Insulin-Related Lipohypertrophy: Ultrasound Characteristics, Risk factors, and Impact of Glucose Fluctuations

Yiyang Lin  
Fujian Medical University

Wei Wang  
Fujian Medical University

Junfeng Hong  
Fujian Medical University

Hua Zeng (✉ 594987034@qq.com)  
Fujian Medical University

Research Article

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Abstract

Background

Lipohypertrophy (LHT) has been suggested as an outcome of adipogenic effects of insulin injection-related tissue trauma. It is common clinically, but the current understanding of LHT by medical staff and diabetic patients is still insufficient, and it has not attracted attention as a research topic.

Objective

The aim of this study was to investigate the ultrasound characterization of LHT, to identify factors associated with the development of LHT by assessing the prevalence of LHT compared to both clinical palpation and ultrasound detection methods, and to further evaluate the possible impact of LHT on patients' blood glucose fluctuations.

Method

A cross-sectional study was established, in which 120 patients with type 2 diabetes were selected. General information was registered in the form of a questionnaire, and the patients were evaluated for LHT by ultrasonography and clinical palpation of the abdomen. Patients were instructed to inject equal amounts of insulin in LHT and normal adipose tissue (NAT) on a non-consecutive 2 d in a selected week, and the possible effect of LHT on patients' blood glucose fluctuations was assessed using a continuous glucose monitoring system.

Results

LHT has special ultrasonic signs. We found a high rate of missed clinical palpation of LHT compared with ultrasonography (P < 0.05). The duration of insulin treatment, whether to rotate the injection site, frequency of needle use, and number of insulin injections per day were the main factors influencing the development of LHT (P < 0.05). Compared to NAT, LHT resulted in elevated largest amplitude of glycemic excursion, mean blood glucose, standard deviation of blood glucose, and postprandial glucose excursion, and large fluctuations in blood glucose (P < 0.05).

Conclusion

Ultrasonography can detect more LHT than can clinical palpation. The development of LHT is related to many factors and can lead to significant blood glucose fluctuations; thus, LHT should be given sufficient attention.

1. Introduction

Insulin, a commonly used hypoglycemic drug, is itself a growth factor with growth-promoting effects, inducing protein and fat differentiation and proliferation. Lipohypertrophy (LHT) can develop when
insulin is repeatedly injected at the same site. LHT mainly presents as rubbery or scar-like lesions with thickened subcutaneous tissue at the injection site [1]. At present, LHT is mostly evaluated by palpation and visualization in clinical treatment, which is a simple, convenient method that is easy to promote. However, this method lacks specificity and can only be detected when the lesion is obvious. It cannot accurately detect early lesions and is not conducive to accurate quantitative assessment, which often leads to missed diagnoses [2]. Ultrasound is a non-invasive test that has a higher specificity for LHT than does clinical palpation [3]. Recent meta-analyses have reported a mean prevalence of 38% for LHT [4], but the incidence of LHT appears to vary widely across studies, ranging from 1.9% [5] to 73.4% [6]. The wide variation in the prevalence of LHT may be explained by the different diagnostic methods used in different studies (some using palpation only, some using ultrasound). There are no guidelines to date that suggest definitive ultrasound diagnostic criteria for LHT, and more studies are needed to verify whether clinical palpation is sufficient to detect LHT or whether color ultrasound would improve the accuracy of LHT assessment.

A univariate analysis study [7] showed that the formation of LHT may be related to the poor manner of insulin injection. However, further research is needed to confirm this. On the other hand, when insulin is injected at LHT areas in diabetic patients, insulin absorption slows and peak levels decrease, resulting in poor glycemic control and an increased incidence of hypoglycemia [8, 9]. A multicenter clinical investigation study in China showed that [10] patients with LHT had a significantly higher HbA1c and greater insulin dosage than patients without LHT. It was further found that LHT can lead to additional insulin consumption at a cost of $2 billion, significantly increasing the economic burden. The relationship between glycemic control and LHT is currently controversial and needs to be validated by further evidence.

This study aimed to explore the ultrasound representation of LHT via a cross-sectional study to identify factors associated with the development of LHT by assessing the prevalence of LHT as detected by both clinical palpation and ultrasound detection methods. The possible impact of LHT on patients’ blood glucose fluctuations was ultimately assessed. The results of this study will help medical staff and patients to properly understand the severity of LHT, thus educating patients to maintain good injection habits during insulin use and minimizing the development of LHT.

2. Research Design And Methods

2.1 Study design

The participants with diabetes on treatment with insulin were enrolled at 900 Hospital of the Joint Logistics Team in China between April 2019 to December 2019. The trial was approved by the local ethics committee (2019–007) and performed under the principles of the revised Declaration of Helsinki. Written informed consent was obtained from the participants before the study. The trial was registered in ClinicalTrials.gov (registration NO. ChiCTR2100048886).

2.2 Eligibility criteria
Inclusion criteria included those aged $\geq 18$ y at screening; male or female; who met the 1999 World Health Organization (WHO) diagnostic criteria for type 2 diabetes; and/or who had been treated with subcutaneous abdominal insulin at least once a day for more than 1 y. Exclusion criteria included those with type 1 diabetes mellitus; a combination of acute infection, ketoacidosis and other serious complications; known presence of abdominal masses, such as lipomas; a combination of inflammatory abdominal skin lesions, such as psoriasis and eczema; known allergy to medical ultrasound couplants; inability to complete general information collection or clinical physical examination; history of abdominal surgical treatment with significant scars; frequent or recent severe hypoglycemia; women during pregnancy; and/or a combination of mental illness and cognitive dysfunction and inability to care for themselves.

2.3 Data Collection

Demographic information, assessment of glycemic control, and insulin injection technique were evaluated through a questionnaire administered by a physician or research nurse. The questionnaire including age, sex, body mass index (BMI), HbA1c, duration of insulin treatment, total daily insulin dose, injection habits (needle length, frequency of needle reuse, and rotation), and unexplained hypoglycemic events.

2.4 Assessment of LHT

Clinical palpation assessment of LHT: A senior nurse with a long history of diabetes education was trained to assess each patient for abdominal LHT [11]. The steps were as follows: 1) the patient was placed in a lying position and instructed to fully relax their abdominal muscles, bend their knees, relax their quadriceps muscles, cross their arms over their chest, and relax their arm muscles, while the examiner took a sitting position for the examination; 2) the examiner used a light source to fully illuminate the area under examination, adjusting the angle so that any subtle elevations or depressions on the skin surface could be seen and using a marker to mark the center of the area where there were bumps or changes in skin color or hair distribution; 3) when palpation revealed initially soft, elastic, subcutaneous fatty tissue that changed to tough, rubbery, or inelastic tissue, the exact location of the lesion was marked on the patient's skin using a marker that was safe for the skin.

Ultrasonography to assess LHT: Ultrasound examination of abdominal skin tissues by an ultrasonographer with a fixed senior title who had been practicing superficial tissue ultrasound for a significant period of time, applying a multi-frequency linear probe (L8-18I, 8–18 MHz). The steps were as follows: 1) the patient was examined in the same position as for clinical palpation; and 2) ultrasound signs at the examination site and the thickness of subcutaneous adipose tissue in the lesion area and of surrounding normal subcutaneous adipose tissue were recorded, and the exact location of the lesion on the patient's skin was marked using a safe skin marker. LHT was considered to be present when the specific criteria for the diagnosis of LHT ultrasound were met [12, 13]: the presence of echogenically heterogeneous nodules in the hyperplastic area with differences in echotexture from the surrounding normal tissue and interstitial edema around the hyperplastic nodules; continuous thick fascial tissue or
interrupted and distorted thin connective tissue around the hyperplastic nodules; and few or no neovascularized echogenicity in the hyperplastic nodules. Common subcutaneous masses, such as subcutaneous hemorrhage and lipoma, were excluded.

### 2.5 Assessment of blood glucose fluctuations

Patients were instructed to inject equal amounts of insulin subcutaneously into LHT and normal adipose tissue (NAT) on a non-consecutive 2 d in a selected week. To avoid the occurrence of hypoglycemia, the insulin injection dose was reduced by 10% compared with the original dose. In order to avoid acute hyperglycemia caused by the reduction of the insulin dose, the participants’ fasting blood glucose should have been controlled at 3.9–7.0 mmol/L on the day before, 2 h postprandial blood glucose should have been less than 10 mmol/L, and the amount of activity and food intake on the 2 d of blood glucose measurement should have been kept approximately the same. CGMS (Medtronic, Dublin, Ireland) was used to measure blood glucose values at morning fasting, 2 h after breakfast, before lunch, 2 h after lunch, before dinner, 2 h after dinner, and at 10:00 p.m. From here, the patients’ blood glucose fluctuations were evaluated. The specific calculation of the intra-day blood glucose fluctuation index was as follows: largest amplitude of glycemic excursion (LAGE): the difference between the maximum and minimum values of blood glucose in 1 d; mean blood glucose (MBG): the mean of blood glucose values in 1 d; the standard deviation of blood glucose (SDBG): the standard deviation of blood glucose values in 1 d; and postprandial glucose excursion (PPBG): the mean of the difference between blood glucose 2 h after three meals and the corresponding pre-meal blood glucose.

### 2.6 Statistical analysis

Data were analyzed using the SPSS 23.0 software (IBM Corp., Armonk, NY, USA). Descriptive data were expressed as means ± standard deviation (SD). The Cohen k statistic was used to determine an agreement between clinical and ultrasound assessment. Consistency in detection between the two methods of evaluation was analyzed using the McNemar test. The differences between both groups were analyzed using a paired Student's t-test for normal continuous variables and a non-parametric Wilcoxon test for non-normal data. Pearson was selected for correlation analysis. Pearson correlation analysis and paired Student's t-test associated with the size of LHT (P < 0.05) were incorporated into a multivariate linear regression model for analysis. Values of P < 0.05 were considered statistically significant.

### 3. Results

#### 3.1 Clinical characteristics

A total of 120 diabetic patients who injected insulin were included in this study, including 70 males and 50 females, with a mean age of 59.21 ± 11.44 years, median years of insulin injection of 6.56 (4.26) years, mean total daily insulin injection of 36.08 ± 19.06 units, and mean HbA1c of 9.45 ± 1.85%. Additional information is shown in Table 1.
Table 1  
Demographic and clinical variables of the study population

| Characteristics                                      | N (n = 120) | (%)  | Mean (SD)     |
|-----------------------------------------------------|-------------|------|---------------|
| **Continuous characteristics (mean ± SD)**          |             |      |               |
| Gender                                              |             |      |               |
| Male                                                | 50          | 41.7 |               |
| Female                                              | 70          | 58.3 |               |
| Age (years)                                         |             |      |               |
| ≤ 60                                                | 63          | 52.5 | 59.21 (14.44) |
| > 60                                                | 57          | 47.5 |               |
| BMI (kg/m\(^2\))                                    |             |      |               |
| ≤ 25                                                | 43          | 35.8 | 24.93 (3.18)  |
| > 25                                                | 77          | 64.2 |               |
| HbA1c (%)                                           |             |      |               |
| ≤ 7                                                 | 114         | 95   | 9.45 (1.85)   |
| > 7                                                 | 6           | 5    |               |
| Duration of insulin treatment (years)                |             |      |               |
| ≤ 10                                                | 29          | 24.2 | 6.56 (4.26)   |
| > 10                                                | 91          | 75.8 |               |
| Total insulin daily dose (unit)                      |             |      |               |
| ≤ 50                                                | 30          | 25   | 36.08 (19.06) |
| > 50                                                | 90          | 75   |               |
| Characteristics                          | N (n = 120) | (%)  | Mean (SD)    |
|-----------------------------------------|-------------|------|--------------|
| Injection habits                        | 46          | 38.3 | 5.68 (1.53)  |
| Needle length (mm)                      | 47          | 39.2 | 4.88 (2.73)  |
| 4                                       | 27          | 22.5 |              |
| 6                                       | 52          | 43.3 |              |
| 8                                       | 57          | 47.5 |              |
| Frequency of needle reuse               | 11          | 9.2  |              |
| <4                                      | 66          | 55   |              |
| 4–8                                     | 54          | 45   |              |
| >8                                      |             |      |              |
| Rotation                                |             |      |              |
| Yes                                     |             |      |              |
| No                                      |             |      |              |
| Unexplained hypoglycemic events         | 63          | 52.5 |              |
| Yes                                     | 57          | 47.5 |              |
| No                                      |             |      |              |

### 3.2 Characteristics of ultrasonic detection of LHT

As shown in Fig. 1, the difference between NAT and LHT under ultrasonography. In normal subcutaneous tissue, the epidermis and dermis have ultrasound features separated from the muscle layer by the subcutaneous layer, and the adipose tissue (hypoechoic) was separated by thin connective tissue and thick myofascia. The distance between the two was the thickness of the subcutaneous fat. The ultrasound features of fatty hypertrophy (Fig. 1B-D) were located in the subcutaneous tissue between the epidermal and muscular layers, and the lesion was a nodular hyperechoic or interstitial edematous hypoechoic area with echogenicity different from the surrounding normal tissue.

### 3.3 Compared with ultrasound examination, the LHT missed rate of clinical examination

Clinical examination and ultrasonography were performed to evaluate LHT in 120 diabetic patients (as shown in Table 2). LHT was detected by clinical examination in 56 (46.6%) patients, LH was detected by ultrasound in 83 (69.1%) patients, and LHT was detected by ultrasound only in 27 (22.5%) patients. Compared to ultrasonography, the rate of LHT missed by clinical examination alone was 32.6%.
Table 2
Compared with US examination, the LHT missed rate of clinical examination

| Variables                      | Palpation (n, %) | US (69.1%) | Missed rate % (Palpation vs US) |
|--------------------------------|------------------|------------|---------------------------------|
| Frequency of LHT               | 56 (46.6%)       | 83         | 32.6%                           |
| Total number of LHT            | 67               | 144        | 53.5%                           |
| The vertical length of LHT (mm)|                  |            |                                 |
| <5                             | 55               | 83         | 32.9%                           |
| 5–10                           | 9                | 10         | 10.0%                           |
| >10                            | 1                | 21         | 95.2%                           |
| The horizontal width of LHT (mm)|                  |            |                                 |
| <5                             | 45               | 2          | 4.2%                            |
| 5–10                           | 2                | 48         | 95.9%                           |
| >10                            | 10               | 39         | 74.4%                           |
| The area of LHT (mm)           |                  |            |                                 |
| <30 mm^2                       | 2                | 2          | 4.2%                            |
| 30–60 mm^2                     | 2                | 48         | 95.9%                           |
| >60 mm^2                       | 10               | 39         | 74.4%                           |

Compared to ultrasonography, the rate of LHT missed by clinical examination alone was 53.5%, with 144 and 67 LHT detected by ultrasonography and clinical palpation, respectively. When the vertical length of LHT was < 5 mm, 5–10 mm and > 10 mm, respectively, the leakage rate of LHT assessed by clinical examination was 94.2%, 32.9% and 10.0%, respectively. When the LHT area was < 30 mm^2, 30–60 mm^2 and > 60 mm^2, the leakage rate was 95.9%, 74.4% and 3.5%, respectively, when LHT was evaluated by clinical examination alone. LHT vertical length, LHT horizontal width and LHT area were strongly correlated with the rate of missed clinical examinations (P < 0.05) (see Table 2).

3.4 Risk factors associated with the influence of abdominal LHT

The incidence of LHT in diabetic patients who had 1, 2 and 4 subcutaneous insulin injections daily was 29.0%, 61.5% and 92.1%, respectively; the incidence of LHT in patients with < 5, 5–10 and > 10 years of duration of insulin treatment was 48.1%, 78.4% and 96.6%, respectively; the incidence of LHT in patients with < 4, 4–8 and > 8 times of needle reuse were 53.1%, 78.9% and 90.9%, respectively, and the incidence of LHT at rotational injection sites and non-rotational injection sites were 60.6% and 79.6%, respectively, with statistically significant differences (P < 0.05) (see Table 3). Gender, age, waist circumference, BMI,
needle length and total daily insulin dosage had no significant effect on LHT (P > 0.05). By bringing the above statistically significant influencing factors into the binary logistic regression model analysis, the results showed that total daily injections and duration of insulin treatment were positively associated factors affecting LHT formation [OR (95% CI) was 2.994 (1.905 ~ 4.704) and 1.385 (1.165 ~ 1.647), respectively, with p-values of 0.000 and 0.000], while rotational injection site was a negative correlate of LHT formation [OR (95% CI) 0.323 (0.103 to 0.715), P = 0.048]. (See Table 4).
| Variables                      | Lipohypertrophy (N = 83) | No lipohypertrophy (N = 37) | $\chi^2$-value | $P$-value |
|-------------------------------|--------------------------|----------------------------|----------------|-----------|
| Gender                        |                          |                            |                |           |
| Male                          | 48 (68.6%)               | 22 (31.4%)                 | 0.028          | 0.867     |
| Female                        | 35 (70.0%)               | 15 (30.0%)                 |                |           |
| Age (years)                   |                          |                            |                |           |
| < 50                          | 20 (74.1%)               | 7 (25.9%)                  | 4.484          | 0.106     |
| 50 ~ 60                       | 20 (55.6%)               | 16 (44.4%)                 |                |           |
| > 60                          | 43 (75.4%)               | 14 (24.6%)                 |                |           |
| waist circumference (cm)      |                          |                            |                |           |
| < 90                          | 30 (60.0%)               | 20 (40.0%)                 | 3.377          | 0.066     |
| ≥ 90                          | 53 (75.7%)               | 17 (24.3%)                 |                |           |
| BMI (kg/m$^2$)                |                          |                            |                |           |
| < 24                          | 35 (63.6%)               | 20 (36.4%)                 | 1.487          | 0.475     |
| 24 ~ 28                       | 35 (74.5%)               | 12 (25.5%)                 |                |           |
| > 28                          | 13 (72.2%)               | 5 (27.8%)                  |                |           |
| Total daily injections        |                          |                            |                |           |
| 1                             | 9 (29.0%)                | 22 (71.0%)                 | 39.611         | 0.000     |
| 2                             | 16 (61.5%)               | 10 (38.5%)                 |                |           |
| 4                             | 58 (92.1%)               | 5 (7.9%)                   |                |           |
| Needle length (mm)            |                          |                            |                |           |
| 4                             | 30 (65.2%)               | 16 (34.8%)                 | 2.483          | 0.289     |
| 6                             | 31 (83.8%)               | 16 (16.2%)                 |                |           |
| 8                             | 22 (81.5%)               | 5 (18.5%)                  |                |           |
| Duration of insulin treatment (years) | 26 (48.1%)    | 28 (51.9%)                 | 22.856         | 0.000     |
| < 5                           | 29 (78.4%)               | 8 (21.6%)                  |                |           |
| 5 ~ 10                        | 28 (96.6%)               | 1 (3.4%)                   |                |           |
| > 10                          |                          |                            |                |           |
Variables | Lipohypertrophy (N = 83) | No lipohypertrophy (N = 37) | $\chi^2$-value | $P$-value |
---|---|---|---|---|
Frequency of needle reuse | | | | |
< 4 | 26 (53.1%) | 23 (46.9%) | 10.762 | 0.005 |
4 ~ 8 | 47 (78.3%) | 13 (21.7%) | | |
> 8 | 10 (90.9%) | 1 (9.1%) | | |
Rotation | | | | |
Yes | 40 (60.6%) | 23 (39.4%) | 5.040 | 0.025 |
No | 43 (79.6%) | 11 (20.4%) | | |
Total insulin daily dose (unit) | | | | |
< 20 | 20 (64.5%) | 11 (35.5%) | 5.395 | 0.370 |
20 ~ 29 | 9 (52.9%) | 8 (47.1%) | | |
30 ~ 39 | 15 (83.3%) | 3 (16.7%) | | |
40 ~ 49 | 19 (79.1%) | 5 (20.9%) | | |
50 ~ 59 | 7 (63.6%) | 4 (36.4%) | | |
≥ 60 | 13 (68.4%) | 6 (31.6%) | | |

Table 4
Correlates of LHT: results of multivariate logistic regression

| Variables | Odds Ratio | 95% CI | $P$-value |
---|---|---|---|
Rotate injection site | 0.323 | 0.103–0.715 | 0.048 |
Total daily injections | 2.994 | 1.905–4.704 | 0.000 |
Duration of insulin treatment | 1.385 | 1.165–1.647 | 0.000 |
Frequency of needle reuse | 1.051 | 0.817–1.350 | 0.700 |

3.5 Relationship between glucose fluctuations and LHT

After 83 LHT patients were injected with equal amounts of insulin at LHT and NAT on two non-consecutive days of the week, LAGE was $5.70 \pm 1.74$ mmol/L and $4.21 \pm 1.29$ mmol/L, respectively, MBG was $10.13 \pm 0.92$ mmol/L and $9.43 \pm 1.16$ mmol/L, respectively, SDBG was $1.96 \pm 0.58$ mmol/L and $1.39 \pm 0.39$ mmol/L, respectively, and PPBG was $3.44 \pm 1.24$ mmol/L and $1.99 \pm 0.64$ mmol/L, respectively, which were significantly higher in the LHT group than in the NAT group, and the differences were statistically significant ($P<0.05$) (see Fig. 2).
4. Discussion

The results of this study showed that there were specific ultrasound signs of LHT, and that ultrasonography could detect more LHT than can clinical palpation and could accurately measure the size and area of LHT. The years of insulin injection, whether to rotate the injection site, the frequency of needle use, and the number of daily insulin injections were the main factors affecting the development of LHT. LHT may influence the control of patients' blood glucose, leading to increased fluctuations in blood glucose.

No other studies to date have proposed specific criteria for detecting LHT with head-to-head comparison and validation of clinical palpation and ultrasound. This greatly limits the use of ultrasonography in LHT. Histopathological examination showed that LHT was formed by adipocytes, which abnormally enlarged to two to three times the size of normal adipocytes, and fibroblasts, and could invade the adjacent reticular fibrous membrane and surrounding connective tissue, though often without neovascularization [14]. In this study, the following LHT ultrasound characteristics were innovatively summarized by combining previous studies in the literature [12, 13]: 1) the hyperplastic area is characterized by echogenic nodules (hyperechoic or isoechoic) with differences in echotexture from the surrounding normal tissue and interstitial edema (hypoechoic) around the hyperplastic nodules; 2) there is continuous thick fascial tissue or interrupted and distorted thin connective tissue around the hyperplastic nodules, though this may not be present in markedly obese individuals; 3) there is little or no neovascularized echogenicity within the hyperplastic nodules; and 4) the edges of the area are clear and there is no envelopment. The ultrasound features summarized in this study will provide a reference for the ultrasound diagnostic criteria of LHT.

In clinical practice, LHT is most commonly assessed by palpation. However, this method is less reliable and is associated with a high level of interclinical variability. Nurses with rigorous training in palpation techniques were able to show a 97% case detection rate, while general nurses demonstrated 34% of missed diagnoses [15]. The high rate of missed clinical palpation examinations for LHT was also suggested in the results of this study, which showed a 32.6% underdiagnosis rate of LHT assessed by physical examination alone compared to that of ultrasonography. Notably, in this study, a total of 144 LHT were detected by ultrasound and only 67 by physical examination, resulting in a 53.5% underdiagnosis rate by physical examination alone compared to ultrasound for LHT. In addition, this study found that when the area of LHT was less than 30 mm², the leakage rate of physical examination was as high as 95.9%. In addition, as the area of hyperplasia decreased, the leakage rate of physical examination increased. Therefore, when LHT lesions are small, physical examination alone often results in a greater degree of underdiagnosis. To the best of our knowledge, this study is the first to explore the leakage rate of ultrasound and clinical palpation in terms of the area size of LHT. In addition to the precise diagnostic aspects, ultrasound better detects the nature and severity of LHT compared to palpation, allowing greater granularity of LHT (size, distribution, and elasticity), thus giving clinicians the opportunity to give more detailed advice to patients [3, 16]. By visualizing LHT tissue, ultrasound images can encourage changes in injection behavior by revealing areas of tissue disruption, inflammation, and
depth of subcutaneous tissue [17]. This has important implications in terms of better implementation of patient education and the subsequent reduction of the incidence of LHT.

Studies related to LHT due to faulty insulin injection techniques have been described in the literature for decades [4]. Unfortunately, most patients do not understand the severity of LHT and prefer to inject insulin in the area associated with LHT instead of the normal injection site because of the reduced pain. Therefore, elucidation of the factors influencing the occurrence of LHT is essential to guide patients on the correct method of insulin injection. Many authors have concluded that LHT is always associated with the following factors: sex, BMI, injection device, whether or not to rotate, injection area, needle length, insulin regimen, and daily total dose of insulin [7]. Among them, correct injection site rotation is the most studied and emphasized method for preventing LHT [18]. However, a number of studies also concluded that the development of LHT is independent of factors such as sex, BMI, and whether needles are used repeatedly [19, 20]. In this study, we measured the subcutaneous fatty hyperplasia tissue using ultrasound and analyzed the main factors affecting the area. The results showed that in addition to the number of years of insulin injection, whether to rotate the injection site and the frequency of needle reuse, the length of the needle was also a factor affecting the size of the hyperplastic tissue, which we speculated might be related to the increase in the damaged area of the subcutaneous tissue when using a longer injection needle. However, the specific factors influencing the onset of development have not yet been elucidated, and further research is needed.

Subcutaneous insulin absorption is one of the key factors affecting glycemic control in insulin-treated diabetic patients. Insulin-induced LHT has been reported to impair normal insulin uptake and affect glycemic control [21]. Almost all early studies reported a significant reduction in insulin uptake (in some cases clearance of radiolabeled insulin at the injection site) and glucose reduction with LHT, and in some cases patients exhibited elevated glycated hemoglobin [22–24]. When subjects with LHT were taught to inject into normal tissues, total daily insulin dose requirements were significantly reduced and glycemic control and variability were improved [25, 26]. In this study design, patients with ultrasound-confirmed LHT were taught to administer equal doses of insulin subcutaneously on LHT and NAT on a non-consecutive 2 d of the week, while the insulin dose was reduced by 10% compared to the original dose to avoid hypoglycemia, in order to assess the effect of LHT on patients' blood glucose fluctuations and insulin dose. The results showed that insulin injections at LHT areas could significantly affect patients' glycemic control and lead to increased blood glucose fluctuations. Therefore, standardized insulin injection education for diabetic patients can help them avoid injecting insulin at LHT areas and can simultaneously achieve the goal of good glycemic management. In addition, the results of this study suggested that when the injection site was changed from fatty hypertrophic tissue to normal tissue, the insulin dose was reduced, avoiding the risk of hypoglycemia on the one hand, and effectively reducing the insulin dosage and mitigating medical costs on the other.

Inevitably, some limitations were present in this study. First, this was a cross-sectional study that only investigated the incidence of LHT in insulin-injected diabetic patients who visited our hospital. Because of the difference in education on injection techniques in different treatment centers, it may not represent
the overall incidence of insulin-injected patients. In addition, the small sample size of this study may have introduced errors in the analysis of factors influencing LHT; thus, more patients need to be included for further analysis. This study is unprecedented in several ways. It provided an innovative summary of the characteristics of LHT under ultrasound and the first detailed comparison of the rate of missed diagnosis between ultrasound and clinical palpation in terms of the area size of LHT.

In summary, LHT is a common comorbidity of long-term insulin therapy in diabetic patients, which not only increases patients' pain and additional financial burden, but also decreases insulin absorption and reduces the efficacy of glycemic control. We strongly recommend training and having experienced health professionals to better identify LHT lesions and apply ultrasound to assist in the diagnosis when necessary. Properly teaching patients the correct way to inject insulin and emphasizing the seriousness of LHT to patients is critical to the long course management of diabetes.

Declarations

Author contributions

Yy Lin contributed to the conception of the study and manuscript. W Wang performed the experiments and analysed the data. Jfeng Hong contributed to perform the experiments. H Zeng contributed to the conception of the study and manuscript preparation.

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Declaration of competing interest

The authors declare no conflict of interests.

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**Figures**
Figure 1

The difference between normal abdominal subcutaneous tissue and LHT under ultrasound. Ultrasound characteristics of normal subcutaneous tissue (1A), epidermal and dermal layers (a) are separated from the muscular layer (c) by the subcutaneous layer (b), adipose tissue hypochoic is separated by thin connective tissue (e) and thicker muscular fascia (d). The distance between the two (+) is the thickness of the subcutaneous fat. The ultrasound characteristics of lipohypertrophy (1B-D) were location in the subcutaneous tissue between the epidermis and muscularis layer, with lesions that were well circumscribed nodular hyperechoic (*) or interstitial edema hypoechoic zone (f), the echo is different from the surrounding normal tissue. Continuous connective tissue around is interrupted (arrow). with little or without vascularized echo (1D).

![Figure 1](image)

Figure 2

Comparison of blood glucose fluctuation indexes when LHT patients inject the same amount of insulin at different parts of NAT and LHT. LAGE: largest amplitude of glycemic excursion; MBG: mean blood glucose; SDBG: standard deviation of blood glucose; PPBG: postprandial glucose excursion; LHT: lipohypertrophy tissue; NAT: normal adipose tissue. **P<0.01, *P<0.05 NAT vs. LHT.

![Figure 2](image)