin name: _____ What is your usual 1st meal/breakfast dose? _____ What is your usual 2nd meal/lunch dose? _____ What is your usual 3rd meal/evening dose? _____
9. Do you use carbohydrate counting?	☐ Yes My insulin / carb ratio is; Breakfast ____ /____ Lunch ____ /____ Dinner ____ /____ ☐ No ☐ don't know what carbohydrate counting means

10. Do you take any other medication?

Name of medication	Dosage	Frequency

11. Do you have any other long term conditions/illnesses?

Condition	Year

12. Are you a right or left-handed? _____

Your diabetes and treatment	
13. When was your diabetes diagnosed?	__ __ __ __ (*Please enter the year)*
14. Have you attended any diabetes education or self-management course/s?	☐ Yes name of course_____ *e.g. DAFNE BERTIE other* ☐ No
15. How many times do you usually check your blood glucose in a day?	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ more than 4 times per day
16. Have you EVER had a low blood glucose where you have needed assistance to get some glucose and recover your hypo - a severe hypo?	☐Yes ☐No (go to Q 18)
17. How many of these severe hypos have you had in the last year?	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ more than 4 times
18. Do you know when your hypos are commencing?	☐ 1 Always aware ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 Never aware
19. Which needle length do you use?	☐ 4 mm ☐ 5 mm ☐ 6 mm ☐ 8 mm ☐ 12.7 mm
20. What and when was your last HbA1c result?	__ __ __ %/mmol date ___/ ___ / ____ ☐ I don't know
21. Do you have any complications related to diabetes?	☐ Kidney problems ☐ Foot problems ☐ Nerve damage ☐ Eye problems ☐ Stroke ☐ Heart disease (e.g. heart attack, heart failure, angina, other) ☐ None

Section 2. This section contains some specific questionnaires about, insulin, diabetes distress, hypoglycaemia, and your health in general

Satisfaction from Your Current Insulin Treatment

The following questions are about your perceptions of your current insulin treatment and how it affects you in your daily life. When you think of your insulin treatment, please keep in mind the type of insulin you take, the dose or amount of insulin, your schedule for taking insulin, and the device or method you use to give yourself insulin.

Please think about your experiences during the **past 4 weeks** when you answer the questions. If you are unsure about how to answer a question, please give the best answer you can.

1. How much of a bother is it for you to take all your daily insulin doses as prescribed?

No bother at all ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 A tremendous bother

2. How much does your current insulin treatment interfere with your ability to enjoy social or leisure activities?

Does not interfere at all ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 Interferes tremendously

3. How much does your current insulin treatment interfere with your work or school activities? (If you do not work or attend school, think about your regular daily activities).

Does not interfere at all ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 Interferes tremendously

4. How much do you have to plan the timing of your meals or snacks around the insulin you currently use?

No planning at all ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 A tremendous amount of planning

5. How much do you have to plan what you eat with your current insulin treatment?

No planning at all ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 A tremendous amount of planning

6. How much do you have to plan your physical activities (such as exercise or strenuous household chores) around your current insulin treatment?

No planning at all — A tremendous amount of planning

☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7

7. How confident are you that you can avoid symptoms of low blood glucose (such as sweating, trembling, dizziness, blurred vision) with your current insulin treatment?

Extremely confident — Not at all confident

☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7

8. How confident are you that you can avoid severe episodes of low blood glucose that result in loss of consciousness (fainting or passing out) with the insulin you currently use?

Extremely confident — Not at all confident

☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7

9. In general, how bothered are you by symptoms of low blood glucose (such as sweating, trembling, dizziness, blurred vision) due to the insulin you currently use?

Not at All Bothered — Extremely Bothered

☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7

10. How much do you feel that the insulin you are currently using increases the chances that you will experience low blood glucose?

Extremely — Not at All

☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7

11. How worried are you about experiencing low blood glucose during the night with the insulin you currently use?

Not at All Worried — Extremely Worried

☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7

12. How confident are you that you can avoid symptoms of high blood glucose (such as dry mouth, thirst, frequent urination, fatigue, increased appetite) with your current insulin treatment?

Extremely confident — Not at All confident

☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7

13. How satisfied are you with the stability of your blood glucose levels with your current insulin treatment?

Extremely satisfied ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 Not at All satisfied

14. Overall, how pleased are you with the blood glucose control you achieve with your current insulin treatment?

Extremely pleased ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 Not at All pleased

15. In general, how stressful is it for you to manage taking your current insulin treatment?

Not at all stressful ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 Extremely stressful

16. How burdensome is it for you to manage your current insulin treatment?

Not at all burdensome ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 Extremely burdensome

The following questions are about your perceptions of your <u>current method of taking insulin</u> and how it affects you in your daily life. For these questions, you should only think about the device or method you use to give yourself insulin.

17. How easy is it for you to take the correct amount of insulin each time with your current method of taking insulin?

Extremely easy ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 Not at all easy

18. How convenient is your current method of taking insulin when you are away from home?

Extremely convenient ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 Not at all convenient

19. How much pain or other physical discomfort do you experience with your current method of taking insulin?

No pain or discomfort ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 A tremendous amount of pain or discomfort

20. How comfortable are you taking insulin in a public place (where people might see you with your current method of taking insulin)?

Extremely comfortable — Not at all comfortable

☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7

21. How much emotional distress or anxiety do you experience related to your method of taking insulin?

No distress or anxiety — A tremendous amount of distress or anxiety

☐ **1** ☐ **2** ☐ **3** ☐ **4** ☐ **5** ☐ **6** ☐ **7**

22. Overall, how satisfied are you with your current method of taking insulin?

Extremely satisfied — Not at all satisfied

☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7

Diabetes Distress Scale

Living with diabetes can sometimes be tough. There may be many problems and hassles concerning diabetes and they can vary greatly in severity. Problems may range from minor hassles to major life difficulties. Listed below are 17 potential problem areas that people with diabetes may experience. Consider the degree to which each of the 17 items may have distressed or bothered you **DURING THE PAST MONTH and circle the appropriate number.**

Please note that we are asking you to indicate the degree to which each item may be bothering you in your life, NOT whether the item is merely true for you. If you feel that a particular item is not a bother or a problem for you, you would circle "1". If it is very troublesome to you, you might circle "6".

	Not a problem	A slight problem	A moderate problem	A somewhat serious problem	A serious problem	A very serious problem
1. Feeling that my doctor doesn't know enough about diabetes and diabetes care.	1	2	3	4	5	6
2. Feeling that diabetes is taking up too much of my mental and physical energy every day.	1	2	3	4	5	6
3. Not feeling confident in my day-to-day ability to manage diabetes.	1	2	3	4	5	6
4. Feeling angry, scared and/or depressed when I think about living with diabetes.	1	2	3	4	5	6
5. Feeling that my doctor doesn't give me clear enough directions on how to manage my diabetes.	1	2	3	4	5	6
6. Feeling that I am not testing my blood sugars frequently enough.	1	2	3	4	5	6
7. Feeling that I will end up with serious long-term complications, no matter what I do.	1	2	3	4	5	6

	Not a problem	A slight problem	A moderate problem	A somewhat serious problem	A serious problem	A very serious problem
8. Feeling that I am often failing with my diabetes routine.	1	2	3	4	5	6
9. Feeling that friends or family are not supportive enough of self-care efforts (e.g. planning activities that conflict with my schedule, encouraging me to eat the "wrong" foods).	1	2	3	4	5	6
10. Feeling that diabetes controls my life.	1	2	3	4	5	6
11. Feeling that my doctor doesn't take my concerns seriously enough.	1	2	3	4	5	6
12. Feeling that I am not sticking closely enough to a good meal plan.	1	2	3	4	5	6
13. Feeling that friends or family don't appreciate how difficult living with diabetes can be.	1	2	3	4	5	6
14. Feeling overwhelmed by the demands of living with diabetes.	1	2	3	4	5	6
15. Feeling that I don't have a doctor who I can see regularly enough about my diabetes.	1	2	3	4	5	6
16. Not feeling motivated to keep up my diabetes self-management.	1	2	3	4	5	6
17. Feeling that friends or family don't give me the emotional support that I would like.	1	2	3	4	5	6

Under each heading, please tick the ONE box that best describes your health TODAY

MOBILITY

I have no problems in walking about ❑
I have slight problems in walking about ❑
I have moderate problems in walking about ❑
I have severe problems in walking about ❑
I am unable to walk about ❑

SELF-CARE

I have no problems washing or dressing myself ❑
I have slight problems washing or dressing myself ❑
I have moderate problems washing or dressing myself ❑
I have severe problems washing or dressing myself ❑
I am unable to wash or dress myself ❑

USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*

I have no problems doing my usual activities ❑
I have slight problems doing my usual activities ❑
I have moderate problems doing my usual activities ❑
I have severe problems doing my usual activities ❑
I am unable to do my usual activities ❑

PAIN / DISCOMFORT

I have no pain or discomfort ❑
I have slight pain or discomfort ❑
I have moderate pain or discomfort ❑
I have severe pain or discomfort ❑
I have extreme pain or discomfort ❑

ANXIETY / DEPRESSION

I am not anxious or depressed ❑
I am slightly anxious or depressed ❑
I am moderately anxious or depressed ❑
I am severely anxious or depressed ❑
I am extremely anxious or depressed ❑

The best health you can imagine

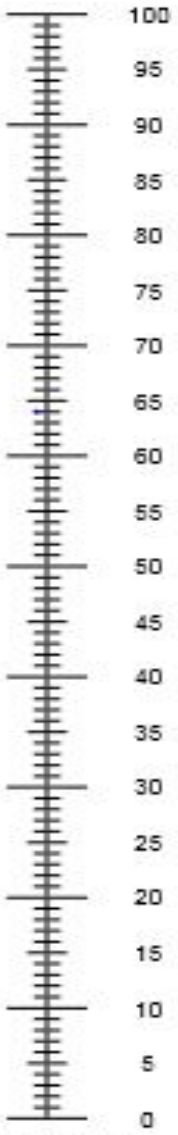
We would like to know how good or bad your health is **TODAY**.

This scale is numbered from **0** to **100**.

100 means the <u>best</u> health you can imagine, **0** means the <u>worst</u> health you can imagine.

Mark an **X** on the scale to indicate how your health is **TODAY**.

Now, please write the number you marked on the scale in the box below.



The worst health you can imagine

YOUR HEALTH TODAY = _____

Please write the number

Thank you for your time in completing this questionnaire

Appendix 9 Clinical Data Forms

Appendix 9.1 Digital Palpation

Appendix 9.2 Ultrasound Examination

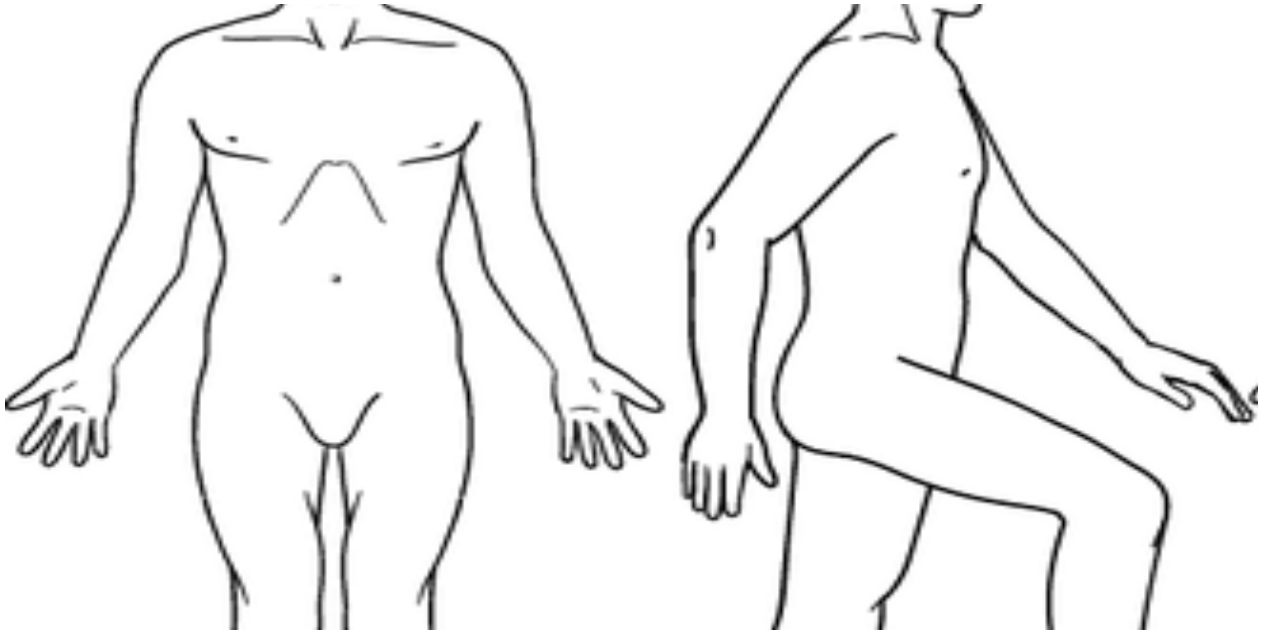
Appendix 9.1 Digital Palpation

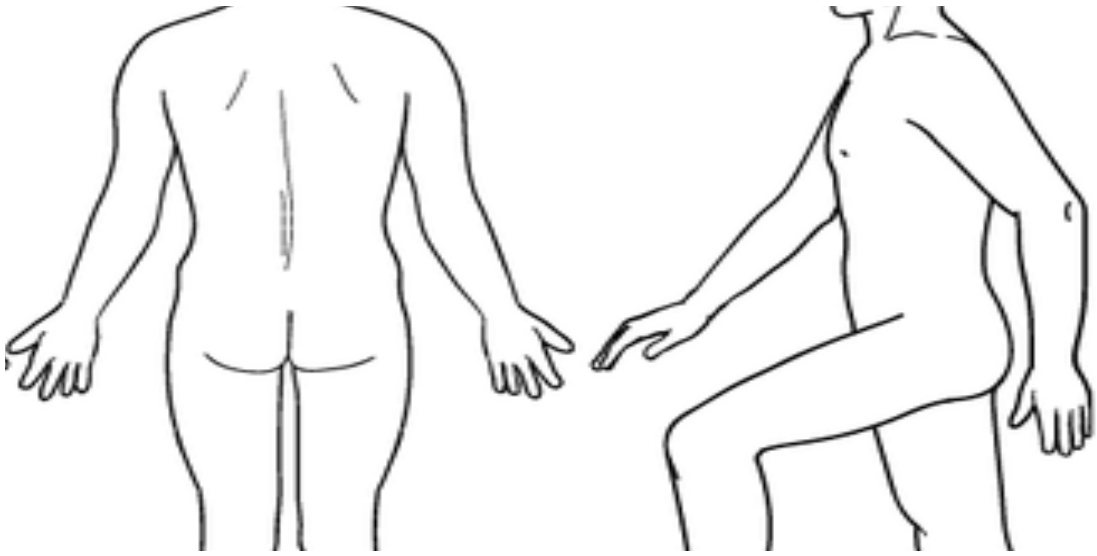
Palpation Examination

Time of the examination: _____/mins

Injection Sites	Palpation with Gel	Palpation without Gel
RTr		
LTr		
RUA		
LUA		
RLA		
LLA		
RAT		
LAT		
RLT		
LLT		
RGM		
LGM		

Body Map:

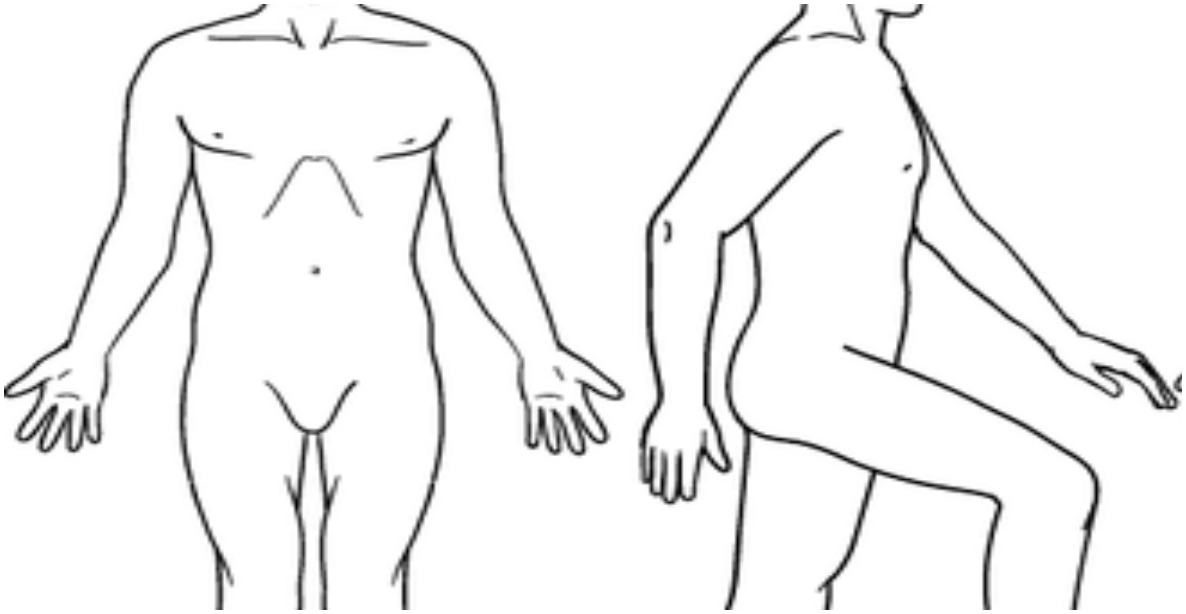




Appendix 9.2 Ultrasound Examination

Skin layer measurement			Location and descriptions of LH
Injection Sites	None Injection area	DL/SC	
RTr			
LTr			
RUA			
LUA			
RLA			
LLA			
RTA			
LTA			
RLT			
LLT			
RGM			
LGM			
RTr, Right Triceps; LTr, Left Triceps; RUA, Right upper abdomen; LUA, Left upper abdomen; RLA, Right lower abdomen; LLA, Left lower abdomen; RTA, Right anterior thigh; LTA, Left anterior thigh; RLT, Right lateral thigh; LLT, Left lateral thigh; RGM, Right gluteal region; LGM, Left gluteal region; DL, Dermis Layer; SC, Subcutaneous			

Body Map:





Appendix 10 Continuous Glucose Monitor Diary

Glucose Variability Study

(Participant Instructions)

Simple tips, instructions and guidelines for continuous glucose monitoring (CGM) sensor and blood glucose (BG) testing

On the first day:

- Take your first BG meter reading 1 hour after the CGM sensor has been inserted
- Take a second BG meter 3 hours after the device has been connected.
- Take a reading before each meal
- Collect at least one-meter reading before bed
- To calibrate properly when downloaded at the end of the week we need at least **four** BG meter readings each day – ideally before breakfast, before lunch, before evening meal and before bed
- Do not change any settings on your meter during the study –even if the clocks go forward or back
- Use the same blood glucose meter for all BG meter readings
- Do not let anyone else use your meter during the study

Diary sheet entries

- Write down your BG meter readings, the time you took your insulin and the dosage, the food (a photo may be easier) and/ or drink you had, and the carb content if you are used to estimating this), any physical activity you do – the time and duration, and any other symptoms or things of note you may experience.

- Keep the diary sheet with you at all times. So, you can write down the information immediately after each event.

Care and wearing of CGM sensor

- Please live your life as you would do normally and do not change the way you manage your diabetes in any way. If you normally exercise, then exercise etc. and do everything as you would usually do.
- Make sure the tape is over the CGM device and sensor to prevent accidental removal or sensor movement. If new tape is needed, just put it over the existing tape.
- If the CGM device looks like it has come apart from the sensor, then please try to gently push it together.
- Check the site for signs of irritation and contact the study nurse (mobile phone No) if you have any concerns regarding this. Removal of the sensor is simple should you need to, in which case, or if it falls out then please place the whole thing tape included into a resalable bag.
- You can bathe, shower and swim while wearing the CGM. The device is watertight at a depth of up to 2.4 meters (8 feet) for 30 minutes. There is no time limit if you are swimming on the surface of a pool in bath or showering.
- The device must be disconnected from the sensor prior to an x-ray, CT scan or MRI, so please contact the study nurse if this is an issue (Mobile phone no).

We know the above is a big ask, but it will help us and you tremendously at the end of the week to see what is happening with your blood sugar, so please do your best. The four blood tests a day are essential.

Glucose Variability Study

(Participant log sheet)

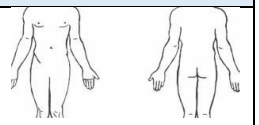
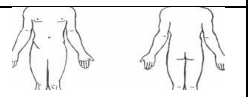
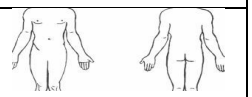
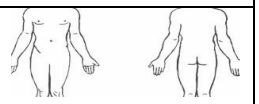
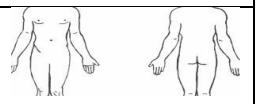
Participant's Study ID_____

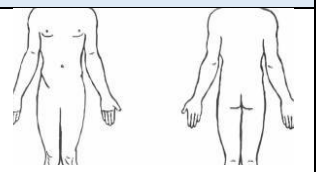
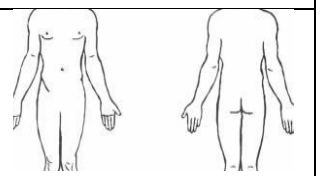
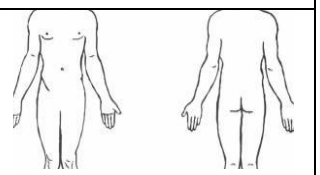
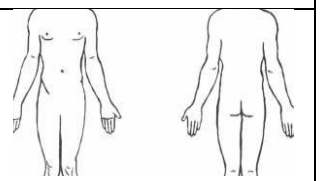
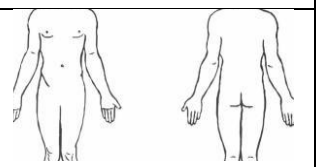
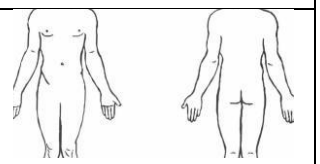
Appt. date_____/_____/_____

For the next six days: please test your blood glucose at least four times and when it might be at its lowest, for example: before breakfast, lunch, dinner, and bedtime.

Please bring your glucose meter with you when you come to have the sensor downloaded.

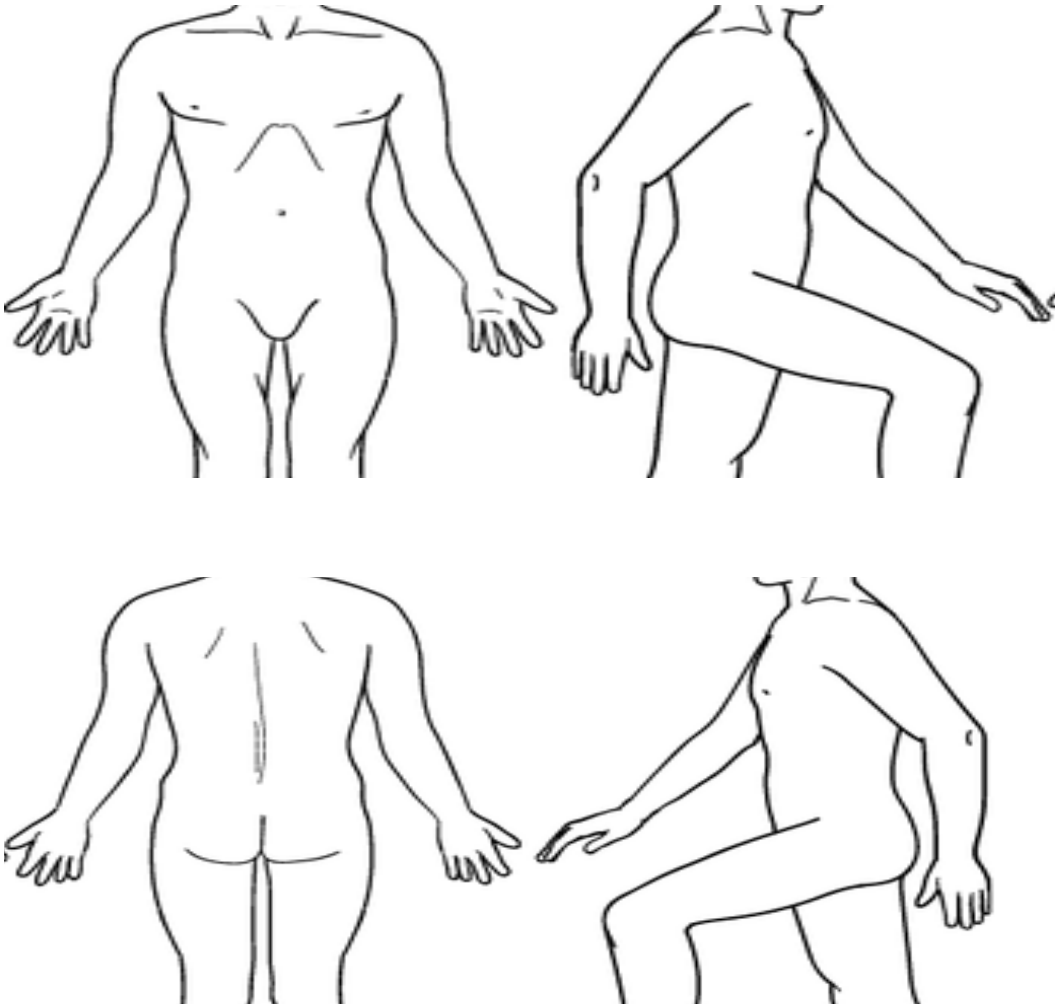
Example

Day 2 **--/--/--** **Time**	**Insulin** **QA LA**	**BG**	**Meals or snacks** Please describe and estimate CHO – e.g. fried fish with mash, grilled fish with chips, or take a picture if that is easier.	**Comments** Exercise, activity or event e.g. hypo, running for 20 mins. Really hot day.	**Please mark roughly where you gave the injection**
09:15	QA7 LA 12	7.2	70g two large slices of toast and some peanut butter	Went swimming for 20 mins	
10:30		4.3	30g Apple and a banana	Going low	
12:00	QA 6	5.5	60g sandwich and crisps	Stressful afternoon, so went for a long walk	
18:00	QA 6	8.2	60g avocado and salad with brown bread		
22:00	QA6 LA 12	9.2	40g cheese and biscuits	Off to bed	

Day 1 **__/__/__** **Time**	**Insulin** **QA LA**	**BG**	**Meals or snacks** Please describe and estimate CHO – e.g. fried fish with mash, grilled fish with chips, or take a picture if that is easier.	**Comments** Exercise, activity or event e.g. Went running for 20 mins. Really hot day. Had a hypo.	**Please mark roughly where you gave the injection**
			On day 1 please test your glucose one hour after the insertion and then two hours after that. Then carry on with pre-meal		
					
					
					
					
					

Appendix 11 Injection Site and Dose Calculation Form

Schema for showing where LH is and where it is not



Dose calculation for use when injection into new area

Current dose	
Agreed dose	
Comment	

Appendix 12 Exit Interview Guide

1. General introduction: *(mention confidentiality*)
   Prompts:
   - Remind me how long have you had Type 1?
   - Where did you have LH (lumps)?
   - Where did we suggest you inject?

   - How many units of long acting are you using now?
   - How many units of short acting are you using now?
   - Is that less than you used to be on? Yes/No
   - Is it more? Yes/No
   - Is it the same? Yes/No

2. Can you tell me about how you found changing your injection sites?
   Prompts:
   - Was it difficult to move? Yes/No
   - Did you feel you wanted to go back? Yes/No
   - Was the new site painful in comparison? Yes/No
   - Have you had less swings? Yes/No
   - Do you feel the insulin is more efficient/reliable? Yes/No

3. What advice or teaching have you been given about your injection sites in the past?
   Prompts: How long ago?

   - Did you use a grid/guide? Yes/No
   - Did you divide your sites into quadrants Yes/No
   - Were you given any fixed/formal? Yes/No

4. When you have attended your routine diabetes visits in the past two years have health professionals….
    - Palpated your sites? Yes/No
    - Looked at your sites visually? Yes/No
    - Asked you about your sites? Yes/No

5. Have you been shown the FIT guidance on injection site management? Yes/No

6. LH takes three months to start building up. If we suggested you inject all over four large sites/areas (e.g. 1) your upper abdomen, or 2) lower abdomen, or 3) flanks, or 4 upper thighs), and inject into one site for three months and then move to the next, and so on, so that each has nine months to recover:

    - Do you think that would be do-able/achievable?
      Yes/No
    - What about using the seasons to remind you to change sites?
      Yes/No

7. Do you have any questions you would like to ask us?

8. *(If the interviewer feels it is appropriate*) Would you like some further support? Would you like us to arrange an appointment for you with the DSN?

Thank you very much for your contribution to our research.

Appendix 13 Standard Operator Procedures

Appendix 13.1 Standard Operator Procedures 1- Physical examination and ultrasound scan technique

Appendix 13.2 Standard Operator Procedures 2- Insertion Technique for the IPro2 Continuous Glucose Monitor

Appendix 13.3 Standard Operator Procedures 3- Identification of lipohypertrophy free injection sites and safety issues regarding use of these new injection sites

Appendix 13.1 Standard Operator Procedures 1- Physical examination and ultrasound scan technique

This SOP describes the procedure of the physical examination and ultrasound scan technique, and the steps that will be followed by the clinicians/researchers, to inform and prepare the participant for the data collection.

A) Physical examination using inspection & digital palpation

The visual inspection and digital palpation will be carried out by one of the two study research nurses.

Equipment (for examination):

- Marker pen
- Gel
- Examination lamp with an adjustable neck

Prior to the examination

- Ensure the room is warm to prevent participant chilling (this ensures participant comfort and also prevents shivering and muscle tension, which can interfere with the examinations).
- Explain the procedure to the participant and check informed consent has been provided
- Reminded the participant they are welcome to ask any questions – before, during or after the procedure – whether they relate to the science or the procedure
- Participants will be made aware that the researchers will adhere to universal precautions to ensure safety during this data collection
- Wash hands thoroughly

Positioning:

- Supine position (lying down):

  The participant should initially be lying down on their back (to relax abdominal muscles) with knees bent (to relax thigh {quadriceps} muscles), and arms folded over chest (to relax arm muscles).

- Standing position: ask the participant to stand and arms folded over chest, to ensure that further LH is not identified.

Method:

- Inspect the site with the lamp first. The light should be shined onto the skin surface at an angle of 30-45 degrees (obliquely – not overhead) adjusting its angle to be able to detect any subtle risings or depressions across the surface of the skin.
- Ensure hands are washed and warm, apply a water based gel to the site to facilitate palpation of the area especially if there are no visible changes or lumps in the site.
- Palpate the area using slow circular and vertical fingertip movements followed by repeated horizontal attempts on the same spot (see figure x).
- Start with light pressure and increase thereafter to ensure that deeper tissue changes are felt.
- Perform the 'pinch' maneuver shown in Figure A to further identify LH changes. Comparing the thickness of the suspected spot to that of surrounding areas
- Record the position of any tissue changes carefully on the case report form.

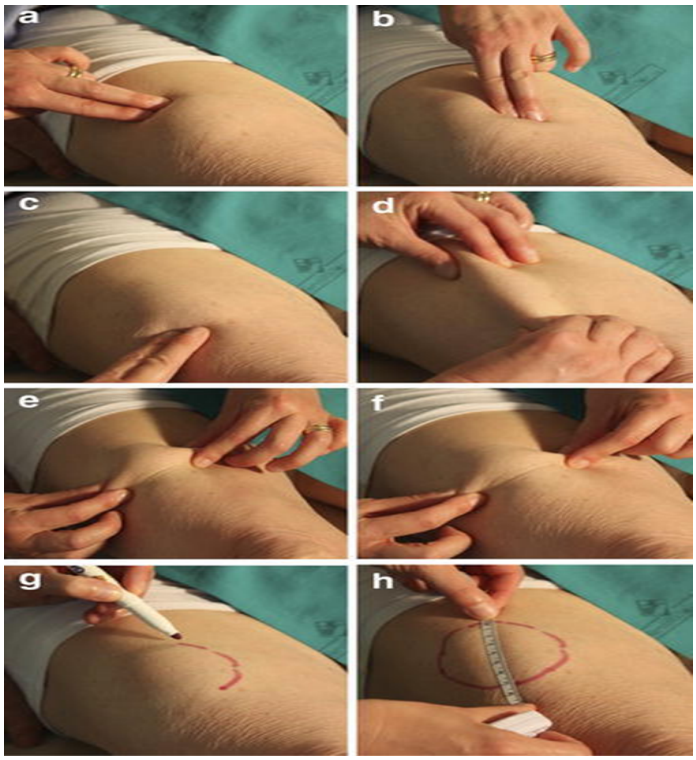


Figure A. Digital palpation of injection sites. Perform repeated vertical and horizontal fingertip movements over and around the area (a–c), 'pinch' gently vertically and horizontally across the area to further facilitate identification of LH (d–f). Marking (g) and measure it if not continuing to ultrasound (h) Gentile et al. (2016a).

Ultrasound Scan

This SOP is to be used only after the researcher/clinician has completed basic training in the use of ultrasound

The ultrasound scans will be performed by a trained and experienced ultrasonographer.

Equipment

- SonoSite X-Porte with high-frequency linear probe (6–13 MHz)
- Ultrasound gel

Scanning technique

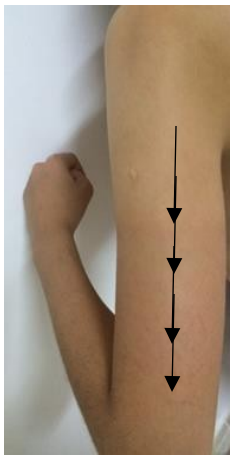
1) Upper arm scan

Start with asking the participant to rest both arms across the chest or, alternatively, both arms resting on the abdomen. Place the transducer in a transverse plane on the posterior and lateral aspect of the upper arm (right and left), two finger-widths below the acromion and sweep up and down to two to four finger-widths (depending on individual limb length and extent of soft tissue in the area) above the elbow (Images A and B).

Image A: Arrows: lateral side of the upper arm; lines: two fingers below acromion and two or four fingers above elbow



Image B: Arrows: posterior side of the upper arm; lines: two fingers below acromion and two or four fingers above elbow



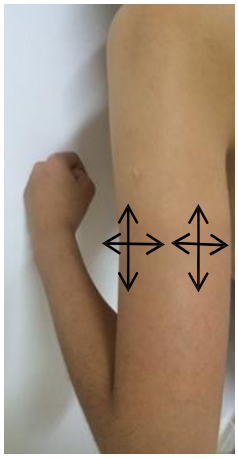


Image C: Arrows: midline of the posterior and lateral aspect of the upper arm

Images will be recorded of any tissue changes thought to be LH. Nodules or diffuse areas will be measured and recorded. An image will also be recorded for the posterior and lateral side of both arms, on the midline of the upper arm (Image C). If the arm is being used as an injection site and tissue changes are measured, then an area showing normal skin and tissue depth and type will also be recorded either above or below the injection site in the midline to collect data on epidermal, dermal and subcutaneous tissue.

2) Abdominal scan

With the individual in a supine position, place the transducer in a longitudinal plane over the outer margin of the right hypochondrial region to examine the upper abdominal area, and then sweep side-to-side to the outer margin of the left hypochondriac region. When the transducer is moved by sliding, the angle of entry should remain fixed so that a series of parallel planes can be scanned.

The transducer should continue to the lower abdominal area and begin scanning from the outer margin of the right lumbar region to the outer margin of the left lumbar region (Image D).

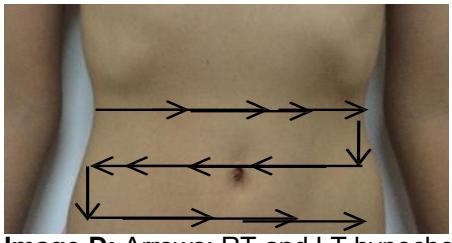


Image D: Arrows: RT and LT hypochondriac region and RT and LT lumbar region

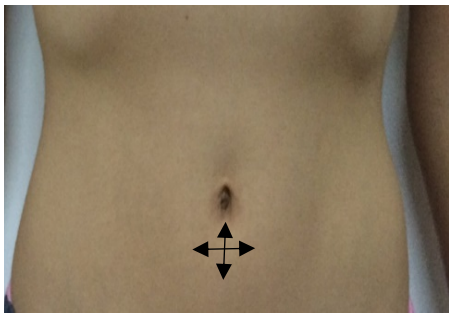


Image E: Arrows: at the midsternal line and the height of the iliac crest avoiding the umbilicus

Images will be recorded of any tissue changes thought to be LH. Nodules or diffuse areas will be measured and recorded. An image will also be recorded and saved of the tissue in the midline at the height of the iliac crest (image E). If this is being used as an injection site – an area of none injected tissue as close as possible to this will be recorded to collect data on normal tissue depth and type in the abdomen.

3) Thigh scan

With the individual in a supine position, place the transducer in a transverse plane, two to four finger-widths above the knee (depending on limb length and soft tissue area), and sweep up and down to the base of the iliac region to scan the anterior side of the thigh (right and left) (Image F).

To scan the lateral side of the thigh (right and left), the individual should be in a lateral recumbent position.

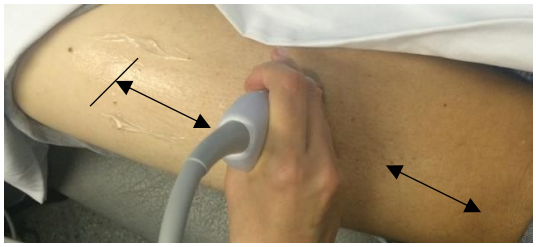


Image F: Arrows: two to four finger-widths above the knee and the base of iliac region

Images will be recorded of any tissue changes thought to be LH. Nodules or diffuse areas will be measured and recorded. An image will also be recorded for the anterior and lateral side of both thighs, on the midline of the midline of the thigh (Image F). If the thigh is being used as an injection site and tissue changes are measured, then an area showing normal skin and tissue depth and type will also be recorded either above or below the injection site in the midline to collect data on epidermal, dermal and subcutaneous tissue.

4) Gluteal region scans:

With the individual on lateral (Right or Left) decubitus position, place the transducer in a longitudinal plane, over the outer side of the right gluteal area and sweep it side to side to the midline. Follow the same technique to scan the left gluteal area (As shown in image G below).

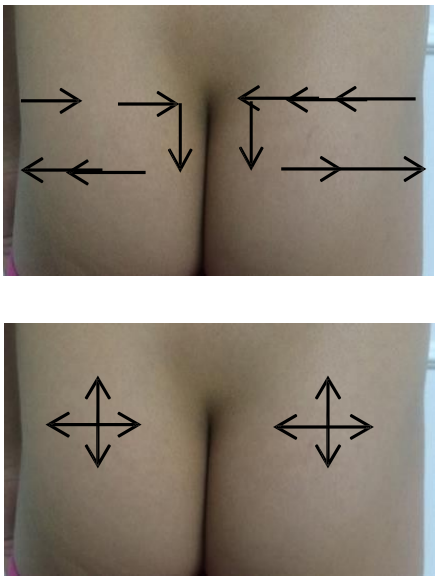


Image H: The midline at the right and left of the posterior inferior iliac spine

Images will be recorded of any tissue changes thought to be LH. Nodules or diffuse areas will be measured and recorded. An image will also be recorded and saved of the tissue in the midline at the right and left of the gluteal area (image H). If this is being used as an injection site – an area of none injected tissue as close as possible to this will be recorded to collect data on normal tissue depth and type in the Gluteal area.

Accuracy of measurement

The distance from skin surface to muscle fascia will be measured from inner to inner side. The normal subcutaneous tissue and skin thickness will be measured for all identified injection site.

Dermis layer: measured from the lower border of the epidermis and to the upper border of the subcutaneous tissue.

Subcutaneous tissue (SC): The layer between the dermis and the muscle fascia. The fat tissue acts to preserve neutral fat, cushioning against external physical pressure, retaining moisture and generating heat.

The subcutaneous layer will be measured from the lower border of the dermis layer to the upper border of the muscle.

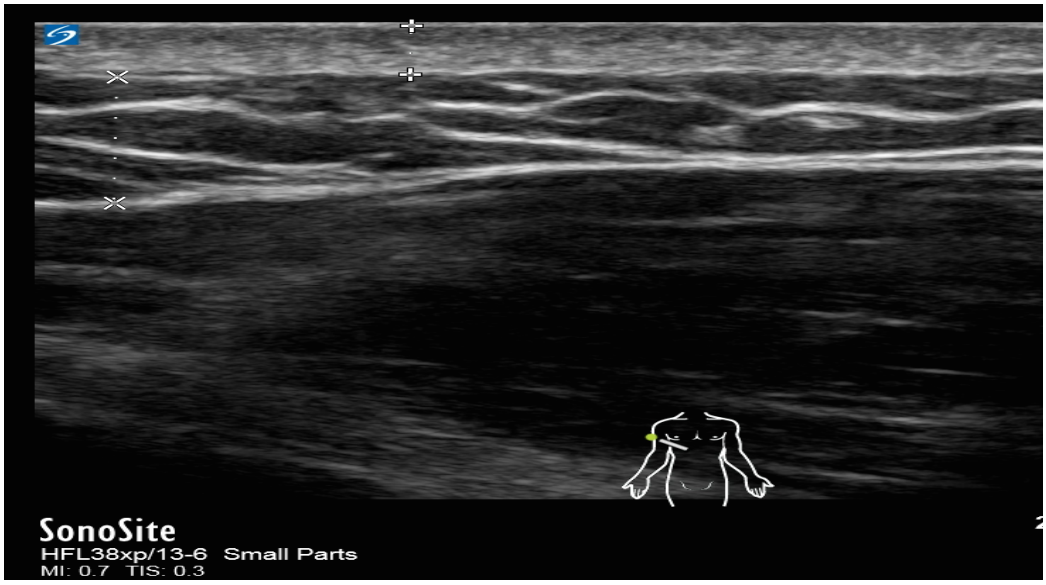


Image I. (+) the distance from the lower border of the epidermis and to the upper border of the SC. (x) the distance from the lower border of the dermis layer to the upper border of the muscle.

Lipohypertrophy (LH): When the site of LH is identified, the nodule may not have clear edges. For that reason, images should be obtained in both longitudinal and transverse planes to maximize information and accuracy of localization (Kaplan et al. 1990).



Image J. LH measure

Risks

Participants-There is a risk of discomfort during the physical examination and ultrasound scan. This discomfort will be related to examination.

Researchers-There are no known risks to the researchers implementing the SOP as a result of the protocol itself, or the equipment.

Ultrasound scan- There are no known risks from the sound waves used in an ultrasound scan. It does not involve exposure to radiation. External ultrasound scans are generally painless

SOP created by:

Prof. Angus Forbes, Dr. Henrietta Mulnier, Ms. Susan Halson-Brown, Rabab Hashem (PhD student).

[X] I acknowledge that as the principal investigator/faculty supervisor I am responsible for updating this SOP and notifying the Ethic committees if any of the procedures as outlined above change or require revision.

Appendix 13.2 Standard Operator Procedures 2- Insertion Technique for the IPro2 Continuous Glucose Monitor

This SOP describes the procedure for inserting the iPro2 continuous glucose monitor (CGM) device, and the steps that will be followed to inform and prepare the participant for the data collection.

Prior to insertion

- Check informed consent has been provided
- Discuss the positioning of the monitor with the participant to ensure it will not interfere with their daily activities. As a guide this should be above the waistband, slightly to one side (5-10cm) and on the opposite side from usual sleep position or bag/briefcase carriage.
- Participants are reminded to ask any questions – before, during or after the procedure – whether they relate to the science or the procedure.
- Participants will be made aware that the researchers will adhere to universal precautions to ensure safety during this data collection.

Equipment (for insertion):

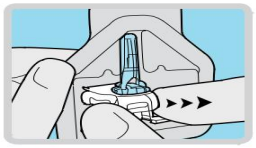
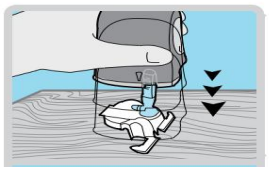
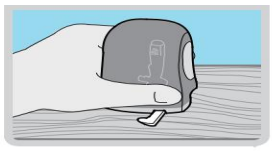
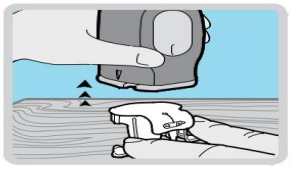
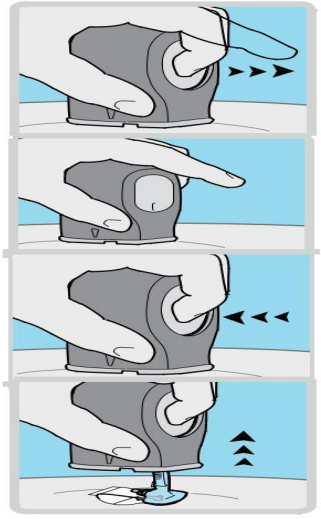
- Gloves
- Glucose sensor and serter (device that introduces the sensor)
- Fully charge recording device
- Sharps bin
- Cannula Dressings x5

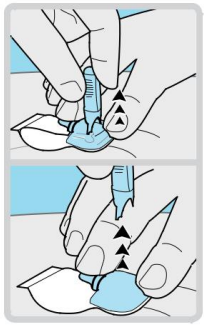
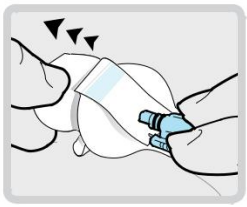
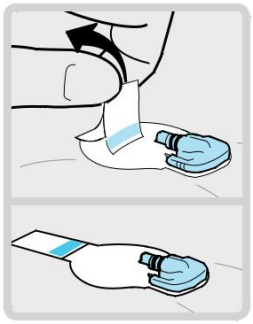
Preparation for sensor insertion

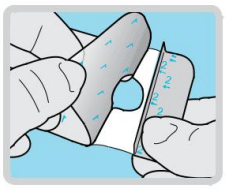
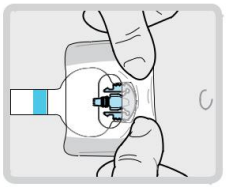
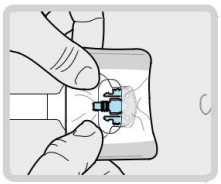
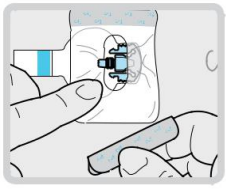
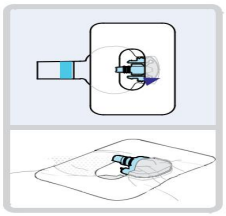
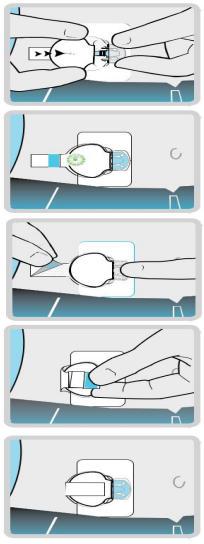
- Wash hands thoroughly
- Wear gloves
- Ask the participant to stand and identify with them where the device should be positioned

- The skin should be socially clean (shower or bath prior to attending clinic is sufficient). If the participant wishes use warm water to clean the area and thoroughly dry before inserting the sensor.

Method:

Part 1. Inserting a new Sensor	
Hold sensor by pedestal and place on table	
To load serter, push serter all the way down onto sensor and pedestal until serter sits on table	
Be careful not to force serter too hard onto sensor/pedestal or it may not load properly	
To remove pedestal, place two fingers on pedestal arms and pull serter straight up	
To insert sensor, press green button in and release it Hold serter against body and wait 5 seconds to allow time for pressure-sensitive adhesive to stick to skin Press and **hold in** green button. While continuing to hold in green button, slowly lift serter away from your body.	

With one hand, hold sensor against your body. With other hand, hold needle housing at the tip Pull needle housing straight out and dispose of into sharps box	
Warning: If bleeding occurs at sensor site (under/around/or on top of sensor), apply steady pressure using sterile gauze or clean cloth placed on top of sensor for up to three minutes. If bleeding does not stop, then remove sensor and apply steady pressure until bleeding stops.	
Remove white paper underneath curved adhesive pad. **Press entire adhesive to skin for several seconds.**	
Flip adhesive tab so it lies flat, but do not remove paper backing yet.	

Part 2. Taping the Sensor	
Remove large paper backing from overtape. Do not remove two smaller paper tabs on sides of overtape.	
Important: Attach overtape to both rounded part of sensor and skin in front of sensor.	
Apply rest of overtape, but do not block sensor connector with overtape. Press overtape to your skin for several seconds.	
Remove two paper tabs from sides of overtape. Press overtape against skin. Overtape covers both sensor and skin	 
Part 3. Connecting the Transmitter	
With one hand, hold sensor in place. With other hand, connect transmitter to sensor You will hear a faint “click” when the two components are connected. Check for green light to flash on transmitter. Remove paper on adhesive tab. Fold adhesive tab over transmitter. Important: Be careful not to pull adhesive tab too tightly	

After insertion

- Make the participant comfortable in a chair.
- Check participant is feeling ok.
- Go through the CGM information sheet and monitoring record with the participant to ensure that they know exactly what they need to do. In particular, they must be informed of what to do should they need to remove the device and also what information they need to record and when. Ensure that they fully understand and appreciate that the interpretation of the data download at the end of the week will only be successful if the blood glucose measurements have been taken as needed on the first day and at least four times a day on subsequent days.
- Make sure the participant can review and reflect what they need to do in the coming week and that they feel confident in what they are doing (achieving 7 on a 0 – 10 scale).
- Ensure they have at least three spare dressings that they can put on top of the originals should they start to peel back.

Equipment (for removal):

- Gloves
- Gauze
- Plaster
- Computer with CareLink iPro software installed
- Download cable
- Docking device for re-charging
- Cleaning plug

Procedure:

- Explain procedure to participant.
- Get participant to sit or lie down.
- Loosen adhesive dressings and when ready remove device and sensor together and place in a receptacle.
- Apply pressure for a short period to the area where the sensor was placed with a piece of gauze.

- Clean the area around sensor of any dried-on blood/dirt with a cleansing wipe.
- Place a small plaster over the puncture area.

Cleaning the iPro2

- Remove the sensor and tape from the device.
- Insert the cleaning plug and use cleaning solution containing alcohol supplied by the manufacturing company.
- Remove the plug and download the data onto the computer.
- Insert cleaning plug back in and ensure thoroughly clean and free of previous adhesive. Remove the cleaning plug and place on the dock to charge for the next participant. Usually 30 minutes.

Risks

- Participants

There is a risk of discomfort during the insertion of the sensor. This discomfort will be similar to the prick of a needle that would be obtained from a glucometer. Irritation and bleeding at the site may also occur but is rare. These risks will have been explained to the participant before consent and before insertion.

- Researchers

  There is a risk of sharps contamination after insertion of the sensor. To reduce this risk the sharp is disposed of directly into a sharps box after insertion.

SOP created by:

Prof. Angus Forbes, Dr. Henrietta Mulnier, Rabab Hashem PhD Candidate.

[X] I acknowledge that as the principal investigator/faculty supervisor I am responsible for updating this SOP and notifying the Ethic committees if any of the procedures as outlined above change or require revision.

Appendix 12.3 Standard operating procedure 3: Identification of lipohypertrophy free injection sites and safety issues regarding use of these new injection sites

This SOP describes the steps that will be followed by the study diabetes specialist nurse (DSN) to ensure participant safety during their transition to injecting into lipohypertrophy (LH) free areas. The DSN will help prepare participants for changing to LH free injection areas, by: agreeing their insulin dose; giving advice on blood glucose monitoring; and ensuring participants are aware of and can access ongoing support. The hazard of greatest risk is hypoglycaemia following a change in injection site. The method for insulin dose calculation and reduction to minimise this risk are set out below. It is important to emphasise that other than agreeing this dose change no additional adjustments will be made to the participant's insulin delivery technique, so that the validity of the study observations is maintained. The DSN will observe the following steps for each participant:

Step 1 Identifying LH sites and LH free areas for future injection

The study DSN will review the ultrasound (US) scan findings with the ultrasonographer. Areas of LH will be identified and marked in detail on the case report form and on a schema for the participant to take home. The US images will also be shown to the participant to reinforce the importance of avoiding these areas. The DSN will also print the participants' CGM traces or for those in the grading arm of the study their blood glucose data, to help further reinforce the need to avoid their LH areas. The DSN will give these to the participant to take home.

Step 2 set new insulin dose

A detailed discussion looking at the participant's glucose monitoring profile and current insulin dose will then take place. A weight based dose will be calculated as it would for a person starting insulin at diagnosis. Injecting into a new site can result in increased insulin sensitivity to a similar state to a new diagnosis, when a safe, but effective dose is calculated and agreed. The participant and DSN with then agree a safe and cautious reduced insulin dose. This will be documented, and a paper version given to the participant to take home for reference. If the participant is at all anxious about the dose adjustment, then the study clinician will be contacted to agree a dose with the participant.

Example dose calculation

Participants current total daily dose (TDD) 50% given as basal	Rapid 8 10 10 Basal 14 14 TDD = 56 units Current basal = 28 (14 units am and 14 units pm)	Weight calculate total daily dose based on 0.6 units per Kilogram 50% given as basal	80Kg x 0.6units = TDD 48 units Basal = 24 (12 units am and 12 units pm
Recommended reduction depending on hypoglycaemia risk 10% to 20%	20%		10%
CHO ratio/usual meal dose	1 unit :8g		1 unit: 10g
Comment	Participant (patient) is confident to take a 10% reduction using the calculated dose based on their weight, and use 1:10g		
Agreed dose	Basal = 22 units and 1 unit to 10g for mealtimes		

Participants will be advised by the DSN to monitor and record their blood glucose levels at least four times per day. They will ensure that the participant has a fully functioning blood glucose monitor and provide them with a second device in case the first device fails.

NB. The DSN will not make any other recommendations or changes to the participant's current insulin delivery model, unless there was a significant clinical hazard in which case the adjustments would be advised and the participant would be clinically followed up, but they would be excluded from the study.

The participants will be given the study DSN's mobile phone number and encourage to ring this number for advice over the following days. This may be held by other experienced DSNs employed by GSTFT to allow cover for holidays and illness. They will also be given a second number, which will be for the on-call diabetes medical team who are available 24hrs 7 days

Step 3 Insulin safety monitoring phase

The DSN will contact participants daily to discuss their glucose readings and make increases or further reductions in the insulin dose if their glucose levels were >12mmols at any time point, <5mmols/l pre-breakfast or <4mmols/l pre meals or <6mmols/l pre-bed. Daily contact will continue until the participant's glucose remains within the parameters for three consecutive days. Participants will then be given a weekly call to reinforce continuing to inject into LH free tissue and to check for any additional hypo or hyperglycaemic episodes. They will also be advised to contact the team should they have any queries or concerns. After five weeks for the GV study they will return for their follow-up CGM insertion. Then at six weeks all the participants will return for a final blood test and the final data collection visit.

NB. While the amount of insulin reduction proposed is not likely to lead to hazardous glucose elevation, participants will be advised to contact the team.

Step 4 Ongoing follow-up

At the final visit the DSN and participant will discuss the current glucose control and insulin doses. Based on this information the DSN will advise the participant on how to prevent further problems with LH and re-view the participants entire injection technique including needle length in case there are any additional changes that could help the participant avoid glucose variability and hypos e.g. taking meal time insulin at the optimal time. With the participants permission, the DSN will prepare a clinical summary of the results and adjustments made for the participant's diabetes team and ongoing follow-up will be organised with the diabetes team as necessary.

Appendix 14 Ethical Approval

Health Research Authority

[redacted]

Please note: This is the favourable opinion of the REC only and does not allow you to start your study at NHS sites in England until you receive HRA Approval

02 August 2017

Professor Angus Forbes
Florence Nightingale Faculty of Nursing and Midwifery, King's College London,
James Clerk Maxwell Building,
57 Waterloo Road, LONDON
SE1 8WA

Dear [redacted]

Study title:	**Ultrasound Classification and Grading of Lipohypertrophy and Its Impact on Glucose Variability in Type 1 Diabetes (The TITANIC studies): a Case Cross-over Study**
REC reference:	**17/LO/1242**
IRAS project ID:	**217187**

The Research Ethics Committee reviewed the above application at the meeting held on 20 July 2017. Please convey the Committee's thanks to Ms Hashem and Dr Mulnier for attending to discuss the application.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a

substitute contact point, wish to make a request to defer, or require further information, please contact hra.studyregistration@nhs.net outlining the reasons for your request. Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

Ethical opinion

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below. .

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

Conditions:

1. **Changes to the participant information sheet**

   a. Please add the following information to explain that a questionnaire will be administered and to explain why psychological measures are being evaluated as part of the study under 'What will happen to me if I take part?'

   'We would like to ask you to complete a few brief questionnaires that assess how people with diabetes feel about their diabetes and its treatment. There are no right or wrong answers to any of these and you can choose to answer all or none of the questions.'

   b. In the GV Study 2A participant information sheet please replace the phrase, 'you may well have lumps, so we would really like to look at your injection sites in detail,' with, 'we would like to look at your injection sites.'

2. **Changes to the consent form**

   a. Please add a yes/no check box next to each consent statement.

   b. Please add a statement by which participants can consent to their medical records being accessed for the purposes of the study.

You should notify the REC once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. Revised documents should be submitted to the REC electronically from IRAS. The REC will acknowledge receipt and provide a final list of the approved documentation for the study, which you can make available to host organisations to facilitate their permission for the study. Failure to provide the final versions to the REC may cause delay in obtaining permissions.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

17/LO/1242	Please quote this number on all correspondence

With the Committee's best wishes for the success of this project.

Yours sincerely

[redacted]

pp. [redacted]
REC Manager

5/22/2019 [redacted]

IRAS 217187 GSTT confirmation of capacity and capability

[redacted]

2 attachments (704 KB)

Sponsored Conditions of GSTFT participation V1 October 2015.docx; EDGE_Quick_Patient_Activity_Guide_v3_(10012017).pdf;

Dear [redacted]

Study Title: TITANIC - Ultrasound Classification and Grading of Lipohypertrophy and Its Impact on Glucose Variability in Type 1 Diabetes
Sponsor: KCL/GSTT co-sponsorship
Chief Investigator: Prof Angus Forbes

Guy's and St Thomas' NHS FT has agreed to host your research study (please see attached the confirmation of capacity and capability email to the Sponsor). Your study can therefore now start at GSTFT.

If the study requires the use of the CRF please contact the R&D office beforehand as we will need to see evidence that the CRF has the capacity to accommodate the research.

Appendix 15: Publications arising from this project

15.1 Journal articles:

Diabetes Ther (2018) 9:1741–1756
https://doi.org/10.1007/s13300-018-0472-7

REVIEW

A Systematic Review of Ultrasound-Detected Lipohypertrophy in Insulin-Exposed People with Diabetes

Haya Abu Ghazaleh · Rabab Hashem · Angus Forbes · Thandiwe Rebecca Dilwayo · Maria Duaso · Jackie Sturt · Susan Halson-Brown · Henrietta Mulnier

Received: May 18, 2018 / Published online: July 16, 2018
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ABSTRACT

Introduction: Lipohypertrophy (LH) is a common complication occurring in diabetes individuals. The most common methods used include palpation, visual examination and/or ultrasound (US). To date, there is limited information on the detection sensitivity among the different techniques used to identify LH. This systematic review aimed to identify studies that examined insulin-related LH using US detection to identify the prevalence, characteristics and morphology of LH, and to compare US and clinical palpation methods for detecting LH.

Methods: Three electronic databases were systematically searched for studies detecting LH using US in insulin users. Articles were screened for eligibility and included studies were appraised using quality assessment tools. The quality of the evidence was evaluated using Grading of Recommendations Assessment, Development and Evaluation, and the extracted data was synthesised narratively.

Results: Sixteen articles were included in the review providing data on 1722 patients. The prevalence of LH prevalence varied from 14.5% to 88% (median 56.6%). Identified risk factors for the development of included insulin injection behaviour such as a lack of injection site rotation and social factors such as low education level. Four studies compared LH detection by US to palpation, providing inconsistent results. One study showed that palpation detected 64% more LH, whilst two studies demonstrated that US identified 50% more sites and extended areas of LH (additional ~ 5 cm^2). Another study provided comparable estimates between palpation and US in clinicians trained to detect LH (97%).

Conclusion: The evidence highlights a lack of congruence in results pertaining to the detection sensitivity of US and palpation for LH sites. More research with robust study design is needed to verify whether clinically palpation is sufficient to detect LH, or whether US would increase the precision of LH assessment to help address this common clinically significant problem.

Enhanced digital features To view enhanced digital features for this article go to https://doi.org/10.6084/m9.figshare.6741602.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13300-018-0472-7) contains supplementary material, which is available to authorized users.

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Keywords: Hypertrophy; Insulin injection; Lipohypertrophy; Palpation; Ultrasound

INTRODUCTION

Lipohypertrophy (LH) occurs in subcutaneous tissue as a result of the lipogenic effect of repeated insulin exposure [1, 2]. The fat cells enlarge and proliferate resulting in thickened tissue, sometimes forming lumps under the skin. LH is associated with suboptimal glycaemic control, with one recent study reporting a threefold higher incidence of LH in patients whose control was above the current national target (HbA1c $\geq$ 7%, 86 mmol/L), compared to those within the target range [3]. Injecting insulin into an LH lesion has been shown to attenuate insulin action with consequent excess glucose exposure, glycaemic variability and increased risk of severe hypoglycaemia [4–6]. Known risk factors for the development of LH include high BMI ($\geq$ 25), frequent needle reuse, failure to rotate insulin injection sites effectively, size of rotation area, level of education, and duration of insulin exposure [3, 7, 8]. It is also likely that patient behaviours are significant mediators in the level of LH observed, with patients reusing sites that are less painful or because a site is more convenient to access [9, 10].

Increasing awareness of the importance of LH in diabetes care has led to the development of international guidelines for managing injection areas and for detecting LH [11, 12]. One recent multicentred UK study demonstrated improved injecting behaviours and metabolic outcomes following implementation of one of these guidelines in about two thirds of those exposed to the guideline [13]. However, the extent to which these guidelines are observed in routine clinical care is unknown, nor is it known how frequently or rigorously LH is assessed. It may be that there is a lack of awareness within the community of diabetes professionals and patients on the prevalence and significance of LH.

In clinical practice, LH is most commonly assessed by palpation. However, the reliability of this method is potentially low, with high levels of inter-clinician variation. This was recently demonstrated by Gentile et al. [14], whereby nurses trained to use a more stringent palpation technique were able to show 97% detection of cases; while the comparator missed 34% of cases. As a consequence, ultrasound (US) has been proposed as a potentially more objective method for detecting LH. US may provide more precise estimates of the true prevalence of LH, as current estimates based on mainly observation or palpation are divergent with estimates ranging from 3.6% to 64%, with a median of 32.8% [14].

The aim of this systematic review was to present a summary of the additional insights into LH contributed by US detection studies by assessing the prevalence of LH, identifying factors associated with the development of LH, and providing some estimation on the sensitivity of palpation versus US in detecting LH.

METHODS

A protocol-based systematic review was used to identify studies using US to detect LH, addressing the following objectives:

- To identify the estimated prevalence, anatomical distribution and the tissue morphology of LH with US and/or palpation assessment
- To identify factors associated with LH in US assessed cases
- To estimate the sensitivity of palpation in LH detection, with US as the reference

Search Strategy

A comprehensive literature search was conducted using three electronic databases (Medline, Embase and Cinahl) to identify articles pertaining to the detection of LH using US in insulin-treated patients with diabetes mellitus. The search used both Medical Subject Headings (MeSH) and free-text synonyms for the following terms: palpation, ultrasonography, lipodystrophy, diabetes mellitus and insulin. An example search protocol is presented in Appendix 1. Additional papers were identified through free-text searches, citation searching and by reviewing secondary references.

Retrieved articles were screened by three independent researchers for their relevance (HA, RH and RD).

Selection Criteria

Studies examining the detection of LH using US with or without palpation techniques in type 1 and type 2 insulin-treated patients with diabetes were included in the literature synthesis. Publications were excluded if they discussed LH in the context of gestational diabetes, were based on visual examination only without palpation, or addressed other causes of lipodystrophy. Only data from primary studies were considered and review articles were excluded.

Data Extraction

Two review authors (HA, RD) independently extracted the data from the included studies. The main outcomes reviewed were the different forms of insulin administration resulting in LH occurrence, risk factors of LH and the detection sensitivity of LH using US and/or palpation. Additional data extracted included study design, study location and year, population, sample size, insulin exposures, patient characteristics and metabolic factors.

Data Synthesis

The data were synthesised using a narrative approach addressing the outlined review objectives. Studies were too heterogeneous to provide a meta-analysis, but study results were tabulated to provide a collective assessment of their findings.

Quality Assessment

The relevant studies identified for inclusion in the review used different methods and designs; hence, multiple appraisal tools were used to assess the scientific rigour and quality of the studies, including the Cochrane Collaboration tool for randomised controlled trials (RCTs) [15], the Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies devised by the National Institutes of Health [16], and the Joanna Briggs Institute critical appraisal checklist for case reports [17]. Two authors assessed the overall quality of the included studies (HA, RD). The Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach was also employed to assess the quality of the estimations provided by the included studies, considering the following criteria: risk of bias; inconsistency, indirectness and imprecision; effect magnitude; dose–response effect; and other sources of potential confounders. The quality assessments were used to rate the quality of the evidence from high to very low [18].

Ethical Considerations

This article does not contain any new studies with human or animal subjects performed by any of the authors and as such no ethical approval was required.

RESULTS

The search strategy yielded 317 citations, duplicates were removed and the remaining articles were subsequently screened for their relevance based on the review inclusion criteria ($n = 284$). Reasons for exclusion included detection method not identified ($n = 5$), study did not address the review objectives ($n = 4$), or repetition of findings in multiple papers ($n = 4$). A total of 17 unique studies reported in 16 papers were selected for inclusion (Fig. 1).

The characteristics of the studies included in the literature synthesis are presented in Table 1. Most studies utilised observational designs to consider the frequency of LH in cohorts of insulin-treated patients [14, 19–30]. Four studies compared the detection sensitivities of palpation and using US as a reference standard [14, 19, 23, 29, 31]. Three individual cases of LH in patients with diabetes were presented [20, 32] and one considered the effectiveness of LH detection using three different methods using a cross-over RCT design [4]. A few studies presented data on glycaemic control and insulin

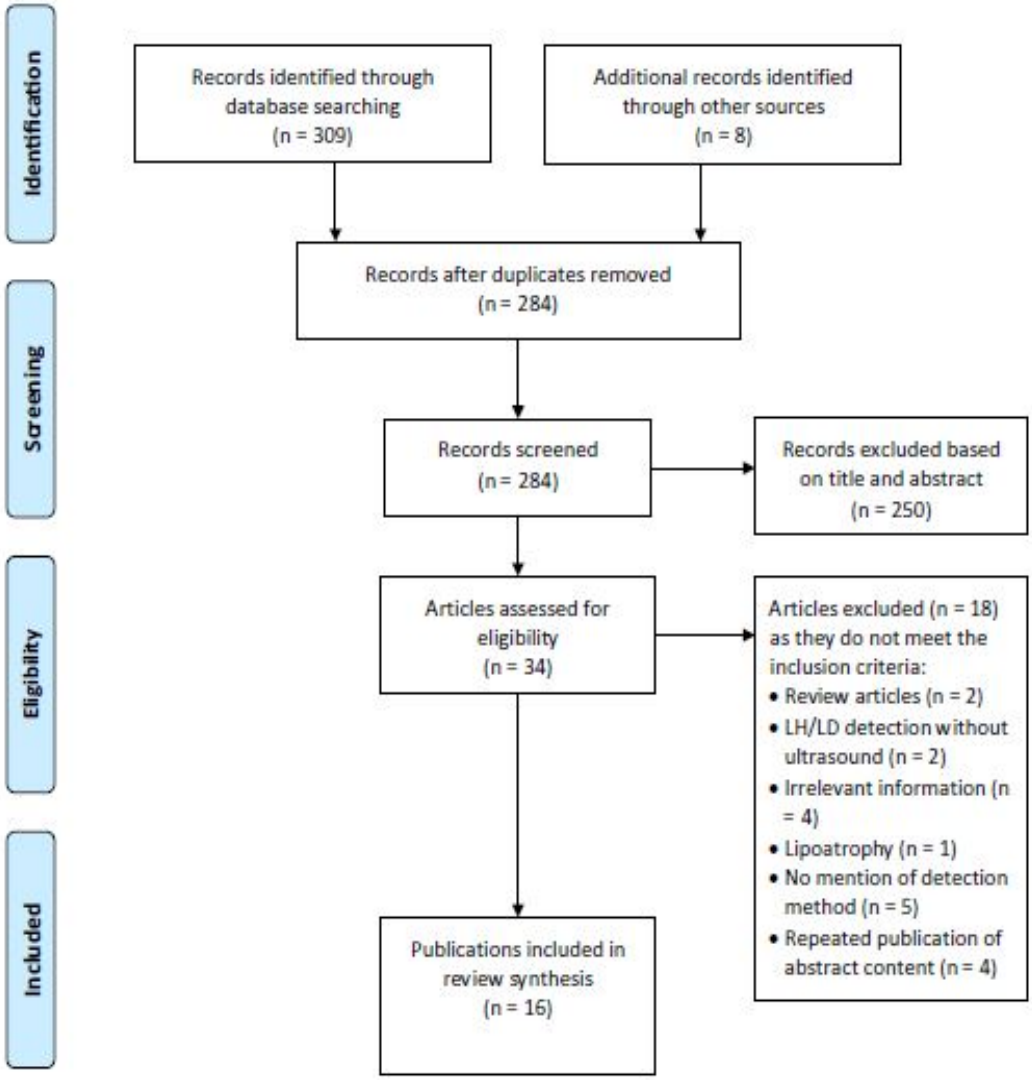


Fig. 1 PRISMA flowchart for inclusion of selected articles in this systematic review

activity following insulin administration in LH regions [4, 19, 20, 32] and risk factors in promoting LH lesions [20–22, 25, 26]. Three studies aimed to define and grade LH using US scanning [19, 24, 33]. Across the studies, insulin exposure was almost exclusively via needle injection. Thirteen studies were carried out in Europe, and two each in North America and Asia.

Quality Appraisal

The overall quality of the observational studies was poor, with information deficits in relation to the power of the analyses undertaken and level of follow-up. Of the studies comparing palpation and US, only two mentioned blinding and only three out of the 12 observational studies were adjusted for confounders. Despite the overall poor quality of the studies, they all yielded data of relevance to the review objectives. The RCT study was identified as having a moderate rating for risk of bias, as there was a lack of information on allocation concealment and participant blinding which may have led to assessor contamination [4]. The findings of the review are presented thematically below, with tables summarising the study findings.

Table 1 Summary of included studies

Author (year), country	Study aim(s)	Sample population	Insulin therapy*	Detection method(s), assessor(s), region(s)	Outcome measure(s)
RCT					
Famulla et al. (2016) [4] Germany	Determine the impact of LH on insulin activity	Sample size: 13 Population: T1DM Mean age (years, SD): 50.1 ± 10.5 Gender (M/F): ND Mean diabetes duration (years, SD): 26.8 ± 6.9	Insulin needle injection	*Detection* Observation, palpation (pre-defined examination procedure) and US *Assessors* 2 independent investigators *Region* Abdomen	LH detection, insulin absorption in LH regions, diabetes outcomes: insulin and glucose levels
Observational studies					
Bertuzzi et al. (2017) [19] Italy	Characterisation of LH using US	Sample size: 20 Population: T1DM Mean age (years, SD): 37 ± 12 Gender (M/F): 8/12 Mean diabetes duration (years, SD): 22 ± 12	CSII, MDI	*Detection* Palpation (pre-defined criteria), US (pre-defined criteria) *Assessors* Investigators *Region* Abdomen, arm, gluteus	LH detection, metabolic parameters: HbA1c
Blanco et al. (2013) [20] Spain	Assess LH prevalence and determine its correlation with clinical and public-health factors	Sample size: 430 Population: T1DM (41%) + T2DM (59%) Mean age (years, SD): 49 ± 22.8 Gender (M/F): 221/202 Diabetes duration (years, range) 6–15	Insulin pen	*Detection* US (pre-defined characteristics) *Assessors* CD *Region* Abdomen	Prevalence of LH, risk factors of LH, diabetes outcomes: hypoglycaemia, glucose variability

Table 1 continued

Author (year), country	Study aim(s)	Sample population	Insulin therapy*	Detection method(s), assessor(s), region(s)	Outcome measure(s)
Conwell et al. (2008) [21] Canada	Describe dermatological changes with CSII therapy	Sample size: 50 Population: T1DM Mean age (years, SD): 13.3 ± 3.5 Gender (M/F): 24/26 Mean diabetes duration (years, SD): 6.5 ± 3.7	CSII	*Detection* Palpation (pre-defined criteria) in all 50 patients, US (pre-defined criteria) in 8 out of 50 patients *Assessors* 1 trained investigator *Region* Abdomen, back/buttock, legs	Frequency of LH, dermatological changes including LH
Davidenko et al. (2014) [22]—abstract, Russia	Develop estimation risk model of insulin induced LH	Sample size: 140 Population: Diabetes (NS) Mean age (years, SD): ND Gender (M/F): 51/89 Mean diabetes duration (years, SD): ND	NS	*Detection* Observation, palpation, US *Assessors* CD *Region* ND	Frequency of LH, estimation of insulin as a risk factor of LH
Gentile et al. (2016) [14] Italy	Identify inexpensive and accurate LH detection method	Sample size: 40 Population: Diabetes (NS) Mean age (years, SD): 54 ± 15 Gender (M/F): 16/24 Mean diabetes duration (years, SD): ND	Insulin needle injection	*Detection* Palpation (pre-defined criteria), US (pre-defined criteria) *Assessors* Trained vs non-trained nurses *Region* Abdomen, arm, thigh	Prevalence of LH, detection accuracy of LH between trained and non-trained HPs

Table 1 continued

Author (year), country	Study aim(s)	Sample population	Insulin therapy*	Detection method(s), assessor(s), region(s)	Outcome measure(s)
Kapeluto et al. (2015) [33]—abstract, Canada	Establish a criteria for LH detection using US	Sample size: 7 Population: Insulin-users Mean age (years, SD): ND Gender (M/F): 6/1 Mean diabetes duration (years, SD): ND	NS	*Detection* Palpation, US *Assessors* Single team + radiologist *Region* Abdomen	LH diagnostic criteria using US
Kasperska-Czyzyk et al. (2000) [23]—abstract, Poland	Determine the robustness of US in the diagnosis and characterisation of LH	Sample size: 30 Population: T1DM (33%) + T2DM (60%) + secondary diabetes (7%) Mean age (years, SEM): 60 ± 2 Gender (M/F): 16/14 Mean diabetes duration (years, SD): ND	Insulin needle injection	*Detection* Palpation, US *Assessors* CD *Region* Abdomen, arms, thighs	Detection accuracy of LH between US and palpation
Mulnier et al. (2017) [24]—abstract, UK	Evaluate the feasibility of US to detect and characterise LH	Sample size: 26 Population: T1DM Mean age (years): 41 Gender (M/F): ND Mean diabetes duration (years): 22.7	Insulin needle injection	Detection: US Assessors: CD Region: CD	LH detection, changes in subcutaneous tissue with insulin use

Table 1 continued

Author (year), country	Study aim(s)	Sample population	Insulin therapy*	Detection method(s), assessor(s), region(s)	Outcome measure(s)
Nasser et al. (2017) [25] Bahrain	Determine LH prevalence and risk factors of LH	Sample size: 95 Population: T1DM (3%) + T2DM (97%) Age (years, range): 40 to ≥ 70 Gender (M/F): 23/72 Diabetes duration (years, range): 5 to ≥ 20	Insulin needle injection	Detection: US Assessors: Clinical nurse Region: Abdomen, arm, thigh	Prevalence of LH, risk factors of LH
Patrakeeva et al. (2014) [26]—Abstract, Russia	Evaluate LH frequency using different insulin regimens and risk factors of LH	Sample size: 29 Population: T1DM Mean age (years, SD): 27 ± 4 Gender (M/F): ND Mean diabetes duration n (years, SD): 13.7 ± 2.1	CSII, MDI	Detection: US Assessors: ND Region: ND	Prevalence of LH, risk factors of LH
Perciun (2010) [27] Romania	Characterise LH sites using US	Sample size: 40 Population: Diabetes (NS) Age (years, range): 15 – 65 Gender (M/F): 14/26 Mean diabetes duration (years, SD): ND	Insulin needle injection	*Detection* palpation (pre-specified criteria), US (pre-specified criteria) *Assessors* ND *Region* Abdomen, arm, thigh, buttocks	Diagnosis and evaluation of LH sites

Table 1 continued

Author (year), country	Study aim(s)	Sample population	Insulin therapy*	Detection method(s), assessor(s), region(s)	Outcome measure(s)
Perciun et al. (2014) [28] Romania	Compare the diagnosis of dystrophies between US and palpation	Sample size: 53 Population: T1DM Age (years, range): 2–15 Gender (M/F): 33/20 Diabetes duration (years, range): 1–13	Insulin needle injection	*Detection* Palpation (pre-specified criteria), US (pre-specified criteria) *Assessors* CD *Region* Abdomen, arm, thigh, buttocks	Frequency of hypertrophic sites
Volkova et al. (2013) [29]—abstract, Russia	Compare the frequency of LH between US and palpation	Sample size: 215 Population: Diabetes (NS) Mean age (years): 46 Gender (M/F): 142/73 Mean diabetes duration (years, SD): ND	Insulin needle injection	*Detection*: Observation, palpation, US *Assessors*: ND *Region*: Paraumbilical/buttocks, hips, shoulders	Detection accuracy of LH between US and palpation
Wang et al. (2014) [30]—abstract, China	Estimation of skin and subcutaneous layer thickness and prevalence of LH	Sample size: 509 Population: T1DM + T2DM (% NS) Age (years, range): 18–85 Gender (M/F): ND Diabetes duration (years, SD): ≥ 1	Insulin needle injection	*Detection* US *Assessors* ND *Region* Abdomen, arm, thigh, buttocks	Prevalence of LH, estimation risk of subcutaneous and intramuscular injection

Table 1 continued

Author (year), country	Study aim(s)	Sample population	Insulin therapy[a]	Detection method(s), assessor(s), region(s)	Outcome measure(s)
Case reports					
Blanco et al. (2013) [20] Spain	Assess diabetes history of LH patient	Sample size: 1 Population: T1DM Age (years): 32 Gender: M Diabetes duration (years): 18	Insulin needle injection	*Detection* Palpation, US Assessors ND *Region* Abdomen	Change in LH, diabetes-related outcomes: HbA1c, insulin use, hypoglycaemic episodes
Perciun et al. (2012) [32] Romania	Analyse cutaneous and subcutaneous dystrophies using two different ultrasounds	Patient 1 Population: Diabetes (NS) Age (years): 55 Gender: M Diabetes duration (years): ND	Insulin needle injection	*Detection* Physical examination, US *Assessors* ND *Region* Abdomen	Presence of cutaneous damage, metabolic control
		Patient 2 Population: Diabetes (NS) Age (years): 30 Gender: F Diabetes duration (years): ND	CSII	*Detection* US *Assessors* ND *Region* ND	

CD cannot determine, *CSII* continuous subcutaneous insulin infusion, *F* female, *HbA1c* glycated haemoglobin, *HPs* health professionals, *LH* lipohypertrophy, *M* male, *MDI* multiple dose injection, *ND* no data, *NS* not specified, *RCT* randomised controlled trial, *T1DM* type 1 diabetes mellitus, *T2DM* type 2 diabetes mellitus, *US* ultrasound

[a] Primary or longest mode of insulin delivery

LH Prevalence, Anatomical Distribution and Tissue Morphology

Nine studies assessed the prevalence of LH lesions. The prevalence of LH ranged from 14.5% to 88%, with a median estimate of 56.6% (Table 2). Two studies considered prevalence in the context of continuous subcutaneous insulin infusion (CSII) delivery and reported respective prevalences of 44% and 76% [21, 26].

Seven studies examined the anatomical distribution of LH sites, with the abdomen, thighs and arms being the most frequently identified (Table 3). Less common areas were the back, buttocks and hips [14, 19–21, 23, 25, 29].

Characterisation of LH Using US

In terms of tissue morphology, the reported characteristics showed mainly increased echogenicity in diffuse areas of the injected subcutaneous tissue, some with clearly defined nodules of different sizes embedded within the area with circumscribed margins [19, 24, 27, 28, 33]. Kapeluto et al. [33] define this further as nodules not having a capsule or vascularity, which differentiates the US signature of LH from haematomas or fluid-filled cysts, which do have capsules. In some cases, the centre or part of these LH nodules could be hypoechogenic possibly representing fluid from oedema or fat necrosis [19, 27, 28]. Perciun and Mihu [28] also showed reduced echogenicity when the sites had been rested for 6 months, suggesting dissipation of the LH, but not in all cases and particularly not in those showing greater fibrosis of the fat tissue (echogenicity), or in those with possible necrosis at baseline scan. The study by Perciun and Mihu [28], which included 10 children (19% of the cohort), reported the presence of LH in cases with insulin exposure of as little as 2–5 months. Thickening of the dermal layer and loss of a clear delineation between the subcutaneous and dermal layer at the injection site was noted in two papers and was identified as a potential inflammatory response to repeated insulin exposure [24, 27].

Four studies attempted to classify LH into types or grades of LH [19, 24, 27, 33]. Perciun [27] included five levels for LH grading: (1) nearly normal, (2) diffuse echogenicity (fibrous tissue) with no well-defined delineation between dermis and subcutis, (3) focal areas within this tissue (nodules within diffuse areas), (4) focal areas with hypoechogenic halos within the nodules, a thickened dermal layer and loss

Table 2 Prevalence of LH depicted by different detection methods

Author (year)	Insulin therapy[a]	LH prevalence (%) based on detection method		
		US	Palpation	Not specified
Blanco et al. (2013) [20]	Pen	64.4		
Conwell et al. (2008) [21]	CSII		44.0	
Davidenko et al. (2014) [22]	NS			84.0
Gentile et al. (2016) [14]	Needle injection			48.8
Nasser et al. (2017) [25]	Needle injection	36.8		
Patrakeeva et al. (2014) [26]	CSII, MDI	76.0		
Perciun (2010) [27]	Needle injection			88.0
Volkova et al. (2013) [29]	Needle injection	86.5	37.0	
Wang et al. (2014) [30]	Needle injection	14.5		

CSII continuous subcutaneous insulin infusion, *LH* lipohypertrophy, *MDI* multiple dose injection, *US* ultrasound

[a] Primary or longest mode of insulin delivery

Table 3 Regional distribution of LH

Author (year)	Insulin therapy[a]	Anatomical distribution of LH			
		Abdomen	Arm	Thigh/gluteus	Other
Bertuzzi et al. (2017) [19]	CSII, MDI	100% (US, Palp.)	25.0% (US), 20.0% (Palp.)	25.0% (US), 20.0% (Palp.)	
Blanco et al. (2013) [20]	Pen	ND[b]			
Conwell et al. (2008) [21]	CSII	SS: 6.1 ± 3.3		SS: 4.9 ± 4.2	
Gentile et al. (2016) [14]	Needle injection	40.0%	35.0%	25.0%	
Kasperska-Czyzyk et al. (2000) [23]	Needle injection	61.8%	17.6%	20.6%	
Nasser et al. (2017) [25]	Needle injection	S/SC: 3.1/ 23.3 mm	S/SC: 2.9/ 12.3 mm	S/SC: 3.2/ 12.3 mm	
Volkova et al. (2013) [29]	Needle injection				Paraumbilical/buttocks: 61.0% Paraumbilical/ buttocks + hips: 15.0% Paraumbilical/ buttocks + shoulders: 11.0%

CSII continuous subcutaneous insulin infusion, *LH* lipohypertrophy, *MDI* multiple dose injection, *ND* no data, *Palp.* palpation, *S/SC* skin/subcutaneous thickness, *SS* severity score, *US* ultrasound

[a] Primary or longest mode of insulin delivery

[b] Authors report that LH sites were most commonly observed in the abdomen

of delineation between the dermis and subcutis layers, (5) nodules with a hypoechogenic necrotic or liquid-filled areas and thickened dermis. More recently, Mulnier et al. [24] further identified a four-level grading scale of LH based on the presence of diffuse areas, nodule size, nodule number and inflammatory changes. Bertuzzi et al. [19] characterised LH on the basis of hyperechogenic regions with prevailing fibrosis, hypoechogenic areas and mixed hypo/hyperechogenicity.

Risk Factors of LH

Five studies considered associations between injecting behaviours and patient characteristics with the presence of LH (Table 4). These findings suggest higher prevalence of LH in relation to the level of site rotation, frequency of injections, needle reuse, needle injection at 90°, injection in the arm and abdomen, and a lower level of general education.

Sensitivity of LH Detection Methods

Four studies compared physical assessment of LH with US detection. The methods used for physical examination varied and included both visualisation and different palpation protocols. Only one of four studies used prespecified criteria to examine areas of LH, including either features of hyperechogenic (fibrosis) or

Table 4 Risk factors of LH

Author (year)	Risk factors of LH	Statistical value
Blanco et al. (2013) [20]	Needle reuse	$P = 0.008$
	Patient-reported injection site rotation	$P = 0.001$
	Nurse-reported injection site rotation	$P = 0.0001$
	Nurse observed + patient claimed injection site rotation	$P = 0.0001$
Conwell et al. (2008) [21]	BMI z-score 0.60 ± 0.76	$r = -0.3$, $P = 0.04$
	Needle insertion angle at 90°	$P = 0.03$
Davidenko et al. (2014) [22]	Insulin use	AUC > 0.5–86.0%
Nasser et al. (2017) [25]	Level of education	$P = 0.02$
	Number of injections	$P = 0.02$
	Injection site: Arm	$P = 0.04$
	Injection site: Abdomen	$P = 0.001$
Patrakeeva et al. (2014) [26]	Glucose variability	$r = 0.8$
	Incorrect insulin injection technique/infusion set changing	$r = 0.7$

AUC area under the curve, *BMI* body mass index, *LH* lipohypertrophy

hypoechogenic (oedema/fluid) lesions [19]; whilst the remaining studies were preliminary and did not mention their protocol in detail. The methods used for palpation detection of LH varied considerably and included a palpable increase in subcutaneous fat [23], an extended version of the FIT guidelines involving a pinch technique to compare the thickness of harder skin to adjacent areas of skin [14], and another examined the shape irregularity of LH areas, as well as assessing texture consistency and area of LH extension [19]. As a result of inconsistences in protocol design in detection tools across the studies, the estimations of LH prevalence varied between studies. One study reported that palpation detected 64% more LH regions compared to US [23]. Conversely, Volkova et al. [29] reported that US scanning detected 56% more LH lesions than with palpation alone. One study included a comparison between routine palpation and palpation by nurses trained to identify LH lesions through a detailed stringent tactile palpation technique with US [14]. They found that while standard palpation methods detected 66% of the US identified lesions, the additionally trained nurses detected 96% of lesions. The fourth study identified overall equivalence in the detection of LH between US and palpation [19]. However, they found that US was able to detect more sites in the arm and gluteus regions than palpation [19]. This study also reported high precision in the US-assessed LH region in relation to the size and distribution of the affected areas. The area of lipohypertrophic extensions was noted to be 5 cm^2 bigger with US (~ 35 ± 10 cm^2) than that recorded by palpation and inspection (~ 30 ± 15 cm^2), suggesting increased sensitivity [19].

DISCUSSION

This is the first systematic review of studies of US assessed LH. The review has identified some potentially important new insights into the distribution and characteristics of LH based on US examination. In terms of regional distribution, LH was predominantly localised in the abdomen, a finding consistent with palpation and possibly associated with patient preference for the abdomen as an injection site. The studies

using US to characterise LH provide a much more detailed perspective on the size and depth of tissue changes observed following repeated insulin exposure and available tissue, suggesting that US could be used to optimise needle length selection.

Findings relating to the comparison between palpation and US for LH sites illustrate a discrepancy in the detection of these sites with one study reporting that palpation produces 65% more false positive results [23], while another demonstrates that US detects 50% more LH cases [29]. It is conceivable that deficits in LH detection reported for palpation are related to inadequate technique. While more rigorous guidelines have been established, most notably the FIT guideline in 2010 [34] which are observed in many countries [35], it was noted that this approach was inferior to an even more rigorous method of palpation with a 60% higher rate of detection [14]. This result may suggest that a rigorous palpation method may be as sensitive as US and if adopted clinically would limit the need to use US in clinical care. However, US has additional advantages over palpation as it can better assign the nature and severity of LH in much more detail compared to palpation, enabling greater granularity in grading the LH (size, distribution and elasticity) [19, 24, 28, 33] and thus giving clinicians the opportunity to give more detailed advice to patients. Through visualisation of the LH tissue, US images may encourage injection behaviour changes by revealing areas of disrupted tissue, inflammation and depth of subcutaneous tissue. This could help inform choice of needle length and reinforce the importance of site rotation and single needle use. Future detailed clinical studies of the impact of the differing types and grades of LH on the insulin action curve and glucose variability could be highly valuable and informative clinically. From a behaviour change perspective, the patient visualising the injection sites on US may act as a strong cue to move sites as well as choose appropriate needle lengths and new injection areas with optimal insulin absorption and action. Overall, the use of US could encourage and reinforce injection techniques, which could support and improve effective self-management of diabetes and help minimise the risk of long-term complications. Finally, the incorporation of US into routine clinical care in the context of the annual review may ensure that LH is screened more objectively, precisely and rigorously.

Strengths and Limitations

As with all reviews, the level of insight gained is predicated by the quality of the evidence and methods of the source studies. There are some shortcomings in the quality of the included studies. A particular weakness was in relation to the studies comparing palpation and US in LH assessment, where there was a high level of heterogeneity in the detection methods observed which may confound the results. Moreover, the lack of detail on the clinician's training level to detect LH may affect the validity of the findings. However, we were able to extrapolate from these studies some important insights into LH by integrating the study findings. Nonetheless, this review has highlighted some important details on the nature of LH and the potential of US in its detection and management, paving the way for further inquiry into this important and neglected aspect of diabetes care.

Implications for Future Research

Currently, there are limited studies that present data on LH detection accuracy from palpation and US assessment. Conducting RCTs that include nurse training to implement the extended FIT guidelines for palpation techniques and interpretation of echogenic US scans of LH sites would provide more credible comparative results on the reliability and sensitivity of each detection method. In addition, economic evaluation of the diagnostic sensitivity of the different methods would ascertain the cost-effectiveness of each. Lastly, information on staff and patients' experiences of LH detection and site management in the avoidance of LH could help us better understand patient injection preferences as well as design a site management method that would help avoid the

build-up of LH and help prevent it in those new to treatment with insulin in the future.

CONCLUSION

The current literature emphasises the knowledge gap in the sensitivity, reliability and accuracy of the different tools used to detect the presence of LH. The existing research highlights the need for further and more robust clinical research to evaluate the feasibility and cost-effectiveness of US in comparison to palpation. Nonetheless, the overall evidence implicates that US scans may provide more accurate results than palpation alone and can report more explicit detailed information that could prompt effective education on injection and site management practices that could potentially improve self-management and diabetes outcomes. The dynamic shift to e-health aimed at improving efficiency and accuracy suggests that introduction of US scanning for LH assessments in routine care is foreseeable.

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Data Availability. Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

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15.2 Published abstracts

The data from this project have been presented at the following conferences as oral and poster presentation

Year	Activity	Title	Organisation
2019	Poster presentation	Characterisation of lipohypertrophy: A case study using Ultrasound to describe lipohypertrophy in different insulin injection sites	FEND*
2017	Oral presentation	Subcutaneous tissue changes and dermal inflammation at insulin injections sites: a feasibility study using ultrasound to describe characterise and grade lipohypertrophy	EASD
2017	Poster presentation	Clinical impact of lipohypertrophy on glycaemic control: A systematic review and meta-analysis	ADA
*FEND, Foundation of European Nurses in Diabetes			

15.3 Publications to prepare

1. Ultrasound characterisation of LH in T1DM
2. Standard operator procedure for using Ultrasound to assess LH
3. Clinical impact of LH on glycaemic control: A systematic review and meta-analysis
4. Impact of LH on glucose variability in T1DM
5. Case report- the impact of LH on glucose variability

Characterisation of lipohypertrophy: A case study using Ultrasound to describe lipohypertrophy in different insulin injection sites

Characterisation of Lipohypertrophy: A Case Study Using Ultrasound to Describe Lipohypertrophy in Different Insulin Injection Sites

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Background

Lipohypertrophy (LH) at injection sites is one of the most frequent complications of insulin therapy. Recent studies have demonstrated that ultrasound imaging provides a detailed assessment of insulin-induced LH. This case study presents the ultrasound examination of injection sites in a 70-year-old woman who has managed her type 1 diabetes for 41 years using many different needle lengths and insulins.

Aim

This case study describes the presentation of LH tissue in different injections sites exposed to various needle lengths and insulins over many years.

Methods

This case was a participant in a larger study aiming to characterise LH using ultrasound imaging. A SonoSite X-Porte scanner with a high-frequency linear probe (6–13 MHz) was used under the supervision of an expert ultrasonographer. All past and current anatomical sites used for insulin injections were scanned.

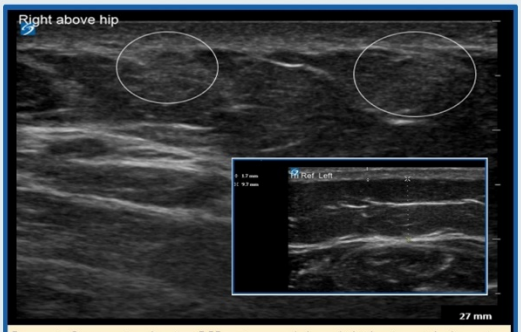


Image 1: Current site showing LH at 6mm and dermal thickening with disruption of the dermal layers. The inset shows triceps tissue not used as an injection site exhibiting normal dermal layers and thickness in the same participant.



Image 2:Upper abdominal site showing LH at around 5mm, which is dissipating in a site not used for two years



Image 3: Lower abdominal site not injected into for eight years, LH continue to be present despite resting for eight years; an 8mm needle was used at the time.



Image 4: An injection site not injected into for very many years and exposed to human insulin with a 12.7mm needle at the time.

Results

LH was seen at all injection sites and matched needle length. The dermal layer was also thicker than in non injection sites and normal delineation of skin layers disrupted; this is suggestive of inflammation (see insect section of triceps tissue to the same scale as the injection site scan; Image 1). The current injection site was the flank area on both sides and showed LH developing at around 6mm plus or minus 2mm (Image 1). In the upper abdomen, which had not been injected into for two years; LH was evident at around 5mm depth despite the site having been rested for two years 9Image 2). The lower abdomen had not been injected into for many years, but continues to show increased echogenicity at around 7mm and thickened dermal layer despite a long recovery period (Image 3). In the right thigh, which had not been injected into for over ten years, there was a large area of LH at around 12mm.

Conclusions

This case illustrates how the depth of LH can be related to needle length. This finding may therefore offer people who inject insulin another plane of site rotation by altering needle length. It may also partly explain improved glycaemic control when new needle lengths are used; the insulin is being delivered into new tissue above or below developed LH. This could be a significant clinical benefit as desensitised 'favourite' areas could continue to be used without compromise to insulin delivery or absorption. The case also highlights that when LH has developed, tissue changes remain evident for longer than has been previously predicted and is an area in need for further clinical investigation.

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Subcutaneous tissue changes and dermal inflammation at insulin injections sites: a feasibility study using ultrasound to describe characterise and grade lipohypertrophy

Background and aims: Subcutaneous (s/c) tissue changes in patients with type 1 diabetes (T1DM) are common and can affect glycaemic control. There are limited data on the utility of ultrasound to describe changes in s/c tissue with insulin use. This study aimed to assess the feasibility of using ultrasound to describe and characterise tissue changes to inform a future lipohypertrophy (LH) grading study.

Materials and methods: Patients with T1DM thought by diabetes clinicians to have LH or with clinical LH on examination were included. A SonoSite X-Porte ultrasound machine was used with a high frequency linear probe (6-13 MHz). Insulin injections sites and 'normal' non-injected adjacent tissues were scanned by a single operator.

Results: We scanned 15 patients with T1DM to establish a standard operating procedure (SOP). Then 11 additional patients with T1DM were scanned in detail using that SOP; mean age 41 years (range 24-59), mean duration of diabetes 22.7 years (range 4 - 49), and mean HbA_{1c} 7.3% (range 5.8 - 9.6%). Ultrasound images exhibited changes in the s/c tissue at a depth corresponding to approximate needle length (± 2-3mm). This tissue appeared as diffuse reflective areas of differing density in all of the 26 patients. More dense areas formed visible lumps: of the 11 patients studied in detail; 4 (33%) had large palpable lumps, 5 (45%) had small or medium nodules some of which were palpable, and 2 (18%) had large regions of diffuse changed tissue across the injection site. Compiling images from the 26 patients, a potential grading system has been proposed: Grade 0 (no evident nodules or diffuse areas of specific density), Grade 1 (small nodule <10mm), Grade 2 (medium nodule 10-20mm), Grade 3 (large nodule >20mm), Grade 4 (general diffuse area of specific density). Grade 1 to 3 can be single or multiple. Nine (82%) of the 11 participants also showed inflammatory tissue in the dermal layers of injections sites, <3mm thickness in 5 participants, and >3mm thickness in the other 4. Similar inflammation was also seen in a proportion of the first 15 patients, so this may be a common occurrence in this group. The inflammatory tissue has been provisionally graded as: 0 = normal, 1 for <3mm and 2 for >3mm. Normal tissue in all patients showed no increased reflectivity and normal dermal and s/c layers above muscle. See figure 1.

Conclusion: This feasibility study has been the first to report and measure dermal inflammation in people injecting insulin. It demonstrates the potential of ultrasound in characterising s/c tissue changes in people who inject insulin. The observations have informed preliminary grading systems, which will be validated in further studies to assess the clinical implication of these tissue changes in relation to glucose variability and insulin action.

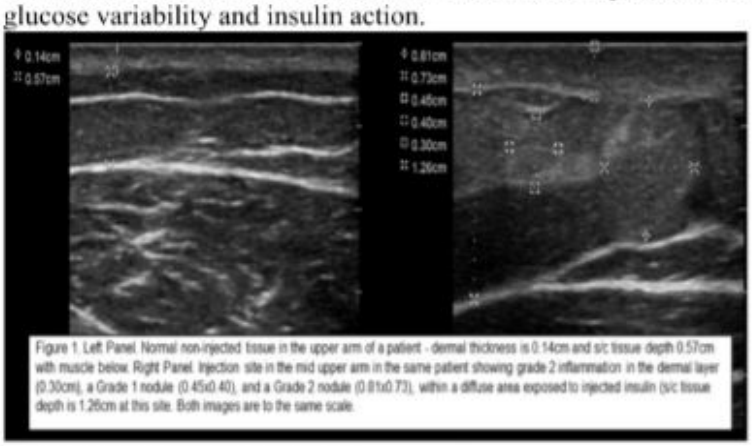


Figure 1. Left Panel: Normal non-injected tissue in the upper arm of a patient - dermal thickness is 0.14cm and s/c tissue depth 0.57cm with muscle below. Right Panel: Injection site in the mid upper arm in the same patient showing grade 2 inflammation in the dermal layer (0.30cm), a Grade 1 nodule (0.45x0.40), and a Grade 2 nodule (0.81x0.73), within a diffuse area exposed to injected insulin (s/c tissue depth is 1.26cm at this site. Both images are to the same scale.

Disclosure: **H. Mulnier:** None.