Quantitative Evaluation of Insulin Injection-induced Abdominal Subcutaneous Dystrophic Tissue Using Shear Wave Elastography

Genki Sato
Division of Diabetes, Metabolism and Endocrinology, Department of Medicine, Toho University

Hiroshi Uchino (✉ h.uchino@med.toho-u.ac.jp)
Division of Diabetes, Metabolism and Endocrinology, Department of Medicine, Toho University

Yosuke Shimizu
Department of Dermatology, Toho University Faculty of Medicine, Tokyo

Junko Tatebe
Department of Laboratory Medicine, Toho University Faculty of Medicine, Tokyo

Toshisuke Morita
Department of Laboratory Medicine, Toho University Faculty of Medicine, Tokyo

Takahisa Hirose
Division of Diabetes, Metabolism and Endocrinology, Department of Medicine, Toho University

Research Article

Keywords: Subcutaneous dystrophic tissue, Shear wave elastography (SWE), Quantitative evaluation, insulin injection

DOI: https://doi.org/10.21203/rs.3.rs-414153/v1

License: ☭ This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Subcutaneous dystrophic tissue produced by injection of insulin causes dysglycemia due to inadequate absorption of insulin. However, precise techniques to measure the dystrophic tissue have not been established. Shear wave elastography (SWE) is an imaging technology that can quantify tissue stiffness. We evaluated insulin injection-induced dystrophic tissue by SWE. We recruited seven patients with type 2 diabetes who had suspicion of the abdominal dystrophic tissue. Using SWE, we measured the shear wave velocity (SWV) of the dystrophic tissue and the normal adipose tissue as a control. Furthermore, two patients underwent whole abdominal SWE examination and we calculated the proportion of dystrophic tissue. We also performed a subcutaneous insulin tolerance test at both dystrophic tissue and control sites. The mean SWV of the dystrophic tissue was significantly higher than the control (2.87 ± 0.24 vs. 1.34 ± 0.11 m/s, p<0.01). The volume of the dystrophic tissue was 2.94 ± 3.21%. The mean area under the curve of subcutaneous injected Insulin Aspart concentration at the dystrophic tissue sites was lower than control (80.3 ± 31.4 vs. 119 ± 34.2 h*mU/L, p=0.10). The results from this study indicate that SWE can be useful in evaluating abdominal subcutaneous dystrophic tissue.

Introduction

Insulin is a hormone that is commonly administered via a subcutaneous injection and has been widely used for blood glucose management in type 1 and 2 diabetics. Insulin therapy is given to mimic the physiological concentration of insulin, however, the absorption of insulin can vary due to the preparation, injection technique, and injection site\textsuperscript{1–6}. Variability in the subcutaneous absorption of insulin is related to glucose variability and can arise as an unpredictable therapeutic response resulting in inadequate glycemic control and increased risk of hypoglycemia\textsuperscript{1,7}. Some studies have shown that subcutaneous injections of insulin may cause dystrophic tissue such as lipohypertrophy, fibrocollagenous scars, and insulin-derived amyloidosis in diabetic patients\textsuperscript{2,8–12}. In addition, dystrophic tissue can cause poor blood glycemic control due to inadequate absorption of insulin\textsuperscript{2,8–11,13,14}.

Subcutaneous dystrophic tissue is typically diagnosed by clinical evidence of a palpable subcutaneous lump and confirmed by pathological examination\textsuperscript{11,15–22}. However, a standard technique to quantify the amount of dystrophic tissue has not yet been established.

Tissue stiffness has known to be associated with underlying pathological states. Thus, the measurement of tissue stiffness may be a useful clinical estimation of the severity of tissue pathology. Ultrasound elastography is an imaging technology sensitive to tissue stiffness that was first described in the 1990s\textsuperscript{23}. Strain elastography was the first introduced ultrasound elastography technique\textsuperscript{24}. Although strain elastography can represent strain measurements as a semitransparent color map overlaid on a B-mode image, it is not usually quantifiable. In contrast to strain elastography, shear wave elastography (SWE) can produce quantitative images of shear wave velocity (SWV). Measurement of the SWV results in qualitative and quantitative estimates of tissue elasticity\textsuperscript{25}. In general, the stiffer the tissue is, the greater the SWV. In addition, it has a high user-independence and reproducibility, and thus, SWE has the
advantage of quantification of the superficial tissue stiffness. Few studies have evaluated subcutaneous dystrophic tissue. In this study, we used SWE to quantitatively evaluate the abdominal subcutaneous dystrophic tissue in patients with type 2 diabetes who injected insulin.

**Materials And Methods**

**Study design**

We conducted a single-center observational proof of principle study to examine the characteristics of subcutaneous dystrophic tissue generated by daily insulin injection in patients with type 2 diabetes.

**Participants**

Patients were enrolled between December, 2018 and December, 2019 and provided fully-informed consent. We enrolled outpatients with type 2 diabetes receiving daily insulin injections attending Toho University Omori Medical Center, Tokyo. Our eligibility criteria included suspicion of subcutaneous dystrophic tissue at the insulin injection site and those aged 20 years or older. We excluded patients with heart failure, a history of heart failure, a medical history of hypersensitivity to any of the ingredients of the study drugs, severe ketosis, a diabetic coma or pre-coma, severe liver dysfunction, severe renal dysfunction, serious infectious disease, pre or post-operative state, serious injury, and those receiving cancer treatment. Seven patients who met these criteria were enrolled in this study.

**Setting**

We measured the shear wave velocity (SWV) in m/s using the Virtual Touch™ Imaging Quantification (VTIQ) mode with the Acuson S3000™ and a 9L4 probe (6.5 MHz ± 20%) (Siemens Medical Solutions, Mountain Veiw, CA, USA) (see Supplementary Fig. S1 online). We embedded a 0.5 cm-thick spacer (Yasojima Proceed Co., Ltd., Osaka, Japan) between the skin surface and ultrasound probe to mitigate the shear wave signal error. The team for each examination consisted of an examiner and a recorder.

**Comparison between the subcutaneous dystrophic tissue and control tissue**

Two independent investigators confirmed the presence of subcutaneous lumps at the site of repetitive insulin injections through visible inspection and palpation\(^2\). A palpable subcutaneous lump was classified as dystrophic tissue and the lateral abdominal normal adipose tissue of the same subject was classified as control tissue. The raw data of the SWV were obtained using a standard protocol as described by the manufacturer’s recommendations (Virtual Touch™ Image Quantification by Siemens). The region of interest (ROI) encompassed the dystrophic tissue and control tissue, which served as the control-specific background value (see Supplementary Fig. S1 online). Three different ROIs were selected at the site of insulin injections and control regions, respectively. SWV was measured at these ROIs and the mean SWV was calculated in m/s. Two or three independent reviewers analyzed the data from these samples to assess reliability and reduce bias associated with the placement of the dystrophic tissue and
the control ROIs. The average SWV was compared between the dystrophic tissue and control sites with an unpaired t-test.

**Proportion of dystrophic tissue in the abdominal wall**

To evaluate the entire abdominal subcutaneous tissue, two patients underwent an entire abdominal SWE examination. We assessed the entire abdominal surface area as a vertical axis between the lower costal margin to the superior anterior iliac spine, and as a horizontal axis between both anterior axillary lines. We obtained VTIQ images with 1 cm step size intervals toward the cranial-to-caudal axis governed by 2 cm-working distance in the horizontal axis (see Supplementary Fig. S2 online). The ROIs were defined as 4 cm x 4 cm areas and each superior margin was mapped at the surface of the epidermis. To reconstruct 3D full-length horizontal abdominal VTIQ images, the individual VTIQ image stacks was rendered by 1 cm-working distance to cover each edge of the images. Imaging analysis was generated with Dragonfly software (Montreal, Quebec, Canada). We set the superior and inferior margin of the subcutaneous tissue as dermis and fascia, respectively. The colored RGB images of VTIQ at the ROIs were changed to SWV data according to the manufacturer's guidelines, using adaptive histogram equalization. The amount of whole abdominal dystrophic tissue was calculated by the equation: percentage of dystrophic tissue = total individual dystrophic tissue SWV density numbers / entire subcutaneous tissue area density numbers. SWV density numbers were calculated by the SWV histogram. We calculated the average of three individual SWV data derived from the dystrophic tissue and control tissue to account for the density numbers of the total individual dystrophic tissue SWV. We used upper and lower margins of the SWV histogram cut-off points as the data between total individual dystrophic tissue SWV density numbers and control SWV density numbers. We evaluated the distribution of the high SWV area in the entire abdominal wall.

**Subcutaneous insulin tolerance test**

Patients arrived at our institution in the morning after an overnight fast and skipped their morning bolus of insulin. To compare insulin absorption, the patients received subcutaneous abdominal injections of 0.1 units/standard body weight (kg) insulin aspart (IAsp) into the dystrophic tissue and control sites. Blood samples were taken before the insulin injection and at every 30 minutes up to 240 minutes after injection. Serum iso-insulin (Mercodia AB, Uppsala, Sweden) and human insulin (Roche Diagnostics, Tokyo, Japan) were measured and serum IAsp concentration were determined with the following formula: Serum IAsp concentration (mU/L) = (serum iso-insulin) – (serum human insulin). Area under the curve of serum IAsp concentration values from 0 to 240 minutes (AUC\textsubscript{IAsp}) was calculated using the trapezoidal method. The mean AUC\textsubscript{IAsp} after insulin injections into the dystrophic tissue versus control sites was compared with an unpaired t-test.

**Statistical analysis**

All statistical analyses were conducted using JMP version 13.0.0. Data are shown as the mean ± standard deviation (SD). The unpaired t-test was used to compare values between the subcutaneous dystrophic tissue and control tissue, with the level of significance set at p < 0.05.
Ethics declarations

All procedures performed in this study were in accordance with the Declaration of Helsinki, as revised in 2013. The Ethics Committee of Faculty of Medicine, Toho University, Tokyo, Japan approved all procedures in this study (No. A18125). Written informed consent was obtained from all individual participants.

Results

Patient characteristics

Table 1 shows the baseline characteristics of the patients. The study population consisted of 4 (57%) men and 3 (43%) women, with a mean ± SD age of 62.1 ± 11.0 years. The mean body mass index (BMI) was 31.0 ± 4.62 kg/m² and the mean HbA1c was 8.13 ± 1.04%, 65.4 ± 11.2 mmol/mol. The mean duration of diabetes was 21.1 ± 5.61 years and the mean duration of insulin therapy was 13.7 ± 11.1 years. All patients had been using insulin analogues for multiple daily insulin injections and the mean total daily insulin dose was 62.1 ± 26.1 U/day.
Table 1

Baseline characteristics and shear wave velocity of dystrophic tissue and control. BMI body mass index, SWV shear wave velocity, * p < 0.01, P value is reported for t-test, comparing the SWV between dystrophic tissue and control.

|                             | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 | Patient 6 | Patient 7 | Mean ± SD |
|-----------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Age (year)                  | 43        | 67        | 58        | 70        | 77        | 56        | 64        | 61.1 ± 11.0 |
| Sex                         | F         | F         | M         | M         | M         | F         | M         |           |
| BW (kg)                     | 71.2      | 63.2      | 103.4     | 75.2      | 76.9      | 82.5      | 71.3      | 77.7 ± 12.8 |
| BMI (kg/m²)                 | 30.8      | 28.5      | 40.4      | 29.4      | 27.2      | 33        | 27.5      | 31.0 ± 4.62  |
| Duration of Diabetes (year) | 22        | 20        | 10        | 22        | 28        | 25        | 21        | 21.1 ± 5.61  |
| HbA1c (%)                   | 9.5       | 9.1       | 7.9       | 7.4       | 8.8       | 7.6       | 6.6       | 8.13 ± 1.04 |
| Duration of Insulin (year)  | 6         | 10        | 8         | 23        | 35        | 5         | 9         | 13.7 ± 11.1  |
| Number of daily insulin injections | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 ± 0.0 |
| Daily Insulin Dose (Unit/day) | 79 | 64 | 106 | 42 | 74 | 32 | 38 | 62.1 ± 26.6 |
| Daily insulin Dose (Unit/kg/day) | 1.11 | 1.01 | 1.03 | 0.56 | 0.96 | 0.39 | 0.53 | 0.80 ± 0.29 |
| SWV (dystrophic tissue) (m/s) | 2.87 | 2.66 | 2.79 | 3.27 | 2.98 | 2.97 | 2.53 | 2.87 ± 0.24* |
| SWV (Control) (m/s)         | 1.5       | 1.2       | 1.28      | 1.23      | 1.41      | 1.44      | 1.29      | 1.34 ± 0.11  |

Comparison of stiffness between dystrophic and control tissue

The SWV of the dystrophic tissue and control tissue is also shown in Table 1. The mean SWV of the dystrophic tissue was significantly higher than the control tissue (2.87 ± 0.24 vs. 1.34 ± 0.11 m/s, p < 0.01).

Proportion of dystrophic tissue in the whole abdominal wall
Figure 1 shows the SWE histogram and percentage of dystrophic tissue. The density numbers of dystrophic tissue / entire subcutaneous tissue area are 39,783 / 5,894,523 in patient 4, and 194,481 / 3,730,419 in patient 7, respectively. The mean proportion of dystrophic tissue volume calculated from entire abdominal subcutaneous tissue was 2.94 ± 3.21%. Whole abdominal VTIQ images highlighted some high SWV lesions during the entire abdominal scanning which was undiagnosed as dystrophic tissue before the examination.

**Subcutaneous insulin tolerance test**

Five of the seven patients’ subcutaneous insulin tolerance test data were available. Two patients’ data were excluded because of data errors and no specimen collection. Figure 2 shows the mean area under the curve of serum IAsp concentration (AUC\textsubscript{IAsp}) over time after IAsp injection into areas with dystrophic tissue and control tissue. Although not significant, the mean insulin subcutaneous absorption tended to be lower after the dystrophic tissue site injection compared with the control site, indicated by a 33% decrease in AUC\textsubscript{IAsp} after IAsp injection into areas with dystrophic tissue (80.3 ± 31.4 vs. 119 ± 34.2 h*mU/L, p = 0.0989).

**Discussion**

In this study, we used SWE to perform a quantitative evaluation of insulin injection-induced subcutaneous tissue change in the abdomen of patients receiving repeated insulin injections. We found that the mean SWV of the insulin injection-induced dystrophic tissue was significantly higher than that of control tissue (2.87 ± 0.24 vs. 1.34 ± 0.11 m/s, p < 0.01). In addition, we calculated the 3D volume of the insulin injection-induced dystrophic tissue from the entire abdominal subcutaneous tissue. The mean volume of the dystrophic tissue was 2.94 ± 3.21%. Although no statistically significant difference was found, the mean insulin subcutaneous absorption was 33% lower at the dystrophic tissue sites than control sites (p = 0.0989).

Although the insulin injection-induced subcutaneous dystrophic tissue, such as lipohypertrophy, fibrotic scar tissue, or amyloid tissue, has been traditionally assessed only by survey or physical examination, previous studies reported that palpable lumps were not observed in all cases of insulin amyloid\textsuperscript{26}. Thus, it is difficult to identify dystrophic tissue only upon physical examination. Some authors reported that subcutaneous dystrophic tissue caused by insulin injections, which could be identified by physical examination, could be recognized by ultrasound\textsuperscript{27,28}. Furthermore, another study showed that ultrasound could be used to detect cases in which subcutaneous lesions were not identified by physical observation\textsuperscript{29}. However, it remains unclear how widespread the insulin injection-induced abdominal subcutaneous dystrophic tissue has been distributed in the entire subcutaneous abdominal wall. To our knowledge, this is the first study to examine the volume and distribution of the abdominal subcutaneous dystrophic tissue using SWE. Previous studies have shown that different types of dystrophic tissue such as lipohypertrophy, fibrotic scars, and amyloid at the site of insulin injection all inhibited absorption of insulin\textsuperscript{2,8,10,11}. In agreement with previous reports, our study showed that the absorption of insulin after
injections into dystrophic tissue sites was reduced when compared with injections into control sites. However, there was no statistically significant difference ($p = 0.0989$). This result can be attributed to the small number of cases.

Our results showed that SWV of the insulin injection-induced dystrophic tissue was more than twice as high as that of the normal adipose tissue. This data may reflect histopathological changes such as fibrosis and amyloid deposits. Although it is necessary to perform histopathological examination to prove our hypothesis, the dystrophic tissue occupied only 2.94% of the volume of the entire abdominal subcutaneous tissue. Thus, our methods could be useful to more precisely identify areas of dystrophic tissue. This data suggests that there are lesions other than the palpable subcutaneous lumps where repeated insulin injections are given. Previous studies showed that non-palpable lesions that were difficult to detect by physical diagnosis were also related to an insulin absorption disorder. Thus, it is important to detect the subcutaneous dystrophic tissue to achieve normal insulin delivery and good glycemic control. In our study, it was difficult to clearly show the cut-off value of SWV that separated the dystrophic tissue from normal tissue, since the number of cases was small. Further large-scale studies are necessary to differentiate quantitatively between the dystrophic tissue and normal tissue with SWE.

Several limitations of this study should be acknowledged. First, the number of cases was small, in particular in the whole abdominal study, which only included two patients. Second, we regarded palpable subcutaneous lumps as dystrophic tissue and defined the average SWV of three different ROIs as the reference value of the dystrophic tissue. However, a previous study using strain elastography reported that the palpable dystrophic tissue was harder than the non-palpable type. Thus, the non-palpable dystrophic tissue might be overlooked in our method. Additional studies are needed to determine whether non-palpable dystrophic tissue has a lower SWV than the palpable type. Third, we did not perform a pathological examination in our study. Thus, we were unable to prove whether the high SWV was histologically associated with the pathological changes such as lipohypertrophy, fibrotic lesions, and amyloid in this study.

Despite undergoing frequent abdominal subcutaneous tissue investigations, many patients with multiple insulin injections have fluctuations in blood glucose and severe hypoglycemia. Therefore, a non-invasive and examiner-independent method with improved diagnostic accuracy is needed to assess the condition of the subcutaneous abdominal tissue. We performed a quantitative evaluation of insulin injection-induced subcutaneous tissue change using SWE. The mean SWV of the insulin injection-induced dystrophic tissue was significantly higher than that of normal adipose tissue. Our findings showed that SWE enables us to calculate the 3D volume of insulin injection-induced dystrophic tissue from the entire abdominal subcutaneous tissue. Although there was no statistically significant difference, the mean insulin subcutaneous absorption was lower in the dystrophic tissue than the normal adipose tissue. In the future, we intend to validate our system with a larger number of patients and automated 3D-imaging software.

Declarations
Data availability

The datasets generated during and/or analyzed during the current study are not publicly available due to concerns about data confidentiality but are available from the corresponding author on reasonable request.

Acknowledgments

This work was supported by funding from the Grant-in-Aid for Scientific Research from the Japan Science and Technology Agency, KAKEN (17K01425, JSPS) to H.U. We thank K. Okui and M. Suzuki for help with obtaining approval by the local ethical committee for the experiments and for providing analysis computers, software and advice.

Author Contributions

G.S and H.U. initiated the original idea. Y.S designed the experiment. J.T. fabricated samples. T.M. and G.S. carried out the measurement and analyzed data. G.S and T.H. performed simulations. G.S and H.U. provided the theoretical explanations. J.T., T.M. assisted in part of the experiment. T.H. and H.U. supervised the project.

Competing interests

The authors declare no competing interests.

References

1. Gin, H. & Hanaire-Broutin, H. Reproducibility and variability in the action of injected insulin. *Diabetes & Metabolism*. **31**, 7-13, [https://doi.org/10.1016/s1262-3636(07)70160-x](https://doi.org/10.1016/s1262-3636(07)70160-x) (2005).

2. Famulla, S. *et al.* Insulin Injection Into Lipohypertrophic Tissue: Blunted and More Variable Insulin Absorption and Action and Impaired Postprandial Glucose Control. *Diabetes Care*. **39**, 1486-1492, [https://doi.org/10.2337/dc16-0610](https://doi.org/10.2337/dc16-0610) (2016).

3. Pozzuoli, G. M., Laudato, M., Barone, M., Crisci, F. & Pozzuoli, B. Errors in insulin treatment management and risk of lypohypertrophy. *Acta Dabetol*. **55**, 67-73, [https://doi.org/10.1007/s00592-017-1066-y](https://doi.org/10.1007/s00592-017-1066-y) (2018).

4. Ter Braak, E. W. *et al.* Injection site effects on the pharmacokinetics and glucodynamics of insulin lispro and regular insulin. *Diabetes Care* **19**, 1437-1440, [https://doi.org/10.2337/diacare.19.12.1437](https://doi.org/10.2337/diacare.19.12.1437) (1996).

5. Frid, A. & Linde, B. Intraregional differences in the absorption of unmodified insulin from the abdominal wall. *Diabet Med* **9**, 236-239, [https://doi.org/10.1111/j.1464-5491.1992.tb01768.x](https://doi.org/10.1111/j.1464-5491.1992.tb01768.x) (1992).

6. Frid, A. H. *et al.* New Insulin Delivery Recommendations. Mayo Clin Proc 91, 1231-1255, [https://doi.org/10.1016/j.mayocp.2016.06.010](https://doi.org/10.1016/j.mayocp.2016.06.010) (2016).
1. Vora, J. & Heise, T. Variability of glucose-lowering effect as a limiting factor in optimizing basal insulin therapy: a review. Diabetes Obes Metab 15, 701-712, https://doi.org/10.1111/dom.12087 (2013).

2. Blanco, M., Hernandez, M. T., Strauss, K. W. & Amaya, M. Prevalence and risk factors of lipohypertrophy in insulin-injecting patients with diabetes. Diabetes Metab 39, 445-453, https://doi.org/10.1016/j.diabet.2013.05.006 (2013).

3. Nagase, T. et al. The insulin ball. The Lancet 373, 184, https://doi.org/10.1016/S0140-6736(09)60041-6 (2009).

4. Wallymahmed, M. E., Littler, P., Clegg, C., Haqqani, M. T. & MacFarlane, I. A. Nodules of fibrocollagenous scar tissue induced by subcutaneous insulin injections: a cause of poor diabetic control. Postgrad Med J 80, 732-733, https://doi.org/10.1136/pgmj.2004.019547 (2004).

5. Nagase, T. et al. Insulin-derived amyloidosis and poor glycemic control: a case series. Am J Med 127, 450-454, https://doi.org/10.1016/j.amjmed.2013.10.029 (2014).

6. Ji, L. et al. Lipohypertrophy in China: Prevalence, Risk Factors, Insulin Consumption, and Clinical Impact. Diabetes Technol Ther 19, 61-67, https://doi.org/10.1089/dia.2016.0334 (2017).

7. Johansson, U. B. et al. Impaired absorption of insulin aspart from lipohypertrophic injection sites. Diabetes Care 28, 2025-2027, https://doi.org/10.2337/diacare.28.8.2025 (2005).

8. Heinemann, L. Insulin absorption from lipodystrophic areas: a (neglected) source of trouble for insulin therapy? J Diabetes Sci Technol 4, 750-753, https://doi.org/10.1177/193229681000400332 (2010).

9. Ansari, A. M., Osmani, L., Matsangos, A. E. & Li, Q. K. Current insight in the localized insulin-derived amyloidosis (LIDA): clinico-pathological characteristics and differential diagnosis. Pathol Res Pract 213, 1237-1241, https://doi.org/10.1016/j.prp.2017.08.013 (2017).

10. Samlaska, C., Reber, S. & Murry, T. Insulin-derived amyloidosis: The insulin ball, amyloidoma. JAAD Case Rep 6, 351-353, https://doi.org/10.1016/j.jdcr.2020.02.011 (2020).

11. Grunes, D., Rapkiewicz, A. & Simsir, A. Amyloidoma secondary to insulin injection: Cytologic diagnosis and pitfalls. Cytojournal 12, 15. https://doi.org/10.4103/1742-6413.161602 (2015).

12. Mayhew, J. M., Alan, T., Kalidindi, V. & Gandamihardija, T. A. K. Isolated insulin-derived amyloidoma of the breast. BMJ Case Rep 2017, bcr2017219491, https://doi.org/10.1136/bcr-2017-219491 (2017).

13. Mangla, A. et al. Localized insulin amyloidosis with use of concentrated insulin: a potential complication. Diabet Med 33, e32-e35, https://doi.org/10.1111/dme.13137 (2016).

14. Shikama, Y. et al. Localized amyloidosis at the site of repeated insulin injection in a diabetic patient. Intern Med 49, 397-401, https://doi.org/10.2169/internalmedicine.49.2633 (2010).

15. Yumlu, S., Barany, R., Eriksson, M. & Röcken, C. Localized insulin-derived amyloidosis in patients with diabetes mellitus: a case report. Hum Pathol 40, 1655-1660, https://doi.org/10.1016/j.humpath.2009.02.019 (2009)
22. Gentile, S. et al. A suitable palpation technique allows to identify skin lipohypertrophic lesions in insulin-treated people with diabetes. *Springerplus* 5, 563, https://doi.org/10.1186/s40064-016-1978-y (2016).

23. Gennisson, J. L., Deffieux, T., Fink, M. & Tanter, M. Ultrasound elastography: principles and techniques. *Diagn Interv Imaging* 94, 487-495, https://doi.org/10.1016/j.diintim.2013.01.022 (2013).

24. Ophir, J., Céspedes, I., Ponnekanti, H., Yazdi, Y. & Li, X. Elastography: a quantitative method for imaging the elasticity of biological tissues. *Ultrason Imaging* 13, 111-134, https://doi.org/10.1177/016173469101300201 (1991).

25. Sigrist, R. M. S., Liau, J., Kaffas, A. E., Chammas, M. C. & Willmann, J. K. Ultrasound Elastography: Review of Techniques and Clinical Applications. *Theranostics* 7, 1303-1329, https://doi.org/10.7150/thno.18650 (2017).

26. D'Souza, A. et al. Localized insulin-derived amyloidosis: a potential pitfall in the diagnosis of systemic amyloidosis by fat aspirate. *Am J Hematol* 87, E131-132, https://doi.org/10.1002/ajh.23334 (2012).

27. Perciun, R. Ultrasonographic aspect of subcutaneous tissue dystrophies as a result of insulin injections. *Medical Ultrasonography* 12, 104-109 (2010).

28. Perciun, R., Telcian, A., & Olariu, L. Ultrasound assessment of cutaneous/subcutaneous dystrophies in insulin-treated patients. A report on two cases. *Medical Ultrasonography* 14, 60-63 (2012).

29. Kikuchi, M. et al. Ultrasonography Improves Glycemic Control by Detecting Insulin-Derived Localized Amyloidosis. *Ultrasound Med Biol* 43, 2284-2294, https://doi.org/10.1016/j.ultrasmedbio.2017.06.011 (2017).

**Figures**
Figure 1

Histogram of the individual shear wave velocity density numbers and % dystrophic tissue. The figure shows a histogram of the individual shear wave velocity (SWV) density numbers and % dystrophic tissue. Upper (a) and lower (b) histogram depict the data of patient 4 and patient 7, respectively.
Figure 2

The mean area under the curve of serum IAsp concentration (AUCIAsp) over time after IAsp injection into areas with dystrophic tissue and control. The figure shows the mean area under the curve of serum IAsp concentration (AUCIAsp) over time after IAsp injection into areas with dystrophic tissue and control. The results are expressed as means ± standard deviations.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Suppl.pdf