Insulin Injection Into Lipohypertrophic Tissue: Blunted and More Variable Insulin Absorption and Action and Impaired Postprandial Glucose Control

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OBJECTIVE

Lipohypertrophy (LHT) is common in insulin-treated patients but its exact impact on insulin absorption and action is unclear.

RESEARCH DESIGN AND METHODS

In this crossover study, 13 patients with type 1 diabetes received subcutaneous abdominal injections of 0.15 units/kg insulin lispro into LHT (confirmed by examination and ultrasound) and normal adipose tissue (NAT). On one day, a euglycemic clamp was performed with two injections each into LHT and NAT, and on another day one injection per region was given before a standardized mixed meal (75 g carbohydrates), all in randomized order.

RESULTS

Compared with NAT, LHT reduced insulin absorption (mean area under the insulin concentration curve [$AUC_{INS0–4h}$] 131 vs. 165 h * mU/L [LHT vs. NAT]; C_{max} 61 vs. 79 mU/L, $P < 0.02$, respectively) and effect (areas under glucose infusion rate [GIR] curves [$AUC_{GIR0–4h}$ 625 vs. 775 mg/kg, $P < 0.05$]) but increased intrasubject variability ([coefficient of variation] $AUC_{INS0–4h}$ 52 vs. 11%, C_{max} 55 vs. 15%, $AUC_{GIR0–4h}$ 57 vs. 23%, all $P < 0.01$). Postprandial blood glucose (BG) concentrations were ≥26% higher with LHT ($AUC_{BG0–5h}$ 731 vs. 513 mg * h/dL, BG_{max} 199 vs. 157 mg/dL, 2-h BG 150 vs. 104 mg/dL, 5-h BG 145 vs. 81 mg/dL, all $P < 0.05$) and maximum concentrations occurred later. Hypoglycemia (BG ≤50 mg/dL) occurred numerically less frequently with LHT injection (two vs. six patients), whereas profound hyperglycemia (BG ≥300 mg/dL) only occurred with LHT injection (two patients). $T_{max-INS}$ did not differ between LHT and NAT in either study.

CONCLUSIONS

Insulin absorption and action are blunted and considerably more variable with LHT injection, leading to profound deterioration in postprandial glucose control.

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Lipohypertrophy (LHT) is a common side effect of long-standing insulin therapy in patients with diabetes characterized by fibrous and poorly vascularized lesions in the subcutaneous adipose tissue (1). Cross-sectional studies from different countries such as Germany, Ethiopia, Turkey, and Spain reported prevalences between 28 and over 64% in patients with both type 1 and type 2 diabetes; it is generally more common in those with type 1 diabetes (2–5). Although the exact etiology of LHT is unclear, predisposing factors include duration of insulin treatment, needle reuse frequency, and especially repeated injection into the same tissue areas due to incorrect injection and site rotation techniques (1,5).

Anecdotal clinical observations strongly suggest impaired insulin absorption from LHT. Patients' blood glucose (BG) control substantially improved with reductions in insulin dosage after re-education in injection site rotation techniques and avoidance of LHT for insulin injection (6). Blanco et al. (4) observed significantly higher insulin dosing in patients with, versus those without, LHT, but did not report A1C levels. On the other hand, no differences were found in diabetes control or insulin demands between patients with and without LHT in one cross-sectional study (2), and an outpatient 3-day study with continuous glucose monitoring failed to show any substantial differences in insulin pharmacokinetics (PK) and BG profiles (7), so those authors felt it unnecessary to advise patients to avoid LHT for insulin injections. In addition, two meal studies with pharmacodynamic (PD) and PK comparisons of insulin injection into LHT versus normal adipose tissue (NAT) showed inconsistent results. Whereas maximum insulin concentrations were reduced by 25–30% with LHT injection, PD variables were not significantly different (7,8). However, systematic studies using the glucose clamp technique have never quantified the impact of LHT on insulin PK/PD, and the variability in insulin PK/PD has not been investigated.

We therefore performed a combined glucose-clamp/meal test study to quantify the impact of LHT on both the extent and the variability of insulin absorption and action.

RESEARCH DESIGN AND METHODS

Trial Design and Trial Population

Thirteen patients with type 1 diabetes participated in this single-center, randomized, open label study; all completed the study. Patients aged 18–64 years had to have diabetes for at least 3 years, A1C $\leq$10% (86 mmol/mol), a BMI of 18.5–30 kg/m^2, and confirmed lipohypertrophic adipose tissue lesions. Patient characteristics are given in Supplementary Table 1. Patients underwent two treatment periods with glucose clamp tests in period 1 and mixed-meal tolerance tests (MMTTs) in period 2. The study was conducted according to the standards of the Declaration of Helsinki and Good Clinical Practice and was approved by the local ethics committee (Ärztekammer Nordrhein, Düsseldorf, Germany). All patients provided their written, informed consent prior to their participation.

During patient screening, two independent investigators confirmed the presence of abdominal LHT through visible inspection, palpation of the abdominal area according to a predefined examination procedure, and ultrasound (Vivid 7; GE Healthcare, Frankfurt, Germany), as described previously (4). The ultrasound pictures of LHT showed heterogeneous hyperechogenic areas with little or no vascularization echogenicity with a clear margin but no indication of a capsule (Supplementary Fig. 2).

During both treatment periods, patients received repeated injections of 0.15 units/kg body weight of insulin lispro (Humalog; Lilly Deutschland GmbH, Bad Homburg, Germany). During the glucose clamps, four dosings were performed, two into LHT and two into NAT, whereas patients received two insulin lispro injections during the MMTT (one each into LHT and NAT, respectively). The injection site (LHT or NAT) was determined by random assignment to 1 of 12 sequences of the six dose administrations during the two separate treatment periods (Supplementary Fig. 3).

Euglycemic Glucose Clamp

Patients arrived at the study site in the morning after an overnight fast of at least 10 h. Patients had to wash out their background insulin so that the minimum time between last injection and dosing on the study day was 72 h for insulin degludec, 48 h for glargine and detemir, 16 h for NPH insulin, and 12 h for bolus injections. Patients could use a maximum bolus of 6 IU of regular human insulin up to 6 h prior to first dosing. Patients on insulin pumps stopped their basal insulin infusion rate 6 h prior to first dosing if they used human insulin, or 3 h with insulin analogs.

Patients were connected to a Biostator (MTB Medizintechnik, Amstetten, Germany) for continuous BG measurement and glucose infusion as described previously (9). Patients' BG was stabilized via intravenous infusion of either glucose or human insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) to the clamp target level of 100 mg/dL with an accepted variability of $\pm$10 mg/dL during the last 30 min prior to dosing. Glucose infusion needed to be stopped for at least 1 h and insulin infusion for at least 20 min prior to dosing.

After the first insulin lispro injection, the Biostator measured BG levels continuously and automatically adjusted glucose infusion rates (GIRs) every minute to keep BG at the target level. The device's BG measurements were checked at least every 30 min with a reference system (Super GL glucose analyzer; Dr. Müller Gerätebau GmbH, Freital, Germany) and adjusted, if needed. Serum samples for determination of insulin lispro levels were obtained predose and at 15, 30, 45, 60, 80, 100, 120, 150, 180, 210, 240, and 300 min after each lispro injection. If patients' BG increased consistently above 118 mg/dL without any GIR during the last 15 min, an intravenous infusion of human insulin was initiated to restabilize BG to target level. When this target ($\pm$10 mg/dL) was re-established without GIR or insulin infusion as described above, the next injection of insulin lispro was given, but not before 6 h after the previous dose administration. PK sampling for insulin lispro was always continued for 5 h after each injection, even in case of "rescue" human insulin infusion.

MMTT

For the MMTT, patients needed to fast for at least 10 h and wash out their background insulin as described for the glucose clamp test. Patients' BG was stabilized to a level of 100 mg/dL $\pm$ 20 mg/dL via intravenous glucose or insulin infusion. Again, no GIR was allowed from 1 h and

no insulin infusion from 20 min prior to insulin lispro administration, which was done immediately before starting the meal ingestion. The meal was identical at both MMTT intervals and consisted of 75 g of predominantly rapidly absorbable carbohydrates (56% of the meal's energy content), 20.2 g fat (33%), and 15.2 g protein (11%). The meal had to be completely consumed within 15 min. No water consumption was allowed from 1 h prior to until 2 h after start of the MMTT, with the exception of the liquids provided with the meal. Samples for BG and PK measurements were measured in frequent intervals until 5 h postdosing. The MMTT was stopped early in case of hypoglycemia (BG $\leq$50 mg/dL) or hyperglycemia (BG $>$300 mg/dL for $>$30 min) in which case patients received "rescue" intravenous infusions of glucose or human insulin. Otherwise, 5 h after the first insulin dosing, the patient's BG level was restabilized to target level via intravenous glucose or human insulin infusion, if needed. The second MMTT was performed at least 6 h after the first insulin lispro dosing and not before BG was stable at the target without GIR and insulin infusion. Patients stayed in a supine/semirecumbent position throughout all experiments.

Analytical Methods

Serum insulin concentrations were determined with a competitive-binding radioimmunoassay specific to insulin lispro (LisPro Insulin RIA Kit; Millipore Inc., St. Charles, MO; limit of detection: 2.50 mU $\cdot$ L^{-1}, lower limit of quantification: 5.00 mU^{-1}, upper limit of quantification: 250.000 mU $\cdot$ L^{-1}, coefficient of variation [CV] $\leq$20%, relative error $\leq$25%).

Statistical Analysis and End Points

WinNonlin (version 6.3) was used for the analysis of PK parameters and SAS System for Windows (version 9.4) for all other statistical calculations. Primary end points were the area under the insulin concentration and GIR curves in the first 4 h postdosing during the clamp test ($AUC_{INS0–4h}$ and $AUC_{GIR0–4h}$). Main secondary end points were $AUC_{INS0–4h}$ and the area under the BG excursion curve ($AUC_{BG0–4h}$) during the MMTT as well as the intrasubject variability (expressed as CV) of $AUC_{INS0–4h}$ and $AUC_{GIR0–4h}$ during the clamp examination. The statistical analysis was based on all randomized patients and experiments (full-analysis set) and on the intention-to-treat principle. The log-transformed primary PK and PD end points were analyzed using a repeated-measures ANOVA with dosing interval and administration area (LHT or NAT) as fixed effects and subject as random effect. GIR profiles were smoothed using a weighted local regression technique (LOESS, smoothing factor 0.3) for the analysis of time-related PD clamp parameters and maximum GIR. MMTT-related PK and PD end points were analyzed using an ANOVA with sequence, dosing interval, and administration area as fixed effects and subject within sequence as random effect. In case of "rescue" intervention due to hypoglycemia or hyperglycemia, a last observation carried forward method was chosen for BG analysis. Intraindividual CVs were calculated for PK and PD end points during the clamp by using a general linear model with subject as a random factor and log-transformed data. Time-related parameters of the MMTT were analyzed nonparametrically using the Wilcoxon signed rank test. An α level of 0.05 was used for the statistical comparison of least squares mean values derived from the ANOVA. Data reported in text and in tables are mean values $\pm$ SD; figures show mean values $\pm$ SE.

The sample size was based on an assumed difference of at least 20% between LHT and NAT injections for the primary end points and a CV of 21% (10). Eleven patients were required to complete the study for the demonstration of a significant difference with a power of 80%. Two additional patients were enrolled to account for potential dropouts.

RESULTS

Euglycemic Clamp Test

Insulin lispro absorption from LHT showed a similar pattern as from NAT in the first 30 min after dosing but was markedly blunted thereafter (Fig. 1*A*). Likewise, the GIR profiles after LHT and NAT injections clearly separated after 30 min with lower PD effects in LHT (Fig. 1*B*). Consequently, the primary PK and PD end points ($AUC_{INS0–4h}$ and $AUC_{GIR0–4h}$) were significantly higher (by 26 and 24%, respectively) with NAT versus LHT injections as were C_{max} (by 28%) and all partial PK AUCs from 1 h after dosing (Table 1). Total bioavailability based on $AUC_{INS0–5h}$ and total biopotency based on $AUC_{GIR0–5h}$ were significantly reduced with LHT injections to 78–80% of the effect seen with NAT injection (Table 1). Although AUCs for the other GIRs and GIR_{max} were numerically lower with LHT injection, none of these differences reached statistical significance (Table 1). Times to maximum insulin concentration were not different between LHT and NHT, whereas maximum action ($T_{max\text{-}GIR}$) trended to be later with LHT injection ($P < 0.10$) (Table 1).

Clamp quality parameters were within ranges reported previously for automated glucose clamps. Mean deviation of BG from target was 2.0 $\pm$ 9.0 mg/dL, CV in BG during the clamp periods was 7.1 $\pm$ 4.1%, and BG was kept mostly stable at the target level of 100 mg/dL postdosing during the complete observation time of 5 h (Supplementary Fig. 1). The slight rise in mean BG levels toward the end of the clamp period in the LHT treatment group is due to an increase in BG concentrations above the predefined stop level of 118 mg/dL, indicating end of insulin action (11). This occurred after five LHT injections (in four patients) but only once with NAT injections.

Intrasubject variability was substantially higher for both PK and PD parameters with LHT than with NAT injections. CVs of PK parameters ranged from 52 to 65% with LHT compared with 11–20% with NAT (Fig. 1*C*). PD variability was generally higher than PK variability, particularly in the 1st hour postdosing when CVs reached 90% with LHT injection versus 66% in NAT. PD variability was significantly different for the primary PD end point $AUC_{GIR0–4h}$ (Fig. 1*D*).

MMTT

Despite more fluctuations, the PK profiles obtained after the MMTT were in general similar to the PK profiles seen in the glucose clamp test (Fig. 2*B*). Again, serum insulin levels were higher after NAT compared with LHT injection, indicated by a 37–39% increase in $AUC_{INS0–4h}$ and $AUC_{INS0–5h}$, and a 43% increase in maximum concentrations, which were

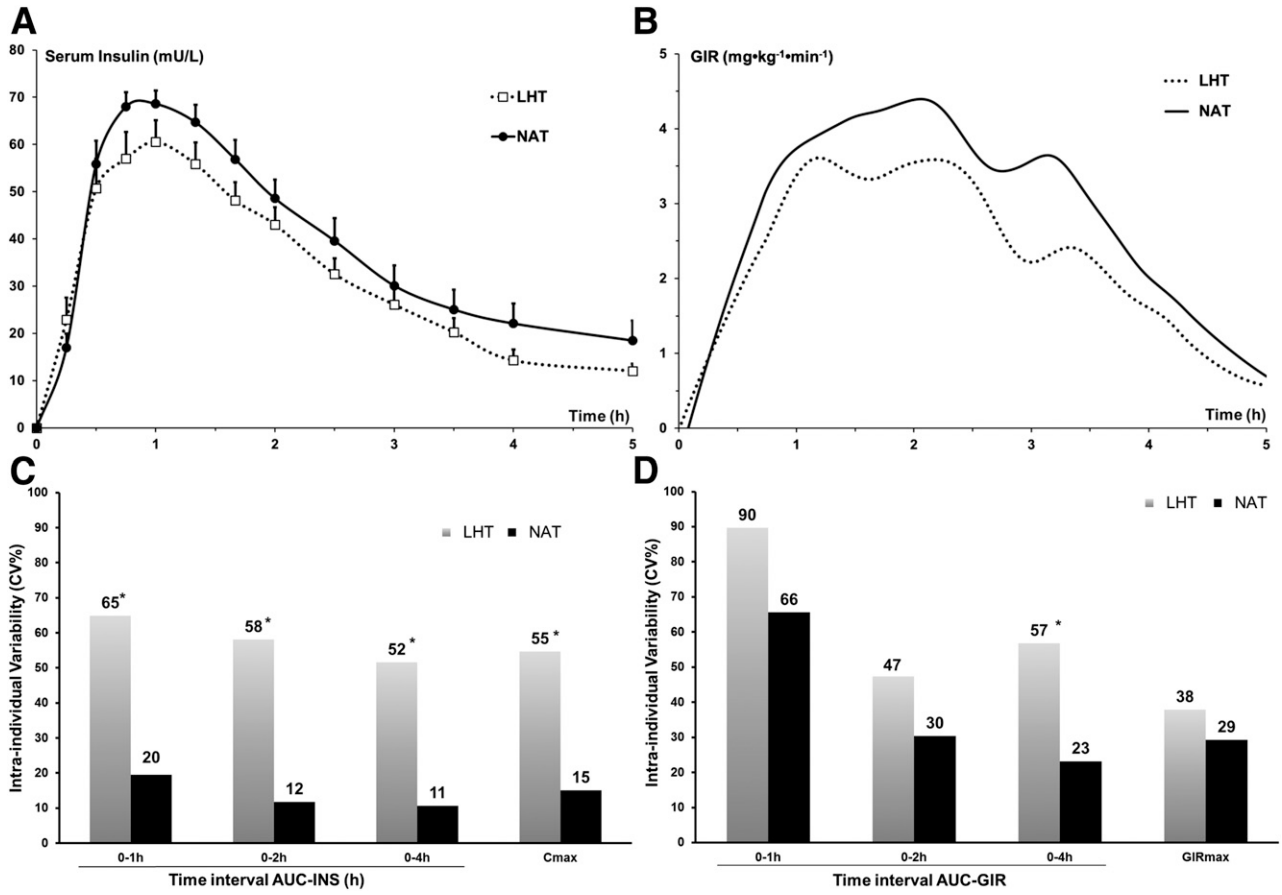

Figure 1—PK and PD results of the glucose clamp experiments. *A*: Baseline-corrected mean serum insulin concentration profiles. *B*: Mean GIR profiles over time after insulin lispro injection into areas with LHT or NAT. Error bars are SEM. The lower panels show the intrasubject variability CVs (%) for PK parameters (*C*) and PD parameters (*D*). Asterisks indicate statistically significant differences ($P < 0.05$).

of borderline statistical significance (Table 2).

In line with the lower serum insulin concentrations, mean postprandial BG concentrations were higher with LHT than with NAT injections (Fig. 2*A*), in particular from 1 h postdosing onwards, indicated by significantly higher AUCs for BG ($AUC_{BG0–2h}$ to $AUC_{BG0–5h}$), higher and later BG_{max}, as well as elevated postprandial BG concentrations and higher AUCs between 2 and 5 h postdosing.

Postprandial hypoglycemia (BG ≤50 mg/dL) occurred numerically more often with NAT injections than with LHT injections (six vs. two patients, $P = 0.2016$, Fisher exact test). In contrast, postprandial hyperglycemia (BG >300 mg/dL) occurred after two LHT injections but was not observed with NAT administrations.

Inspection of individual PK data showed two patients that consistently had no or only very low insulin lispro concentrations in both clamp examinations and MMTT with LHT injection, with absorption patterns comparable to the other patients' NAT injections (data not shown). These two patients were the only subjects who experienced hyperglycemia with BG >300 mg/dL in the MMTT and needed "rescue" insulin infusions.

Table 1—PK and PD end points of the euglycemic-hyperinsulinemic glucose clamp experiments

PK end point	LHT	NAT	*P* value	PD end point	LHT	NAT	*P* value
$AUC_{INS0–0.5h}$ (h * mU/L)	11.7 ± 9.3	11.2 ± 6.5	0.7526	$AUC_{GIR0–0.5h}$ (mg/kg)	29.4 ± 30.6	22.3 ± 21.3	0.2734
$AUC_{INS0–1h}$ (h * mU/L)	38.2 ± 23.5	43.8 ± 13.4	0.0156	$AUC_{GIR0–1h}$ (mg/kg)	104.1 ± 59.6	118.0 ± 88.4	0.6355
$AUC_{INS0–2h}$ (h * mU/L)	84.3 ± 46.1	103.9 ± 22.9	0.0030	$AUC_{GIR0–2h}$ (mg/kg)	313.3 ± 145.0	365.2 ± 187.5	0.2330
$AUC_{INS0–3h}$ (h * mU/L)	114.3 ± 61.2	142.3 ± 40.0	0.0024	$AUC_{GIR0–3h}$ (mg/kg)	498.7 ± 268.3	594.8 ± 256.2	0.0934
$AUC_{INS0–4h}$ (h * mU/L)	131.1 ± 72.4	164.9 ± 57.8	0.0021	$AUC_{GIR0–4h}$ (mg/kg)	625.1 ± 330.8	775.1 ± 288.5	0.0390
$AUC_{INS0–5h}$ (h * mU/L)	138.2 ± 79.7	175.4 ± 72.2	0.0020	$AUC_{GIR0–5h}$ (mg/kg)	685.7 ± 364.5	853.9 ± 285.2	0.0261
$C_{max-INS}$ (mU/L)	61.3 ± 30.3	78.6 ± 19.2	0.0014	GIR_{max} (mg/kg/min)	4.9 ± 2.1	5.4 ± 2.4	0.3776
$T_{max-INS}$ (min)	51.0 ± 23.8	50.0 ± 28.3	0.3481	$T_{max-GIR}$ (min)	97.8 ± 56.6	130.0 ± 67.3	0.0967

Results are given as mean ± SD.

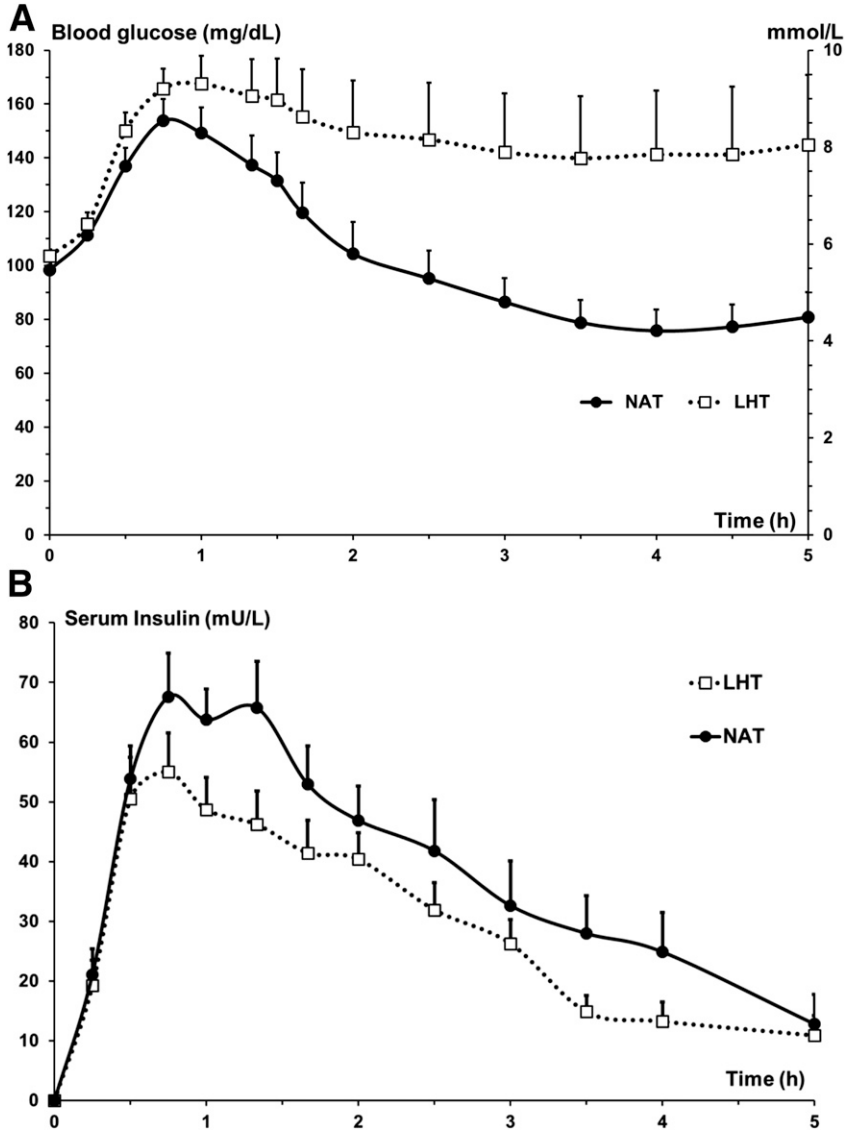

Figure 2—PK and PD results of the MMTTs. *A*: Mean BG profiles. *B*: Mean serum insulin concentration profiles over time after insulin lispro injection into areas with LHT or NAT. Error bars are SEM.

CONCLUSIONS

The aim of this study was to precisely quantify the impact of LHT on PK/PD properties of subcutaneously injected insulin lispro and its variability. In comparison with NAT injections, both the absorption and action of insulin lispro were blunted and variability was substantially increased with LHT injections, leading to significantly higher postprandial glucose excursions after a mixed meal.

The notion that insulin absorption from LHT is blunted is supported by older data with radioactively labeled insulin that is cleared more slowly from sites with than sites without LHT (12). Furthermore, several case studies reported substantial improvements in glycemic control (A1C reductions of >2%) with lower insulin doses (up to 10%) when patients were instructed to avoid LHT areas for insulin injection (6,13,14). Indeed, Grassi et al. (15) recently reported a nearly 0.6% decrease in A1C, and a small (2 unit) decrease in insulin dosing over 3 months, in patients with LHT taught to avoid these areas and rotate injection sites. However, the study was uncontrolled, and there was a 25% loss of study participants to follow-up.

Other previously published studies did not detect a relevant impact of LHT on diabetes control. Hauner et al. (2) reported similar A1C levels and insulin demands in patients with and without LHT and two other meal test studies in small numbers of patients with type 1 diabetes (n = 8 and n = 9) only showed small, mostly nonsignificant differences between LHT and NAT injections (7,8). These studies were likely prone to a type 2 statistical error because of their limited sample size and large differences in pretest BG levels (7).

Our study not only had higher statistical power due to the larger sample size, it also used the euglycemic glucose clamp technique as a state-of-the-art method for the assessment of insulin PD (17), which has higher precision than meal tests. Furthermore, we replicated injections into LHT and NAT during the clamp tests, which further increased statistical power and permitted determination of intrasubject variability. We also standardized predosing conditions by a consistent washout protocol for ongoing insulin therapy, and in particular by adjusting BG tightly to a premeal level of 100 mg/dL. In the MMTTs, we observed a separation of the postmeal BG curves after 30 min leading to significant differences in BG levels from 2 h onwards, and in maximum postprandial BG concentrations. These BG differences highlight the clinical impact of the glucose clamp data in our study, i.e., a blunted PK/PD response and considerably higher insulin variability with LHT injections. A recent observational study in 401 patients in four Chinese cities found 53.1% LHT prevalence; the LHT patients had 0.5% higher A1C levels despite taking nearly one-third more (0.13 units/kg, 31.7%) insulin daily than patients without LHT (16), consistent with impaired insulin uptake from LHT areas as demonstrated here.

In individual patients, the impact of LHT on insulin PK/PD and consequently on diabetes control could be even more extreme. Two of our patients had almost no PK/PD response with LHT injections, neither in the clamp nor in the MMTT. In contrast, their PK/PD response with NAT injections was similar to that observed in other patients. Both patients experienced hyperglycemia after LHT injection in the MMTT, which underlines the potential impact that an avoidance of LHT lesions for insulin injection could have for diabetes control, at least in these individuals.

Potentially adverse clinical outcomes with insulin injections into LHT might be

Table 2—PK and PD end points of the MMTTs

PK end point	LHT	NAT	*P* value	PD end point	LHT	NAT	*P* value
$AUC_{INS0–0.5h}$ (h * mU/L)	10. 5 ± 6.7	11.9 ± 5.7	0.3236	$AUC_{BG0–0.5h}$ (h * mg/dL)	60.7 ± 7.4	57.3 ± 6.7	0.1827
$AUC_{INS0–1h}$ (h * mU/L)	34.6 ± 18.8	43.5 ± 12.8	0.1320	$AUC_{BG0–1h}$ (h * mg/dL)	141.9 ± 20.1	131.5 ± 20.1	0.1121
$AUC_{INS0–2h}$ (h * mU/L)	74.7 ± 39.4	101.3 ± 31.4	0.0727	$AUC_{BG0–2h}$ (h * mg/dL)	301.5 ± 67.8	259.9 ± 56.4	0.0411
$AUC_{INS0–3h}$ (h * mU/L)	102.3 ± 56.9	139.6 ± 53.7	0.0619	$AUC_{BG0–3h}$ (h * mg/dL)	448.0 ± 138.9	355.3 ± 92.0	0.0265
$AUC_{INS0–4h}$ (h * mU/L)	117.9 ± 67.6	161.3 ± 71.2	0.0571	$AUC_{BG0–4h}$ (h * mg/dL)	588.9 ± 216.3	435.2 ± 116.5	0.0181
$AUC_{INS0–5h}$ (h * mU/L)	124.3 ± 75.5	172.2 ± 83.6	0.0528	$AUC_{BG0–5h}$ (h * mg/dL)	731.3 ± 299.8	513.0 ± 138.3	0.0160
C_{max} (mU/L)	53.9 ± 27.1	76.9 ± 28.2	0.0715	BG_{max} (mg/dL)	199 ± 58	157 ± 33	0.0431
$T_{max-INS}$ (min)	46.5 ± 28.8	58.1 ± 30.8	0.2803	T_{max-BG} (min)	106 ± 92	51 ± 12	0.0393
				BG_{2h} (mg/dL)	150 ± 69	104 ± 42	0.0401
				BG_{5h} (mg/dL)	145 ± 94	81 ± 34	0.0167
				$AUC_{BG2–5h}$ (h * mg/dL)	429.8 ± 242.6	253.1 ± 89.9	0.0123

Results are given as mean ± SD.

due not only to impairments in insulin absorption and action (which could be addressed by using higher insulin doses) but could also include increased variability in insulin absorption, leading to erratic and unpredictable BG concentrations impeding insulin titration (4,6,14,18). The worsened intrasubject insulin variability seen in this study is quite substantial. PK variation seen with NAT injections (11–20%) was similar to previously reported data for rapid-acting insulin analogs (10) but increased by a factor of three to nearly five with LHT injections. High glucose variability has been identified as an important predictor for severe hypoglycemia (19), and indeed frequent unexplained hypoglycemia was approximately sixfold more common in patients with LHT in the study by Blanco et al. (4), consistent with the PK/PD observations reported here. Our data indicate that a relatively simple procedure, i.e., educating patients to avoid insulin injections into LHT and to practice good injection site rotation, could both improve insulin absorption (lowering daily insulin consumption) and reduce variability of insulin action; the latter seems to be essential to safely tighten glucose control (20).

Histological findings in LHT lesions are characterized by hypertrophic adipocytes (13) and usually reduced vascularization (1,21) and lower capillary density (22,23). Fewer capillaries at injection sites may contribute to impaired insulin absorption and also increased variability (insulin uptake might vary with the proximity of the subcutaneous insulin depot to capillaries). A higher fat density is also indicated by the diffuse, hyperechogenic appearance of LHT lesions in the ultrasound examinations used in the screening procedures. LHT is usually characterized by palpable subcutaneous lesions that may or may not be visible. In long-term insulin users, it can be quite difficult to distinguish between LHT and NAT just through palpation. It has therefore been proposed to add ultrasound to diagnose LHT (4,24). We included only patients with clear LHT lesions (confirmed by both palpation [performed by two different investigators] and ultrasound) in this study, but further work is needed to formally address the usefulness of ultrasound to diagnose LHT or monitor changes in lesions.

In addition to including only patients with palpable LHT lesions confirmed by ultrasound, study strengths include use of modern methodology such as the euglycemic clamp and the rigorous standardization of experimental procedures. A limitation of this study could be the relatively small sample size (13 patients), in particular in the MMTT, which only included one injection per NAT and LHT region. Furthermore, it is always difficult to transfer results from glucose clamps into clinical practice. This was one reason for adding a meal test to the study design. We recognize that the high standardization of baseline conditions during the MMTT is not directly comparable to the daily life of patients, so that larger, "real world" clinical studies are needed to further quantify the impact of LHT on clinical outcomes. In addition, studies are needed to investigate if LHT also influences the PK/PD effect of basal and bolus insulin administered via insulin pump, as data on the incidence and impact of LHT in pump users are scarce (1). Similar effects with infused insulin could impact closed-loop artificial pancreas effectiveness. Another limitation of this study was the application of repeated insulin doses during both clamp and MMTT periods. This was done to avoid too many visits by study participants to the study site but might have led to slightly different baseline conditions prior to each injection, particularly during the MMTT. Whereas the first dosing was done after a prolonged fasting period of at least 10 h, the second dosing was usually done "only" 6 h after ingestion of the first test meal. Nevertheless, although a bias due to the different feeding conditions cannot be excluded, this should have led to an underestimation of the observed differences in PK and PD. We also randomized the order of regional injections in the MMTTs.

In conclusion, lispro injection into LHT results in substantial impairment and increased variability of both insulin absorption and action in euglycemic clamps, confirmed with MMTTs showing prolonged deteriorations in postprandial BG control. The magnitude of our findings stresses the significance of educating patients in proper injection technique, preventing and avoiding LHT for all insulin injections.

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BD had no role in the study execution, analyses, interpretation of the data, or decision to submit results, but L.Hi. made major scientific contributions to the manuscript.

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