Systemic and regional free fatty acid metabolism in type 2 diabetes

ANANDA BASU, RITA BASU, PANKAJ SHAH, ADRIAN VELLA, ROBERT A. RIZZA, AND MICHAEL D. JENSEN
Endocrine Research Unit, Division of Endocrinology and Metabolism, Mayo Clinic and Foundation, Rochester, Minnesota 55905

Received 2 October 2000; accepted in final form 14 February 2001

ELEVATED FREE FATTY ACID (FFA) CONCENTRATIONS have been shown to impair muscle glucose uptake (4, 10, 23, 28) and impair insulin's ability to suppress hepatic glucose production (7, 11). Persons with type 2 diabetes have increased plasma FFA concentrations in the postabsorptive (31) and postprandial states (13, 25), suggesting that dysregulation of FFA metabolism could play a role in the development of hyperglycemia. Insulin resistance with respect to antilipolysis has been previously reported to occur in type 2 diabetes (8, 31) and to a lesser extent in obesity (9). Thus adipose tissue insulin resistance (as regards lipolysis) may contribute to insulin resistance with respect to glucose metabolism in type 2 diabetes via the provision of excess FFA.

It is now evident that body fat is not a uniform tissue but rather exhibits depot-specific variation in its responses to insulin (19, 26). These variations may explain the association of an upper body/visceral body fat distribution with insulin resistance and type 2 diabetes (5, 22, 24). There are reasons to suspect that some of the regional differences in the ability of insulin to suppress FFA release may be of particular import in type 2 diabetes. We found that splanchnic FFA release, a minimal estimate of visceral adipose tissue lipolysis, contributed only ~15% of systemic FFA under basal conditions in nonobese, nondiabetic humans but that it accounted for ~40% of systemic FFA during hyperinsulinemia (19). Given that obese type 2 diabetic adults typically have much more visceral fat than their lean, nondiabetic counterparts (5, 17, 33), this depot may potentially contribute relatively large amounts of FFA to the portal and systemic circulation during hyperinsulinemia. If excessive amounts of FFA are released from visceral (omentumal and mesenteric) fat, this could explain the association of visceral adiposity and the components of the metabolic syndrome (22, 24, 33). The site(s) of adipose tissue insulin resistance with regard to FFA release in type 2 diabetes has not been identified, however.

Not all investigators attribute the metabolic complications of obesity to visceral adiposity. Abate et al. (1) examined insulin-mediated glucose disposal in healthy middle-aged men with varying degrees of adiposity. They concluded that subcutaneous abdominal fat was more related to glucose kinetic responses than was visceral fat. Along this line, we found that upper body nonsplanchnic FFA release accounted for the majority of the excess FFA in postprandial upper body obese women (18). If excess adipose tissue FFA release contributes to insulin resistance in obesity, our data suggest that visceral fat may not be the source of excess FFA during hyperinsulinemia. This may not be the case in type 2 diabetes, however, given the greater amounts of visceral fat present in this condition than in uncomplicated upper body obesity. We therefore wished to test the hypothesis that the greater visceral
fat associated with type 2 diabetes is the source of excess FFA during hyperinsulinemia.

**RESEARCH DESIGN**

**Subjects.** After approval from the Mayo Institutional Review Board, 14 nondiabetic subjects and 14 subjects with type 2 diabetes gave informed written consent to participate in the study. All subjects were in good health and at a stable weight. None regularly engaged in vigorous physical exercise. None of the first-degree relatives of nondiabetic subjects had a history of diabetes. Five of the diabetic subjects were being treated with diet alone and the other nine with either a sulfonylurea or metformin. Both drugs were discontinued at least 3 wk before the study. Subjects were on no medications at the time of study other than either thyroxine or estrogen replacement therapy. All subjects were instructed to follow a weight maintenance diet containing 55% carbohydrate, 30% fat, and 15% protein for at least 3 days before the day of study.

**Experimental design.** The research protocol and these volunteers were part of another experiment described in detail elsewhere (3). Briefly, subjects were admitted to the Mayo Clinic General Clinical Research Center at 1700 on the evening before the study. A standard 10 cal/kg meal (55% carbohydrate, 30% fat, and 15% protein) was eaten between 1817 and 1800. After the meal, an 18-gauge catheter was inserted into a forearm vein, and an infusion of insulin was started in the diabetic subjects (100 U regular human insulin in 1 liter of 0.9% saline containing 5 ml of 25% human albumin) and saline in the nondiabetic subjects. The insulin infusion rate was adjusted to maintain plasma glucose concentrations at ~5 mmol/l during the night.

The next morning, the subjects were moved to the interventional radiology suite at ~0800. Femoral arterial, femoral venous, and hepatic venous catheters were placed as previously described (3, 19). The arterial catheter was used for blood sampling and the arterial sheath for infusion of indocyanine green (Akorn, Buffalo Grove, IL) at 0.25 mg/min. The sheath of the venous catheter was used for sampling of blood draining the leg, and the hepatic venous catheter was used to sample blood draining the liver.

At 0930 (t = 0 min), infusions of somatostatin (60 ng·kg<sup>-1</sup>·min<sup>-1</sup>), growth hormone (3 ng·kg<sup>-1</sup>·min<sup>-1</sup>), and glucagon (0.65 ng·kg<sup>-1</sup>·min<sup>-1</sup>) were started and continued until the end of the study. Insulin was infused at a rate of 0.25 mU·kg<sup>-1</sup>·min<sup>-1</sup> from 0 to 180 min and 0.5 mU·kg<sup>-1</sup>·min<sup>-1</sup> from 181 to 360 min. [9,10-<sup>3</sup>H]palmitate (New England Nucleic, Boston, MA) was infused at 0.3 μCi/min from 120 to 180 min and from 300 to 360 min to measure palmitate turnover. A dextrose infusion was also begun at 0930, and the infusion rate was adjusted to maintain plasma glucose concentrations at ~9.3 mM (~165 mg/dl) over the next 6 h.

**Analytical techniques.** All samples were placed in ice, centrifuged at 4°C, and separated. Plasma indocyanine green concentration was measured spectrophotometrically at 805 nm on the day of the study, as previously described (19). All other samples were stored at ~20°C until analysis. Plasma glucose was measured by a glucose oxidase method with the use of a YSI glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). [9,10-<sup>3</sup>H]palmitate specific activity (SA) was measured as previously described (20). [3-<sup>3</sup>H]gluco- cose SA was estimated as described previously (27). Plasma insulin was measured using a chemiluminescence method with the Access ultrasensitive immunoenzymatic assay system (Beckman, Chaska, MN). C-peptide and glucagon concentrations were assayed by radioimmunoassay (RIA, Linco Research, St. Louis, MO). Growth hormone was measured with the Access human growth hormone two-site immunoenzymatic assay (Beckman). Body composition (including fat-free mass, total fat mass, visceral fat area, and superficial and deep abdominal subcutaneous fat content) was measured using dual-energy X-ray absorptiometry (DPX-IQ scanner, Lunar Radiation, Madison, WI) combined with a single-slice computerized tomography (CT) scan at the L2-L3 interspace as previously described (14). A fascial plane divides abdominal subcutaneous fat into a superficial and a deep component, which may have physiologically different functions. These compartments were individually measured with use of a software program (Solaris Operating System, Sun Microsystems, Palo Alto, CA). By use of this program, the fascial plane was first delineated all around the circumference of the abdominal wall. Areas where the delineation of the fascial line was indistinct were joined using a curvilinear line to connect the edges of the visible fascia. Superficial abdominal subcutaneous fat was then calculated as the difference between total abdominal fat content and the fat content limited by this fascial plane.

**Calculations and statistics.** Splanchnic plasma flow was calculated by dividing the indocyanine green infusion rate by the arterial-hepatic venous concentration gradient of the dye. Likewise, leg plasma flow was calculated by dividing the dye infusion rate by the corresponding gradient across the leg. Systemic palmitate flux was estimated using isotope dilution techniques and steady-state equations. The regional palmitate balances across the splanchnic and leg tissues were calculated as described elsewhere (18). Briefly, the equations used were as follows:

\[
\text{splanchnic palmitate uptake} = \frac{\text{FAPalm} \times Q_{\text{HV}}}{1 - \left(\frac{\text{HVSA}}{\text{FASA}}\right)}
\]

(1)

where FAPalm and HVPalms are the mean palmitate concentrations in the femoral artery and the hepatic vein, respectively; FA<sub>SA</sub> and HV<sub>SA</sub> are the mean specific activities of [9,10-<sup>3</sup>H]palmitate in the femoral artery and hepatic vein, respectively; and Q<sub>HV</sub> is the median of quadruple estimates of splanchnic plasma flow

\[
\text{splanchnic palmitate release} = \frac{\text{Q}_{\text{HV}}}{1 - \left(\frac{\text{FA}_{\text{Palm}}}{\text{FAPalm}}\right)} + \text{SPU}
\]

(2)

where SPU is splanchnic palmitate uptake as obtained from Eq. 1. For calculating leg palmitate turnover, the corresponding venous concentrations and specific activities were obtained from femoral vein measurements. Nonplanchnic upper body palmitate uptake and release were calculated by subtracting the corresponding splanchnic and leg turnover data from whole body palmitate flux. Glucose turnover data were calculated as described (3).

Between-group differences in subject characteristics were tested for statistical significance with Student's unpaired t-test. Comparisons of turnover data were performed using the nonparametric Mann-Whitney rank sum test. A P value <0.05 was considered statistically significant.

**RESULTS**

**Subject characteristics.** The characteristics of the subjects are listed in Table 1. The two groups did not differ in age, fat-free mass, body mass index, or percent
body fat. Total abdominal fat area by CT was, however, greater ($P < 0.02$) in the diabetic than in the nondiabetic subjects. This difference was attributed entirely to a greater abdominal visceral fat content in the diabetic individuals, because the abdominal subcutaneous fat (deep and superficial) was comparable between groups. As anticipated, both fasting glucose and glycosylated hemoglobin concentrations were higher in the diabetic than in the nondiabetic subjects.

There was no correlation ($r = 0.15, P = 0.5$) between visceral fat content and percent body fat, either when both groups were taken together or when the diabetic and nondiabetic subjects were analyzed separately. On the other hand, there was a correlation ($r = 0.4, P = 0.05$) between percent body fat and abdominal deep subcutaneous fat content when all subjects were taken together. In contrast, there was no correlation between these parameters when the groups were analyzed separately.

**Arterial glucose, insulin, and palmitate concentrations.** Before initiation of the somatostatin, insulin, and glucose infusions at time 0, arterial glucose concentrations were slightly higher in the diabetic than in the nondiabetic subjects (7.1 ± 0.6 vs. 5.1 ± 0.1 mM, $P < 0.01$). The intravenous glucose infusion promptly increased arterial glucose levels to ~9.3 mM in both groups. Arterial glucose concentrations during the low-dose insulin infusion were slightly higher in the diabetic than in the nondiabetic subjects (10.3 ± 0.03 vs. 9.2 ± 0.03 mM, $P < 0.01$) but did not differ during the high-dose insulin infusion (9.4 ± 0.02 vs. 9.3 ± 0.04 mM) between groups (Fig. 1, top).

Likewise, arterial insulin concentrations (Fig. 1, middle) were higher in the diabetic than in the nondiabetic subjects during baseline, low, and high insulin infusion rates (baseline: 175 ± 15 vs. 131 ± 8; low: 76 ± 17 vs. 35 ± 9; and high: 38 ± 11 vs. 13 ± 2 μmol/l, $P < 0.01$). We also examined the relationship between palmitate concentrations and the other major FFA in all subjects. Palmitate was ~23% of total FFA in both groups and did not differ between groups.

**Systemic and regional palmitate release.** Systemic palmitate flux was greater in the diabetic than in the nondiabetic subjects during both the low (245 ± 71 vs. 109 ± 40 μmol/min, $P = 0.05$) and high (94 ± 19 vs. 38 ± 5 μmol/min, $P < 0.01$) insulin infusions. When the insulin infusion rate was increased from low to high, palmitate flux was suppressed ($P < 0.04$) in both groups (Fig. 2, top left). Splanchnic palmitate release was greater in the diabetic than in the nondiabetic subjects both during the low (20 ± 4 vs. 7 ± 1 μmol/min, $P < 0.01$) and high (11 ± 2 vs. 5 ± 1 μmol/min, $P < 0.02$) insulin infusion rates and was suppressed ($P < 0.01$) when the insulin infusion rate was increased from low to high (Fig. 2, bottom left) in both groups.

Palmitate release from the upper body nonsplanchnic region (Fig. 2, top right) tended to be greater in diabetic than in the nondiabetic subjects during the low-dose insulin infusion (195 ± 64 vs. 89 ± 36 μmol/min, respectively, $P = 0.08$) and was significantly greater during the high-dose (69 ± 15 vs. 30 ± 4 μmol/min, respectively, $P < 0.01$) insulin infusion. When the insulin infusion rate was increased from low to high, palmitate flux was suppressed ($P < 0.05$) in

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**Table 1. Volunteer characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Nondiabetic ($n = 14$, 11F/3M)</th>
<th>Diabetic ($n = 14$, 5F/9M)</th>
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</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>50 ± 3</td>
<td>55 ± 2</td>
</tr>
<tr>
<td>Blood pressure, mmHg</td>
<td>116 ± 76</td>
<td>130 ± 76</td>
</tr>
<tr>
<td>Fat-free mass, kg</td>
<td>46 ± 3</td>
<td>51 ± 3</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>28 ± 1</td>
<td>30 ± 1</td>
</tr>
<tr>
<td>Percent body fat, %</td>
<td>34 ± 2</td>
<td>33 ± 2</td>
</tr>
<tr>
<td>Total abdominal fat, cm²</td>
<td>299 ± 46</td>
<td>465 ± 38*</td>
</tr>
<tr>
<td>Visceral fat, cm²</td>
<td>98 ± 21</td>
<td>230 ± 21*</td>
</tr>
<tr>
<td>Deep SC abd fat, cm²</td>
<td>97 ± 20</td>
<td>108 ± 13</td>
</tr>
<tr>
<td>Glyco Hb, %</td>
<td>5.2 ± 0.2</td>
<td>8.0 ± 0.4*</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>5.3 ± 0.3</td>
<td>5.1 ± 0.4</td>
</tr>
<tr>
<td>HDL-C, mmol/l</td>
<td>1.4 ± 0.1</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>LDL-C, mmol/l</td>
<td>3.2 ± 0.3</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>1.5 ± 0.2</td>
<td>1.9 ± 0.2*</td>
</tr>
</tbody>
</table>

Values are means ± SE. SC abd, subcutaneous abdominal; Glyco Hb, glycosylated hemoglobin; HDL-C and LDL-C, high- and low-density lipoprotein cholesterol, respectively. *$P < 0.05$ diabetic vs. nondiabetic.

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**Fig. 1.** Arterial plasma glucose, insulin, and palmitate concentrations obtained in diabetic and nondiabetic subjects during the study.
both groups. Likewise, palmitate release across both legs was higher in the diabetic than in the nondiabetic subjects during both the low (15 ± 5 vs. 6 ± 2 μmol/min, respectively, P = 0.05) and high (7 ± 2 vs. 2 ± 0 μmol/min, respectively, P < 0.03) insulin infusions. Palmitate release was suppressed when the insulin infusion rate was increased (P < 0.02) from low to high (Fig. 2, bottom right) in both groups.

Fractional regional palmitate release. Regional palmitate release was also expressed as a percentage of the systemic palmitate flux (Fig. 3; fractional rates only during high insulin infusion shown). The fractional contribution of leg, splanchnic, and nonsplanchnic upper body lipolysis to systemic palmitate flux did not differ between groups during either the low- or the high-dose insulin infusions. In both groups, nonsplanchnic upper body region was the major contributor (74–78%) to systemic palmitate flux during the hyperinsulinemic intervals.

Correlation between abdominal fat compartments and systemic palmitate release. There was significant correlation (low: r = 0.4, P = 0.03; high: r = 0.6, P = 0.001) between visceral fat content and systemic palmitate release (Fig. 4, left, top and bottom, respectively) when data from both the diabetic and nondiabetic subjects were included in the analysis. However, when the diabetic and nondiabetic subjects were analyzed separately, there was no correlation between the parameters (data not shown). In contrast, there was no correlation (low: r = 0.35, P = 0.08; high: r = 0.22, P = 0.3) between abdominal deep subcutaneous fat content and systemic palmitate flux.
DISCUSSION

Because of our previous findings of selective splanchnic insulin resistance with respect to lipolysis in lean nondiabetic individuals (19), we hypothesized that splanchnic palmitate release would be a greater proportion of systemic release in viscerally obese type 2 diabetic subjects compared with equally obese nondiabetic subjects. Instead, we found that, in the presence of similar hyperglycemia and physiological hyperinsulinemia, type 2 diabetes resulted in a generalized impairment of insulin’s ability to suppress systemic lipolysis. This impairment existed equally across the splanchnic, leg, and nonsplanchnic upper body regions. There was no evidence for additional resistance of splanchnic tissues to insulin suppression of lipolysis. We found a significant correlation between visceral fat area and systemic palmitate release when both groups were combined for analysis. We used the glucose turnover data from these volunteers (3) to examine the relationship among deep abdominal subcutaneous fat, visceral fat, and glucose disappearance. Although there was a significant correlation between deep abdominal subcutaneous fat and whole body glucose disappearance during the high insulin infusion rate, this appeared to be accounted for by the correlation between visceral fat and deep abdominal subcutaneous fat.

There is some controversy regarding insulin’s effect on antilipolysis in type 2 diabetes (13). Some in vitro studies (2, 34) have found normal sensitivity of adipocytes obtained from people with type 2 diabetes to the antilipolytic effects of insulin, whereas others (15) have shown resistance to the antilipolytic action of insulin. In vivo studies have shown resistance to insulin suppression of lipolysis in type 2 diabetes. Euglycemic stepwise insulin clamp studies have shown reduced insulin suppression of lipolysis in nonobese subjects with type 2 diabetes (12). In the postabsorptive state, rates of lipolysis have been shown to be higher in obese diabetic than in nondiabetic subjects (21). However, plasma glucose, and more importantly plasma insulin concentrations, were not matched between groups in some of these reports. In the present experiments, plasma insulin and glucose concentrations were as similar as possible between groups. We confirmed that, under our experimental conditions of hyperglycemia and physiological hyperinsulinemia (conditions that frequently exist in individuals with type 2 diabetes between meals and at night), rates of systemic palmitate release were significantly greater in the diabetic than in equally obese nondiabetic subjects. This observation was especially relevant given that the insulin concentrations were slightly but significantly higher in the diabetic than in the nondiabetic subjects.

In an effort to determine the relative contributions of the leg, splanchnic, and nonsplanchnic upper body regions to systemic lipolysis, we calculated palmitate kinetics across these regions by a combination of isotope dilution and arteriovenous balance techniques. We have demonstrated that the fractional contribution...
of these regions to systemic lipolysis was comparable between groups during both the low and high insulin infusion rates. Although we hypothesized that individuals with type 2 diabetes would have an even greater contribution by the splanchnic tissues to systemic lipolysis, this was not the case. Note that the absolute rate of splanchnic lipolysis was greater in diabetic than in nondiabetic subjects. This, coupled with higher arterial palmitate concentrations, would result in greater hepatic FFA delivery in diabetic than in nondiabetic individuals. Despite this, the calculated rate of splanchnic glucose production was comparable between groups during the high-dose insulin infusion (3). This indicates that, under our experimental conditions of physiological hyperinsulinemia and hyperglycemia, greater FFA delivery to the diabetic liver did not lead to greater splanchnic glucose production. This concurs with similar observations, albeit by use of different techniques (29).

Abdominal visceral fat content has been associated with markers of insulin resistance and the metabolic syndrome (24, 32). Vague (33) was the first to report that upper body distribution of fat was strongly correlated with the obesity-related adverse health and metabolic consequences. An upper body fat distribution signifies fat present in the visceral and subcutaneous abdominal regions. In particular, abdominal visceral fat content is most strongly associated with the presence of insulin resistance (5) and the components of the metabolic syndrome. We (3) and others (1, 5, 16) have reported an inverse relationship among whole body glucose disappearance, insulin sensitivity index, and visceral fat content in nondiabetic and diabetic individuals. In these studies, we also found a direct relationship between abdominal visceral fat content and systemic and splanchnic rates of lipolysis during hyperinsulinemia. Perhaps more intriguing was the fact that, quantitatively, upper body nonsplanchnic tissues contributed a “lion’s share” (~75%) to systemic rates of lipolysis in both diabetic and nondiabetic volunteers. Taken together, our results indicate that, although abdominal visceral fat content is a marker of altered glucose and FFA metabolism, the nonsplanchnic upper body region supplies by far the major quantity of FFA to the systemic circulation.

Recent reports (16) suggest a relationship between abdominal deep subcutaneous fat content and insulin resistance. We corroborated this finding by demonstrating an inverse relationship between whole body glucose disappearance and abdominal deep subcutaneous fat content during the high-dose insulin infusion. However, visceral fat content was a better predictor of whole body glucose disappearance than deep abdominal subcutaneous fat, and in a multivariate analysis, deep abdominal subcutaneous adipose area did not add to visceral fat area in predicting glucose disappearance.

Like all experiments, this experiment also has limitations. These studies were performed during hyperglycemic conditions. Although glucose per se has not been shown to affect FFA flux (6), recent reports (30) suggest the presence of a “reverse fatty-acid glucose cycle” in the presence of hyperglycemia and hyperinsulinemia in nondiabetic subjects. However, in those experiments, the insulin concentrations were higher than those achieved in our present study, and the reduction of fat oxidation obtained could also have been due to hyperinsulinemia rather than to hyperglycemia per se.

In conclusion, we found that, during modest hyperglycemia and physiological hyperinsulinemia, there is a generalized resistance to insulin suppression of lipolysis in type 2 diabetes compared with equally obese, nondiabetic individuals. This resistance was uniform across the leg, splanchnic, and nonsplanchnic upper body regions such that there was no evidence of a further resistance of splanchnic tissues to insulin’s antilipolytic effect in people with type 2 diabetes. Although abdominal visceral fat was a correlate of increased systemic and splanchnic rates of lipolysis, upper body nonsplanchnic tissue was unquestionably the major contributor to systemic lipolysis. We conclude that, although visceral fat is a marker of increased lipolysis and impaired systemic glucose uptake, the major contributor to whole body lipolysis and, hence, systemic FFA delivery is nonsplanchnic upper body adipose tissue in both diabetic and nondiabetic subjects. Hence, therapeutic agents targeted at this adipose compartment would potentially have the most benefit in improving the metabolic milieu.

We thank C. Stacey-Oda, C. Etter, T. Madson, and B. Dicke for technical assistance, J. Feehan, B. Norby, and the staff of the Mayo General Clinical Research Center for assistance in performing the studies, and S. Leachman for assistance in preparation of the manuscript.

The study was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-29953 (R. A. Rizza) and DK-40484 (M. D. Jensen), the Minnesota Obesity Center (DK-50456), and the Mayo Foundation. P. Shah was supported in part by a research grant from Novo-Nordisk.

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