Effects of Free Fatty Acids on Glucose Uptake and Utilization in Healthy Women

Carol J. Homko, Peter Cheung, and Guenther Boden

To study effects of sex on free fatty acid (FFA)-induced insulin resistance, we have examined the effects of acute elevations of plasma FFA levels on insulin-stimulated total body glucose uptake in nine healthy young women. Euglycemic-hyperinsulinemic (~500 pmol/l) clamps were performed for 4 h with infusion of either lipid/heparin (L/H) to acutely raise plasma FFA levels (from ~600 to ~1,200 µmol/l) or saline/glycerol (S/G) to lower fatty acids (from ~600 to ~50 µmol/l). L/H infusion inhibited insulin-stimulated glucose uptake (determined with [3-3H]glucose) and glycogen synthesis by 31 and 40%, respectively (P < 0.01), almost completely abolished insulin suppression of endogenous glucose production (EGP) (13.6 vs. 10.0 µmol · kg⁻¹ · min⁻¹, NS), prevented the insulin induced increase in carbohydrate oxidation (8.1 vs. 7.4 µmol · kg⁻¹ · min⁻¹, NS), and stimulated fat oxidation (from 3.6 to 5.1 µmol · kg⁻¹ · min⁻¹, P < 0.01). These data showed that acute increases in plasma FFA levels inhibited the actions of insulin on glucose uptake, glycogen synthesis, and EGP in women to a degree similar to that previously reported in men. We conclude that at insulin and FFA levels in the postprandial range, women and men were susceptible to FFA-induced peripheral and hepatic insulin resistance. Diabetes 52:487–491, 2003

There is strong support for the notion that free fatty acids (FFAs) are an important link between obesity, insulin resistance, and type 2 diabetes. The evidence can be summarized as follows: (1) most obese people have increased plasma FFA levels (1,2); (2) acutely raising plasma FFA levels causes acute insulin resistance in nondiabetic and diabetic individuals (3–7); (3) chronically elevated plasma FFAs also produce insulin resistance. This could be shown when chronically elevated plasma FFA levels were normalized for 12 h, which increased insulin sensitivity by ~50% in obese nondiabetic and diabetic individuals (8). Until recently, not much attention had been paid to the possibility that sex may play a role in the effects of FFAs on insulin action. In fact, many of the studies that demonstrated that FFAs acutely inhibited insulin-stimulated glucose uptake were performed almost exclusively in men (3,5). In a recent paper, however, Frias et al. (9), while confirming the inhibitory effect of acute elevations of plasma FFAs on insulin-stimulated glucose uptake in men, failed to find similar effects in women. Because of the importance of their findings with respect to the role of FFAs in the pathogenesis of insulin resistance/type 2 diabetes (10) and because they seemed to be in conflict with the results of other studies that were performed either exclusively (7) or predominantly with women (8,11), we felt that it was important to reexamine the effects of acute elevation of plasma FFAs on insulin action in healthy women using the same experimental protocol that we and others have used previously to demonstrate FFA-induced insulin resistance in men (3,5).

RESEARCH DESIGN AND METHODS

Subjects. Ten healthy normal weight women were studied. Eight were studied twice (1–2 months apart), and the remaining two were studied once. The clinical characteristics of the study participants are shown in Table 1. None of the subjects had a family history of diabetes or other endocrine disorders, and none were taking any medications. All women were studied in the follicular phase of their menstrual cycle. All subjects were seen by the General Clinical Research Center biornutritionist before the studies to standardize their diet, which contained a minimum of 250 g carbohydrate for at least 2 days before the studies. Informed written consent was obtained from all participants after explanation of the nature, purpose, and potential risk of the study. The study protocol was approved by the Institutional Review Board for Human Research of Temple University School of Medicine.

Experimental design. All study participants were admitted to the General Clinical Research Center of Temple University Hospital in the afternoon of the day before the studies. They received a physical examination, blood was obtained for routine screening tests, and body composition was determined. At 6:00 P.M., all volunteers received their last meal before the studies, consisting of ~55% carbohydrate, 30% fat, and 15% protein. At ~6:00 A.M. the next morning, a short polyethylene catheter was inserted into one antecubital vein for infusion of all test substances, while another catheter was placed into the contralateral forearm vein for blood sampling. This arm was kept at ~70°C with a heating blanket to arterialize venous blood. An infusion of [3-3H]glucose for measurement of glucose turnover was begun at ~6:00 A.M., followed 2 h later by infusion of insulin and glucose (euglycemic-hyperinsulinemic clamp) together with infusions of either lipid/heparin (L/H) or saline/glycerol (S/G). (The addition of glycerol to the saline infusion was needed as a control for the presence of glycerol in the lipid solution.)

Procedures

Euglycemic-hyperinsulinemic clamps. Regular human insulin (Humulin R; Eli Lilly, Indianapolis, IN) was infused intravenously at a rate of 7 pmol · kg⁻¹ · min⁻¹ for 4 h starting at 0 min. Glucose concentrations were maintained at ~5 mmol/l by a variable rate infusion with 20% glucose.

L/H and S/G infusions. During L/H infusions, Liposyn II (Abbott Laboratories, North Chicago, IL), a 20% triglyceride emulsion containing 10% safflower, 10% soybean oil, and 25 g glycerol/100 ml plus heparin (0.4 units · kg⁻¹ · min⁻¹), was infused at a rate of 1.5 ml/min for 4 h. During S/G infusions,
Plasma insulin concentrations rose during L/H infusions, plasma FFA concentrations decreased from S/G infusions, corrected for the differences in baseline FFA and glycerol was infused at a rate of 2.25 g/h to simulate the infusion of glycerol contained in Liposyn II.

**Measurements.** Glucose turnover was determined with [3-3H]glucose as previously described (12). [3-3H]glucose infusion (a bolus of 40 μCi followed by a continuous infusion of 0.4 μCi · min⁻¹ · 6 h) was started 2 h before initiation of the clamp to assure isotope equilibration. Glucose was isolated from blood for determination of [3-3H]glucose specific activity as described (12). Changes in specific activity due to infusion of unlabeled glucose were prevented by addition of the isotope to the unlabeled glucose infused to maintain euglycemia. Rates of total body glucose appearance (Gapp) and disappearance (Gdis) were calculated using Steele’s equation for steady-state conditions (13).

The rate of glycolysis was obtained by dividing the whole-body 3H2O production rate by the specific activity of its precursor, plasma [3-3H]glucose, as described (14). Glycogen synthesis rates were obtained by subtracting rates of glycolysis from rates of glucose uptake (glycogen synthesis = Gsyn – glycolysis). Rates of EGP were obtained by subtracting the rates of glucose infusions needed to maintain euglycemia (GIR) from the isotopically determined Gapp (EGP = Gapp – GIR).

Respiratory gas exchange rates were determined at 30- to 60-min intervals with a metabolic measurement cart as described (15). Rates of protein oxidation were determined with Lusk tables, which are based on a nonprotein respiratory quotient of 0.707 for 100% fat oxidation and 1.00 for 100% carbohydrate oxidation.

**Body composition.** Body composition was assessed by biompedance analysis (BIL Systems, Clinton Township, MO) (17).

**Analytical procedures.** Plasma glucose was measured with a glucose analyzer with the oxidase method. Free insulin concentrations were determined in serum after deproteinization by radioimmunoassay with an antibody that cross-reacts only minimally (<0.2%) with proinsulin (Linco, St. Charles, MO). Plasma fatty acids were determined enzymatically in plasma containing a lipoprotein lipase inhibitor (Paroxam) with a kit from Wako (Richmond, VA). Glycerol was measured enzymatically.

**Statistical analysis.** Statistical analysis was assessed using two-way repeated measures ANOVA and two-tailed Student’s t test where indicated. All data are expressed as means ± SE.

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### RESULTS

**Glucose, insulin, and FFAs.** Plasma glucose concentrations were clamped at ~5 mmol/l and did not differ in the L/H and S/G studies. Plasma insulin concentrations rose from ~60 to ~500 pmol/l in both the L/H and S/G studies. Plasma FFA levels increased from 611 ± 500 pmol/l during L/H infusions (P < 0.001). During S/G infusions, plasma FFA concentrations decreased from 589 ± 89 to 45 ± 11 μmol/l (P < 0.001) (Fig. 1).

**Effects of FFAs on G_Rd: glycogen synthesis, and glycolysis.** Baseline G_Rd were similar in the L/H and S/G studies (14.0 ± 1.0 vs. 12.4 ± 0.8 μmol · kg⁻¹ · min⁻¹, NS). In response to insulin, the increase in G_Rd was nearly identical during the first 2 h of L/H and S/G infusions. Thereafter, the curves diverged so that by 240 min, G_Rd was 31% lower during L/H than during S/G infusion (31.9 ± 2.3 vs. 46.0 ± 4.5 μmol · kg⁻¹ · min⁻¹, P < 0.001). Corrected for the differences in baseline G_Rd, the L/H induced inhibition of insulin-induced G_Rd was 47% (Fig. 2).

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**FIG. 1.** Plasma glucose, insulin, and FFA concentrations in nine healthy women before and during 4 h of euglycemic-hyperinsulinemic clamping with coinfusion of either Liposyn II (90 ml/h) plus heparin (0.4 units · kg⁻¹ · min⁻¹) (●) or saline plus glycerol (2.25 g/h) (○). Shown are means ± SE.

Basal rates of glycogen synthesis and the glycogen synthesis response to insulin during the initial 2 h were the same in both studies. After 4 h, however, glycogen synthesis was 47% lower during L/H than S/G (13.6 ± 1.9 vs. 25.5 ± 4.1 μmol · kg⁻¹ · min⁻¹, P < 0.001).

Glycogen synthesis increased in response to insulin from 13.3 ± 1.5 to 18.4 ± 1.4 μmol · kg⁻¹ · min⁻¹ during L/H infusion (P < 0.01) and from 12.7 ± 1.3 to 20.5 ± 1.6 μmol · kg⁻¹ · min⁻¹ during S/G infusion (P < 0.001). The differences between the two studies were not significant.

**EGP.** EGP was completely suppressed by insulin during the S/G infusion (from 13.3 ± 0.8 to 25.7 ± 1.7 μmol · kg⁻¹ · min⁻¹ at 240 min, P < 0.001). L/H infusion severely inhibited the insulin-induced suppression of EGP; in fact, the decrease from 13.6 ± 0.9 to 10.0 ± 2.8 μmol · kg⁻¹ · min⁻¹ was not statistically significant (Fig. 3).

**Fat and carbohydrate oxidation.** Fat oxidation increased during L/H infusion from 3.6 ± 0.5 to 5.1 ± 0.7 μmol · kg⁻¹ · min⁻¹ (P < 0.01) and decreased during S/G infusion from 3.4 ± 0.4 to 0.9 ± 0.4 μmol · kg⁻¹ · min⁻¹ (P < 0.01) (Fig. 4).

Carbohydrate oxidation did not change during L/H infusion (8.1 ± 1.3 vs. 7.4 ± 2.6 μmol · kg⁻¹ · min⁻¹) but

### TABLE 1

Characteristics of study subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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<tbody>
<tr>
<td>n</td>
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</tr>
<tr>
<td>Race (Caucasian/African-American)</td>
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<tr>
<td>Age (years)</td>
<td>24.8 ± 1.5</td>
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<td>Height (cm)</td>
<td>162.3 ± 2.0</td>
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<td>Weight (kg)</td>
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<td>BMI (kg/m²)</td>
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<tr>
<td>Fat (%)</td>
<td>26.2 ± 2</td>
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Data are means ± SE.
increased during S/G infusion (from 9.3 ± 1.4 to 21.4 ± 1.3 μmol·kg⁻¹·min⁻¹, P < 0.001).

**DISCUSSION**

The results of this study showed that acute elevations of plasma FFAs into a high physiological range for 4 h resulted in a ~40% inhibition of insulin-stimulated glucose uptake and glycogen synthesis and an almost complete inhibition of insulin suppression of EGP in healthy normal weight women. These results were similar to those obtained by us and others in men (3,5,18–20) as well as in women (4,7,8). For instance, in a study with seven pregnant women, FFA inhibited insulin-stimulated glucose uptake by 43% (7). In another study with seven patients with type 2 diabetes (five women, two men), an acute increase in plasma FFA levels induced a 46% decrease in insulin-stimulated glucose uptake in the five women (4). In a third study with four healthy women and three men, FFA inhibited insulin-stimulated glucose uptake by 43% in the four young women (11). Thus, there is a considerable body of evidence to indicate that acute increases of plasma FFA levels into the high physiologic range inhibit glucose uptake stimulated by insulin levels in the postprandial range (400–600 pmol/l) to a similar degree in all individuals regardless of sex, age, presence or absence of diabetes, or pregnancy. These results, however, seem to disagree with data recently published by Frias et al. (9), who found that acute elevations of plasma FFAs affected insulin-stimulated glucose uptake in men but not in women while inhibiting insulin-mediated suppression of inhibited insulin-stimulated glucose uptake by 43% in the four young women (11). Thus, there is a considerable body of evidence to indicate that acute increases of plasma FFA levels into the high physiologic range inhibit glucose uptake stimulated by insulin levels in the postprandial range (400–600 pmol/l) to a similar degree in all individuals regardless of sex, age, presence or absence of diabetes, or pregnancy. These results, however, seem to disagree with data recently published by Frias et al. (9), who found that acute elevations of plasma FFAs affected insulin-stimulated glucose uptake in men but not in women while inhibiting insulin-mediated suppression of increased during S/G infusion (from 9.3 ± 1.4 to 21.4 ± 1.3 μmol·kg⁻¹·min⁻¹, P < 0.001).

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EGP in both men and women. These discrepancies, however, may be more apparent than real and can probably be explained by differences in protocol design and methods. Frias et al. used two different insulin infusion rates. One rate was comparable to that used in many other studies (40 mU·m⁻²·min⁻¹); but in that study, lipid and insulin were infused together for only 3 h, which was probably not long enough (4). For instance, as seen in Fig. 2, FFA-induced inhibition of insulin-stimulated glucose uptake of the women in the current study, as well as in pregnant women (7) and in men (3) from previous studies, did not become statistically significant until after 3.5 or 4 h. Moreover, Frias et al. used a lower-than-usual lipid infusion rate (50 vs. 90 ml/h of a 20% lipid emulsion). This difference may be important because FFA-induced insulin resistance is FFA dose dependent (3). Unfortunately, the true plasma FFA levels in the Frias study are not known. They were probably much lower than those reported because of in vitro lipolysis (no lipoprotein lipase inhibitor seems to have been added to the test tubes). Lastly, men were not studied with this protocol. Since it is not certain that under these conditions (3 h clamps, low lipid infusion) insulin resistance would have been detectable in men, no conclusions with respect to a sex difference can be drawn from this study.

In a different protocol (9), Frias et al. used a higher insulin infusion rate (80 vs. 40 mU·m⁻²·min⁻¹) than most other investigators, together with a lower lipid infusion rate (60 vs. 90 ml/h of a 20% lipid emulsion) in men and women, and showed that FFA produced peripheral and hepatic insulin resistance in men and hepatic but not peripheral insulin resistance in women. This suggested that the high insulin levels were able to compensate for the peripheral insulin resistance produced by relatively low FFA levels in women but not in men.

The physiological significance of this sex difference, which was apparent at relatively high insulin and low FFA levels, is uncertain. Particularly, in view of the results of the current and other studies that have demonstrated, in women and men, that serum insulin concentrations in the postprandial range (400–600 pmol/l) are unable to overcome insulin resistance produced by plasma FFA levels ranging from ~600 to 1,200 μmol/l. Of particular interest in this respect is the study by Santomauro et al. (8), who have shown that lowering plasma FFA levels from ~600 to ~300 μmol/l for 12 h resulted in a ~50% increase in insulin-stimulated glucose uptake in obese diabetic and nondiabetic women. This demonstrated that the chronically elevated FFAs had been responsible for insulin resistance in these women.

The molecular/biochemical causes for sex differences in FFA action have not been explored. It has recently been shown that the mechanism by which FFAs cause insulin resistance in men (21) and rats (22) involved accumulation of diacylglycerol and activation of protein kinase C in skeletal muscle. Conceivably, this complex process could be influenced at various steps by female sex hormones.

In summary, we found that when studied with plasma FFA and insulin levels in the postprandial range, young healthy women developed FFA-induced peripheral and hepatic insulin resistance that was similar in degree and time of onset to the insulin resistance previously reported in men under the same conditions. These data, however, do not exclude the possibility of a sex difference in susceptibility to FFAs, which becomes apparent at higher insulin concentrations. Thus, further studies are needed to define dose-response relationships between plasma FFA and insulin concentrations and the development of insulin resistance in men and women.

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