The Importance of Palmitoleic Acid to Adipocyte Insulin Resistance and Whole-Body Insulin Sensitivity in Type 1 Diabetes


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Context: Type 1 diabetes is an insulin-resistant state, but it is less clear which tissues are affected. Our previous report implicated skeletal muscle and liver insulin resistance in people with type 1 diabetes, but this occurred independently of generalized, visceral, or ectopic fat.

Objective: The aim of the study was to measure adipose tissue insulin sensitivity and plasma triglyceride composition in individuals with type 1 diabetes after overnight insulin infusion to lower fasting glucose.

Design, Patients, and Methods: Fifty subjects (25 individuals with type 1 diabetes and 25 controls without) were studied. After 3 d of dietary control and overnight insulin infusion, we performed a three-stage hyperinsulinemic/euglycemic clamp infusing insulin at 4, 8, and 40 mU/m²·min. Infusions of [1,1,2,3,3-2H2]glycerol and [1-13C]palmitate were used to quantify lipid metabolism.

Results: Basal glycerol and palmitate rates of appearance were similar between groups, decreased more in control subjects during the first two stages of the clamp, and similarly suppressed during the highest insulin dose. The concentration of insulin required for 50% inhibition of lipolysis was twice as high in individuals with type 1 diabetes. Plasma triglyceride saturation was similar between groups, but palmitoleic acid in plasma triglyceride was inversely related to adipocyte insulin sensitivity. Unesterified palmitoleic acid in plasma was positively related to insulin sensitivity in controls, but not in individuals with type 1 diabetes.

Conclusions: Adipose tissue insulin resistance is a significant feature of type 1 diabetes. Palmitoleic acid is not related to insulin sensitivity in type 1 diabetes, as it was in controls, suggesting a novel mechanism for insulin resistance in this population. (J Clin Endocrinol Metab 98: E40–E50, 2013)

Type 2 diabetes is well known to involve whole-body and tissue-specific insulin resistance, most notably in adipose tissue, liver, and skeletal muscle (1–4). Despite evidence that type 1 diabetes is also an insulin-resistant state, far less is known about the site(s) of insulin resistance (5–11). Previous work from our group highlighted whole-body insulin resistance, involving both skeletal muscle and liver, in a cohort of individuals with type 1 diabetes (7, 12). Interestingly, the mechanisms underlying insulin resistance in type 1 diabetes appeared discrete from that in type 2 diabetes. Specifically, increased adiposity—whether generalized, visceral, or ectopic—was not seen in our participants with type 1 diabetes. This observation led to the speculation that fat may be handled differently in the pathogenesis of insulin resistance in people with type 1 diabetes. For example, if adipocytes themselves are insulin...
resistant, chronic elevation of free fatty acids (FFAs) may perpetuate insulin resistance, atherogenesis, and cardiovascular disease (CVD) independent of body mass index (BMI).

To date, few investigations have examined the contribution of the adipocyte to the pathogenesis or complications relevant to type 1 diabetes. Although not all studies agree (13), adipocyte insulin resistance in type 1 diabetes has been reported in children using microdialysis (14) and in adults using FFA and glycerol concentrations during a multistage insulin clamp, as well as a graded insulin infusion (7, 15). These studies lend support for the notion that altered adipocyte action exists in type 1 diabetes, yet questions remain as to whether the alterations relate to changes in the regulation of lipolysis, pathways of reesterification, or plasma-borne by-products of either. Given the innumerable downstream effects of adipokines, delineation of altered adipocyte action may be key in understanding the largely unexplained CVD risk in people with type 1 diabetes.

Some of the increased CVD risk in individuals with type 1 diabetes may be related to alterations in plasma triglyceride composition, which is related to whole-body insulin resistance (16). However, little is known regarding the composition of plasma triglyceride or FFAs in individuals with type 1 diabetes. One adipose tissue-derived fatty acid that has received recent interest is palmitoleic acid (16:1n-7), which was described as an insulin sensitizer (17). This FFA promoted insulin sensitivity when given to muscle cells and when infused into rodents, in addition to decreasing hepatic steatosis. As a result of the ability of this adipose tissue-derived FFA to influence signaling in other tissues, it was suggested to be termed a “lipokine.” There are no publications reporting plasma palmitoleic acid concentration or the relationship to insulin sensitivity in individuals with type 1 diabetes. The exact nature of insulin resistance in type 1 diabetes is unclear, and not simply a result of glucose toxicity (12). Alterations in the plasma concentration or sensitivity to plasma palmitoleic acid in type 1 diabetes could play a role in insulin resistance that has so far gone unnoticed.

The aim of the current study was to quantify adipocyte insulin sensitivity in a group of individuals with type 1 diabetes compared with a control group (CON) using state-of-the-art stable isotope techniques. We hypothesized that individuals with type 1 diabetes would have decreased adipocyte insulin sensitivity, greater plasma triglyceride saturation, and decreased plasma concentration of palmitoleic acid compared with nondiabetic CON.

Subjects and Methods

Of the 87 individuals who completed the Coronary Artery Calcification in Type 1 diabetes (CACTI) insulin clamp substudy, isotope tracer data were analyzed from 50 subjects (25 people with type 1 diabetes and 25 matched people without diabetes). The CACTI study enrolled 1416 adults between 19 and 56 yr of age—652 with type 1 diabetes, and 764 without any history of diagnosed diabetes. Study participants completed a fasting examination, including the measurement of lipids, blood pressure, glucose, glycosylated hemoglobin (HbA1c), and anthropometric measurements, including height, weight, and waist and hip circumference. All study participants had coronary artery calcium measured using electron beam computed tomography. All study participants were invited to return for follow-up visits 3 and 6 yr after the baseline examination, and all measures were repeated at each visit (18). Inclusion criteria for initial enrollment of type 1 diabetic subjects in the CACTI study were: age, 19–56 yr; no history of CVD; on insulin therapy within 1 yr of diagnosis and current insulin therapy; diagnosed before age 30; and/or with positive antibodies; and diabetes duration of at least 10 yr. Within this substudy, inclusion criteria included those listed above as well as HbA1c no greater than 9.5%, albumin excretion rate below 200 μg/min, triglycerides below 400 mg/dl, blood pressure below 160/100 mm Hg, and a coronary artery calcium measurement at the 6-yr (visit 3) CACTI follow-up. All participants provided written informed consent, and the study was approved by the Colorado Multiple Institutional Review Board.

Screening visit

After signing the informed consent, subjects made a preliminary visit to the Clinical Translational Research Center (CTRC). This visit included a medical history questionnaire, blood draw for TSH, medication inventory, diet questionnaire, insulin record, alcohol and tobacco use, and physical activity questionnaire. A pregnancy test was administered for all female participants. Body composition was determined using dual energy x-ray absorptiometry analysis (Lunar DPX-IQ; Lunar Corporation, Madison, WI).

Diet and activity control

Subjects were provided a diet with a standardized macronutrient composition (50% carbohydrate, 20% protein, 30% fat) for 3 d before their study day and were asked to refrain from vigorous physical activity, smoking, and alcohol. Daily energy requirement was estimated from the dual energy x-ray absorptiometry measurement of fat free mass using the equation: daily energy intake = 1.4 kcal/d × [372 + (23.9 × fat free mass)], and the analysis of dietary records (19). Premenopausal women were scheduled for their study during the follicular phase of their menstrual cycle (d 2–10) to reduce this confounder of insulin sensitivity (20).

Hyperinsulinemic-euglycemic clamp visit

Subjects were admitted to the inpatient CTRC the evening before their study. Subjects with type 1 diabetes were instructed to take their last long-acting insulin injections at least 12 h before admission (24 h before the start of the clamp protocol). Dinner was provided on the unit, and subjects then fasted overnight and through the clamp protocol. Subjects with type 1 diabetes were given bolus insulin for dinner per their usual regimen.
individuals with type 1 diabetes were maintained overnight on iv regular insulin with adjustments to achieve euglycemia by morning. Intravenous insulin was titrated to a goal of 100–150 mg/dl from 2000–0400 h, and then 80–110 mg/dl from 0400–0700 h. The mean final insulin infusion rate before beginning the basal period averaged 7.1 mU/m² · min.

On the morning of the clamp study at approximately 0700 h, two antecubital catheters were placed on the same arm—one for infusions of stable isotopes of glucose, and the other to infuse dextrose, insulin, and potassium during the insulin clamp. Additionally, a hand vein was catheterized on the contralateral arm for blood draws during the study using the heated hand vein technique. At 0800 h, a primed, continuous infusion of [1,1,2,3,3-2H₂]glycerol was initiated at 0.011 mg/kg · min, and a continuous infusion of [1-13C]palmitate was initiated with no prime at 0.04 μmol/kg · min and continued throughout a 2-h basal lead in period and the insulin clamp. Glucose kinetics was measured using [6,6-2H₂]glucose, and those data have been reported (12). For individuals with type 1 diabetes, the overnight insulin infusion was continued from 0700 h through the basal period until the first stage of the insulin clamp at 1000 h. Resting metabolic rate measurement and blood samples for determination of baseline hormones and substrates were performed over the final 30 min before the clamp. At 1000 h, a three-stage hyperinsulinemic euglycemic clamp was initiated and continued for the next 4.5 h using the method of DeFronzo et al. (21). Briefly, a primed continuous infusion of insulin was administered at 4 mU/m² · min for 1.5 h, 8 mU/m² · min for 1.5 h, and then 40 mU/m² · min for the final 1.5 h. A variable infusion of 20% dextrose was administered to maintain blood glucose at approximately 90 mg/dl. Arterialized blood was sampled every 5 min for bedside determination of glucose concentration (Analog Instruments USA, Inc., Lunenburg, MA), and the dextrose infusion was adjusted as necessary. During the last 30 min of each stage of the clamp, measurements of respiratory gas exchange were made via indirect calorimetry, and arterialized blood was taken for hormone and substrate measurements.

**Computed tomography**

Abdominal computed tomography scans for calculation of abdominal visceral fat area and liver to spleen density ratios as a relative measure of liver fat content (22) were performed within 1 yr of the clamp study.

**Plasma triglyceride saturation**

The screening visit blood draw was used to measure plasma triglyceride saturation because subjects were not on diet control. Plasma lipids were extracted and triglyceride isolated using solid-phase extraction cartridges as previously described (23). Extracted triglycerides were converted to fatty acid methyl esters using sodium methoxide and analyzed by gas chromatography/mass spectrometry. Peak identities were determined by retention time and mass spectra compared with standards of known composition.

**Isotope analysis**

Glycerol and palmitate isotopic enrichment were measured using gas chromatography/mass spectrometry (GC model 6890 and MS model 5973A; Hewlett-Packard, Palo Alto, CA). Glycerol was analyzed using [U-13C]glycerol as an internal standard and the triacetate derivative. Methylation and extraction of plasma palmitate were performed as previously described (24). Enrichments were calculated based on a standard curve of known enrichments and corrected for variations in abundance (25). Peak identities were determined by retention time and mass spectra compared with standards of known composition.

**Calculations**

Rates of appearance (Ra) and disappearance (Rd) of glycerol and palmitate were calculated using the Steele equation modified for stable isotopes (26). Volumes of distribution used in the calculations were 180 ml/kg for glycerol and 40 ml/kg for palmitate. The IC₅₀ values for inhibition of lipolysis were determined individually by log transforming insulin concentrations and using linear curve fitting to describe the relationship between log insulin and glycerol Ra. FFA Ra was calculated by dividing palmitate Ra by the percentage of total FFA accounted for by palmitate. Intracellular reesterification rates were calculated by subtracting glycerol Ra × 3 from FFA Ra. Extracellular reesterification was calculated by subtracting FFA Rd from whole-body fat oxidation.

**Statistical analysis**

Data are presented as mean ± SEM. Differences between groups were analyzed using a one-way ANOVA (SPSS Inc., Chicago, IL) with adjustments for multiple comparisons using Bonferroni for triglyceride composition analyses. Differences from basal to clamp stage within individuals were determined using a paired t test. Relationships between variables were determined using Pearson’s correlation coefficient. Differences in gender distribution between groups were determined using a x² test for categorical variables. An α level of 0.05 was used throughout.

**Results**

Demographic information for subjects is shown in Table 1. Groups did not significantly differ by age, BMI, percentage body fat, high-density lipoprotein cholesterol, estimated physical activity level, visceral fat content, liver density, or liver/spleen density ratio. HbA1c was significantly higher, and total cholesterol and triglyceride concentration were significantly lower in individuals with type 1 diabetes compared with CON (Table 1). The overnight insulin infusion resulted in steady-state insulin concentration during the basal period, with a mean glucose concentration of 115 ± 9 mg/dl before starting the insulin clamp (Supplemental Table 1, published on The Endocrine Society’s Journals Online web site at http://jcem.endojournals.org).

Plasma IL-6, TNF-α, and adiponectin were analyzed to determine whether systemic inflammation could explain insulin resistance in individuals with type 1 diabetes. We found no differences in these inflammatory markers between groups (Table 2). We found no significant difference in overall plasma triglyceride saturation between groups. After adjusting for multiple comparisons with Bonferroni, the only fatty acid making up triglyceride that
Glycerol concentration during the final 40 mU/m2

**TABLE 2.**

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>Type 1 diabetes</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>25</td>
<td>25</td>
<td>0.93</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>46.0 ± 1.5</td>
<td>45.0 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>% Males (n)</td>
<td>40 (10)</td>
<td>56 (14)</td>
<td>0.17</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.9 ± 0.8</td>
<td>26.6 ± 0.9</td>
<td>0.53</td>
</tr>
<tr>
<td>% Fat</td>
<td>30.0 ± 1.6</td>
<td>27.9 ± 1.4</td>
<td>0.27</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.4 ± 0.06</td>
<td>7.7 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>166 ± 5</td>
<td>141 ± 7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.005</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>55 ± 3</td>
<td>53 ± 3</td>
<td>0.68</td>
</tr>
<tr>
<td>Estimated physical activity (kcal/wk)</td>
<td>2,705 ± 364</td>
<td>1,786 ± 352</td>
<td>0.08</td>
</tr>
<tr>
<td>Visceral fat area (mm³)</td>
<td>48,716 ± 4,992</td>
<td>48,959 ± 5,128</td>
<td>0.97</td>
</tr>
<tr>
<td>Liver density (HU)</td>
<td>62.4 ± 1.3</td>
<td>61.1 ± 1.4</td>
<td>0.49</td>
</tr>
<tr>
<td>Liver/spleen density ratio</td>
<td>1.3 ± 0.03</td>
<td>1.3 ± 0.02</td>
<td>0.40</td>
</tr>
<tr>
<td>Diabetes duration (yr)</td>
<td></td>
<td>23.2 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>On hypertension medications (%)</td>
<td>8 (2)</td>
<td>44 (11)</td>
<td>0.002</td>
</tr>
<tr>
<td>On statins (%)</td>
<td>8 (2)</td>
<td>64 (16)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM. HDL, High-density lipoprotein.

<sup>a</sup> Significantly different than CON, P < 0.05.

was significantly different between groups was palmitoleic acid.

Figure 1 shows the results of the isotope tracer analysis for glycerol and palmitate concentration and enrichment. The overnight insulin infusion normalized plasma glycerol concentration with no difference between groups in the basal period (Fig. 1A; P = 0.19). As previously reported (12), the insulin infusion to lower blood glucose in individuals with type 1 diabetes resulted in significantly greater insulin concentrations compared with controls under basal conditions (Supplemental Table 1; P = 0.001). In individuals with type 1 diabetes, glycerol concentration was significantly higher than control subjects during the 4 mU/m²·min (P < 0.0001), but not the 8 (P = 0.11) or 40 mU/m²·min (P = 0.32) insulin infusions. Palmitate concentration was also not different between groups during the basal period (Fig. 1C; P = 0.26). However, palmitate concentration was significantly higher in individuals with type 1 diabetes compared with controls during the 4 (P < 0.0001) and 8 mU/m²·min (P < 0.0001) insulin infusions. There were no differences in palmitate concentration between groups during the 40 mU/m²·min insulin infusion (P = 0.13). Both palmitate and glycerol concentrations increased in individuals with type 1 diabetes during the 4 mU/m²·min insulin infusion because this stage represented a decrease in the mean insulin infusion compared with the basal period. Palmitate enrichment followed a similar pattern, with no difference between groups during the basal period (Fig. 1D; P = 0.10), and lower palmitate enrichment in individuals with type 1 diabetes compared with controls during the 4 (P = 0.0004) and 8 mU/m²·min

**TABLE 2.** Baseline plasma inflammatory markers and lipid content

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>Type 1 diabetes</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (pg/ml)</td>
<td>14.8 ± 2.0</td>
<td>22.8 ± 4.3</td>
<td>0.10</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>9.1 ± 1.7</td>
<td>9.7 ± 1.7</td>
<td>0.79</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>9.8 ± 1.2</td>
<td>12.5 ± 1.2</td>
<td>0.11</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>103 ± 9</td>
<td>77 ± 8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.03</td>
</tr>
<tr>
<td>Plasma TG saturation (%)</td>
<td>39.3 ± 1.0</td>
<td>36.7 ± 1.2</td>
<td>0.11</td>
</tr>
<tr>
<td>TG % composition myristic acid</td>
<td>2 ± 0.3</td>
<td>2 ± 0.2</td>
<td>0.20</td>
</tr>
<tr>
<td>TG % composition palmitic acid</td>
<td>28 ± 1</td>
<td>24 ± 1</td>
<td>0.04</td>
</tr>
<tr>
<td>TG % composition palmitoleic acid</td>
<td>3 ± 0.2</td>
<td>2 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.003</td>
</tr>
<tr>
<td>TG % composition stearic acid</td>
<td>10 ± 0.8</td>
<td>11 ± 0.8</td>
<td>0.26</td>
</tr>
<tr>
<td>% Fat</td>
<td>39 ± 2</td>
<td>42 ± 2</td>
<td>0.31</td>
</tr>
<tr>
<td>TG % composition linoleic acid</td>
<td>16 ± 1</td>
<td>16 ± 2</td>
<td>0.86</td>
</tr>
<tr>
<td>TG % composition linolenic acid</td>
<td>2 ± 0.5</td>
<td>2 ± 0.6</td>
<td>0.87</td>
</tr>
<tr>
<td>TG % composition arachidonic acid</td>
<td>1 ± 0.1</td>
<td>1 ± 0.1</td>
<td>0.86</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM. TG, Triglyceride.

<sup>a</sup> Significantly different than CON, P < 0.05.
There were no differences in palmitate enrichment during the 40 mU/m²·min (P = 0.43) insulin infusion.

Whole-body insulin sensitivity has been previously reported for the entire CACTI substudy (7), and our group recently presented data on skeletal muscle and hepatic insulin resistance in a subset of this cohort (12). Rates of adipocyte lipolysis are shown in Fig. 2, measured by glycerol and palmitate Ra. Figure 2A shows no differences in basal glycerol Ra between CON and type 1 diabetes (P = 0.54). Glycerol Ra is significantly greater in type 1 diabetes compared with CON during insulin infusion at 4 mU/m²·min (P = 0.006), but not different at 8 (P = 0.11) or 40 mU/m²·min (P = 0.27). Palmitate Ra is shown in Fig. 2B, with no significant difference in basal Ra between groups (P = 0.55). Palmitate Ra was significantly higher in type 1 diabetes compared with CON at 4 (P < 0.0001) and 8 mU/m²·min (P < 0.0001), with no significant differences between groups at 40 mU/m²·min (P = 0.40). The concentration of insulin required for 50% inhibition of lipolysis measured by glycerol Ra (IC50) was significantly greater in type 1 diabetes compared with CON (Fig. 2C; P = 0.001), and the relationship between plasma insulin concentration and glycerol Ra for both groups is shown in Fig. 2D. As shown in Fig. 2D, for any submaximal dose of insulin, glycerol Ra was higher in individuals with type 1 diabetes. Glycerol IC50 was significantly positively related to HbA1c (r = 0.543; P < 0.0001) and inversely related to percentage palmitoleic acid composi-
FIG. 2. A and B, Glycerol Ra (A) and palmitate Ra (B) during the basal period and insulin infusions of 4, 8, and 40 mU/m²/min. C, The concentration of insulin required for 50% inhibition of lipolysis (glycerol IC50). D, Relationship between insulin concentration and glycerol Ra in controls and individuals with type 1 diabetes. E and F, Relationship between glycerol IC50 and percentage esterified palmitoleic acid in plasma triglyceride (E) and percentage unesterified palmitoleic acid in plasma FFA (F). Values are expressed as means ± SEM. ¥, Significantly different than CON; §, significantly different than basal, P < 0.05.
tion of plasma triglyceride (Fig. 2E; \( r = -0.495; P = 0.002 \)) and plasma FFA (Fig. 2F; \( r = -0.357; P = 0.02 \)).

FFA Ra is shown in Fig. 3A, with no differences in the basal state, significantly higher Ra in type 1 diabetes compared with CON at 4 (\( P < 0.0001 \)) and 8 mU/m²·min (\( P < 0.001 \)), and no differences between groups at 40 mU/m²·min. There were no differences in intracellular FFA reesterification between groups at basal (\( P = 0.32 \)), 4 (\( P = 0.14 \)), 8 (\( P = 0.51 \)), or 40 mU/m²·min (\( P = 0.07 \)) (Fig. 3B). Whole-body fat oxidation is shown in Fig. 3C, with no significant difference in the basal state (\( P = 0.21 \)) but significantly higher rates of fat oxidation in individuals with type 1 diabetes at 4 (\( P = 0.003 \)), 8 (\( P = 0.0004 \)), and 40 mU/m²·min (\( P = 0.002 \)). Extracellular FFA reesterification was not different under basal conditions (\( P = 0.31 \)), was significantly increased in individuals with type 1 diabetes at 4 mU/m²·min (\( P = 0.001 \)), not significantly different at 8 mU/m²·min (\( P = 0.06 \)), and significantly lower at 40 mU/m²·min (\( P = 0.01 \); Fig. 3D).

The relationship between plasma palmitoleic acid (as a FFA not incorporated into triglyceride) and muscle insulin sensitivity is shown in Fig. 4. Plasma unesterified palmitoleic acid concentration was not significantly different between groups (CON, 15.8 ± 2 \( \mu \)mol/liter; type 1 diabetes, 11.5 ± 2 \( \mu \)mol/liter; \( P = 0.11 \)). However, there was a significant positive relationship between plasma unesterified palmitoleic acid concentration and glucose Rd during the high-dose insulin clamp (Fig. 4A; \( r = 0.64; P = 0.001 \)); no such relationship was observed in individuals with type 1 diabetes (Fig. 4B; \( r = -0.05; P = 0.93 \)).

**Discussion**

Well-established, but far less publicized than in type 2 diabetes, is the finding that adults and children with type 1 diabetes exhibit whole-body insulin resistance compared with their euglycemic peers (1, 5, 7, 10, 11, 27, 28). Our previous work elaborated on these findings and highlighted insulin resistance in skeletal muscle and liver in adults with type 1 diabetes (12). Unlike type 2 diabetes, neither skeletal muscle nor liver insulin resistance in type
1 diabetes related to whole-body, visceral, or ectopic fat content. Therefore, we hypothesized that fatty acid handling may be different in individuals with type 1 vs. type 2 diabetes. Major findings from this study demonstrate adipocyte insulin resistance in our human volunteers with type 1 diabetes, which related to the contribution of palmitoleic acid to plasma FFA and triglyceride. Furthermore, whole-body insulin resistance in individuals with type 1 diabetes appeared related to the loss of unesterified plasma palmitoleic acid to sensitize tissues to insulin. Together, these findings support unique mechanisms of insulin resistance in type 1 diabetes.

Despite being only modestly overweight (BMI, 26.6 kg/m²) and matched to controls, we found adipocyte insulin resistance in our volunteers with type 1 diabetes. Collectively, higher glycerol and palmitate Ra during the early stages of the clamp suggested insulin resistance in adipose tissue, but it was the significantly higher insulin concentration required to suppress adipocyte lipolysis 50% (IC₅₀) that confirmed adipocyte insulin resistance in our volunteers with type 1 diabetes. Lower adipocyte insulin sensitivity, measured via adipocyte glucose uptake and FFA release, has been reported in type 1 diabetes in some (29–31), but not all studies (32). Inhibition of lipolysis occurs at a low concentration of insulin; hence, this aspect of insulin sensitivity can be overlooked in human studies of type 1 diabetes where only single or higher doses of insulin are used (33). As shown in Supplemental Table 1, basal hyperinsulinemia was required to control blood glucose in individuals with type 1 diabetes, which has been shown to promote adipocyte insulin resistance to lipolysis in cell culture (34). Therefore, chronic hyperinsulinemia is one possible culprit in adipocyte insulin resistance in individuals with type 1 diabetes. Other possible mechanisms for the adipocyte insulin resistance observed include chronic hyperglycemia (35), as well as decreased insulin receptor number and binding reported in adipose tissue biopsies from individuals with type 1 diabetes (6). Moreover, our data suggest that failure to suppress lipolysis in response to insulin could result in increased plasma FFA concentrations habitually throughout the day and night, resulting in a more atherogenic plasma milieu and increased cardiovascular disease risk (36).

Increasing evidence supports the notion that lipid and lipoprotein composition, beyond concentration, dictates its biological action. Kotronen et al. (16) recently reported that whole-body insulin resistance in healthy humans related to triglyceride in plasma low-density lipoprotein, intermediate-density lipoprotein, and very low-density lipoprotein containing more saturated acyl groups, and not total triglyceride concentration. Therefore, we examined plasma triglyceride composition to determine whether differences between groups could explain insulin resistance in individuals with type 1 diabetes. Although no differences in overall plasma triglyceride saturation between groups were noted, two important observations were made. First, adipocyte insulin sensitivity related to the proportion of palmitoleic acid in plasma triglyceride. Second, subjects with type 1 diabetes had less palmitoleic acid in their plasma triglyceride. This raises the possibility that greater lipoprotein-derived palmitoleic acid delivery to adipose tissue promotes insulin sensitization, similar to that found in skeletal muscle (17). In the fasting state, the majority of triglyceride is contained in the very low-density lipoprotein and intermediate-density lipoprotein fractions (16), and the fatty acid composition of triglyceride

FIG. 4. Relationship between fasting plasma palmitoleic acid concentration and rate of glucose disposal measured during the 40 mU/m² · min clamp in nondiabetic controls (A) and individuals with type 1 diabetes (B).
represents hepatic synthesis and dietary fat intake from the preceding days (37). Therefore, our data suggest that individuals with type 1 diabetes may have reduced dietary palmitoleic acid intake or hepatic synthesis and desaturation. Whether dietary palmitoleic acid supplementation can enhance adipose tissue insulin sensitivity is unknown, but it is ripe for future study.

Lower esterified palmitoleic acid in plasma triglyceride in individuals with type 1 diabetes led us to question whether unesterified palmitoleic acid in plasma FFA may also be lower in this group. Several lines of evidence support a role of unesterified plasma palmitoleic acid in the regulation of insulin sensitivity, whether by a direct effect or by impacting adipocyte inflammation and/or insulin signaling in myocytes (17). Plasma palmitoleic acid has also been related to insulin sensitivity in humans at risk of developing type 2 diabetes (38), although this is not universally observed (39). It should be pointed out that unesterified plasma palmitoleic acid represents less than 5% of plasma FFA, and therefore small changes may have potent downstream effects. Although dietary intake clearly plays a role, unesterified palmitoleic acid is thought to be derived mainly from adipose tissue lipogenesis, with esterified palmitoleic acid derived from hepatic lipogenesis (17). Thus, any change in the circulating concentration of palmitoleic acid in these plasma pools may represent an additional mechanism by which this fatty acid influences insulin action. The inverse relationship between adipocyte insulin sensitivity and plasma palmitoleic acid in FFA and triglyceride pools suggests decreased esterified palmitoleic acid delivery, and/or decreased adipocyte palmitoleic acid storage may impact insulin sensitivity in this tissue. This is consistent with a previous study reporting a significant relationship between adipose tissue stearoyl-coenzyme A desaturase 1, adipose tissue palmitoleic acid content, and plasma unesterified palmitoleic acid content (39). Additional analysis revealed a significant positive relationship between plasma unesterified palmitoleic acid content and glucose Rd (a measure predominantly of skeletal muscle insulin sensitivity) only in control subjects, lending support for a role of palmitoleic acid in additional insulin-sensitive tissues. In sum, diminished palmitoleic acid synthesis and loss of insulin sensitization by plasma palmitoleic acid may be a novel mechanism of insulin resistance in type 1 diabetes.

It is unclear why palmitoleic acid would not be related to insulin sensitivity in individuals with type 1 diabetes as it was in those without diabetes. Similar plasma concentration in each group eliminates differences in fat distribution playing a role because gluterofermal sc adipose tissue produces more palmitoleic acid compared with abdominal sc adipose tissue (40). Palmitoleic acid may alter liver metabolism because it is a transcription factor that binds to a polyunsaturated fatty acid element to regulate hepatic stearoyl-coenzyme A dehydrogenase expression (17). Additionally, palmitoleic acid can bind peroxisome proliferator activated receptor-γ response element, which is enriched in adipocytes, to modulate transcription of genes involved in adipocyte insulin sensitivity (41). Therefore, it is possible that individuals with type 1 diabetes have decreased binding or sensitivity to palmitoleic acid stimulation of hepatic polyunsaturated fatty acid element or adipocyte peroxisome proliferator activated receptor-γ response element compared with nondiabetic controls. Although the exact mechanism(s) is unconfirmed, palmitoleic acid is not related to insulin sensitivity in individuals with type 1 diabetes, as in controls. Identification of such a mechanism may lead to novel therapeutics for insulin sensitization.

There are several limitations to our study that are important to present. Fasting hyperglycemia was reduced in individuals with type 1 diabetes by an overnight insulin infusion to prevent measuring insulin resistance associated with acute glucose toxicity. A similar protocol has been used by others (8, 10), but it is possible that the infusion was not long enough to normalize hyperglycemia-induced insulin resistance. Additionally, it is possible that the overnight insulin infusion may have led to desensitization of insulin receptors promoting insulin resistance as has been reported in cultured adipocytes (35). For individuals with type 1 diabetes, the first insulin dose during the clamp was less than the mean overnight insulin infusion rate. As a result, individuals with type 1 diabetes experienced a decrease, rather than an increase, in insulin concentration during the first stage of the clamp. This difference between groups may have influenced the progression of insulin action on tissues and the calculation of insulin sensitivity. The presence of anti-insulin antibodies is unlikely to explain the insulin resistance observed in individuals with type 1 diabetes because it has been shown that anti-insulin antibodies were unrelated to insulin resistance in individuals with type 1 diabetes (42). Our methods to determine plasma triglyceride saturation did not allow us to determine triglyceride molecular species from individual lipoprotein subfractions, so we may have missed important species differences between groups related to insulin resistance.

In summary, these data demonstrate that individuals with type 1 diabetes are insulin resistant at the level of the adipocytes. Systemic inflammation and plasma triglyceride saturation are not likely to explain this effect. Adipocyte insulin resistance may result in a more atherogenic plasma milieu over the course of a day, which may help explain the increased risk of cardiovascular disease in in-
individuals with type 1 diabetes. Lower palmitoleic acid esterified in plasma triglyceride was found in individuals with type 1 diabetes, was related to adipocyte insulin resistance, and may indicate decreased dietary intake or decreased hepatic synthesis and/or desaturation of this fatty acid. We also found that the positive relationship between plasma palmitoleic acid and insulin sensitivity observed in control subjects was lost in individuals with type 1 diabetes. Mechanisms explaining this phenomenon in type 1 diabetes are unknown but reveal a unique pathway for insulin resistance and, potentially, insulin sensitization and cardiovascular risk reduction in this population.

Acknowledgments

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