Elevated Free Fatty Acids Impair Glucose Metabolism in Women

Decreased Stimulation of Muscle Glucose Uptake and Suppression of Splanchnic Glucose Production During Combined Hyperinsulinemia and Hyperglycemia

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The present study sought to determine whether elevated plasma free fatty acids (FFAs) alter the splanchnic and muscle glucose metabolism in women. To do so, FFAs were increased in seven women by an 8-h Intralipid/heparin (IL/hep) infusion, and the results were compared with those observed in nine women who were infused with glycerol alone. Glucose was clamped at ~8.3 mmol/l and insulin was increased to ~300 pmol/l to stimulate both muscle and hepatic glucose uptake. Insulin secretion was inhibited with somatostatin. Leg and splanchnic glucose metabolism were assessed using a combined catheter and tracer dilution approach. The glucose infusion rates required to maintain target plasma glucose concentrations were lower ($P < 0.01$) during IL/hep than glycerol infusion (30.8 ± 2.6 vs. 65.0 ± 7.9 μmol · kg^{-1} · min^{-1}). Whole-body glucose disappearance (37.0 ± 2.2 vs. 70.9 ± 8.7 μmol · kg^{-1} · min^{-1}; $P < 0.001$) and leg glucose uptake (24.3 ± 4.2 vs. 59.6 ± 10.0 μmol · kg fat-free mass of the leg^{-1} · min^{-1}; $P < 0.02$) were also lower, whereas splanchnic glucose production (8.2 ± 0.8 vs. 4.3 ± 0.7 μmol · kg^{-1} · min^{-1}; $P < 0.01$) was higher during IL/hep than glycerol infusion. We conclude that in the presence of combined hyperinsulinemia and hyperglycemia, elevated FFAs impair glucose metabolism in women by inhibiting whole-body glucose disposal, muscle glucose uptake, and suppression of splanchnic glucose production. *Diabetes* 52: 38–42, 2003

Elevated plasma free fatty acid (FFA) concentrations are common in both obesity and type 2 diabetes (1–4). Currently, there is substantial evidence that FFAs may contribute to insulin resistance in both of these conditions (5–11). Elevated FFAs can decrease muscle glucose transport and muscle glycogen synthesis by inhibiting intracellular signaling (5,7,10,12). FFAs can also stimulate gluconeogenesis and impair insulin-induced suppression of hepatic glucose production (6,13). Therefore, the recent article by Frias et al. (14), showing that elevated FFAs lowered whole-body glucose uptake during a euglycemic-hyperinsulinemic clamp in men but not in women, was intriguing and potentially of considerable importance. In clinical settings hyperinsulinemia commonly occurs in conjunction with hyperglycemia and high FFA levels. The role of high FFAs in the above situation remains to be defined.

It is well established that fat metabolism differs in men and women (15–18). FFA release is greater and insulin-induced suppression of lipolysis is less in upper body fat than in lower body fat (19). Men have a greater portion of visceral and upper body fat than women (20–22). Insulin action, assessed using a variety of techniques, has been reported to be greater (15,23), lower (24), or no different (18,23,25,26) in women than men. On the other hand, hormone replacement therapy has been associated with increased (27), reduced (28), or unaltered (29) insulin concentrations.

We have recently reported that, in the presence of hyperglycemia and hyperinsulinemia, elevated FFAs impair muscle glucose uptake and stimulate hepatic glucose production but do not alter splanchnic glucose uptake (11). Because 16 of the 23 participants in this protocol were women, reanalysis of the data gave us an opportunity to determine whether the lack of effect of FFAs on whole-body glucose disposal in the presence of hyperinsulinemia and euglycemia, as reported by Frias et al. (14), also occurred during combined hyperglycemia and hyperinsulinemia. Furthermore, we directly measured leg and splanchnic glucose balance in those experiments. This enabled us to determine whether the similar rates of whole-body glucose disposal in women during the Intralipid and saline infusions reported by Frias et al. truly represented an absent response to FFAs or whether they were caused by offsetting changes in muscle and hepatic glucose metabolism. We report that in the presence of combined hyperglycemia and hyperinsulinemia, elevated FFAs impair whole-body glucose disposal, muscle glucose uptake, and suppression of splanchnic glucose production in women.

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FFA, free fatty acid; FFM, fat-free mass; IL/hep, Intralipid/heparin.

TABLE 1
Subject characteristics

Group	n	Age (years)	Weight (kg)	Percent fat	BMI (kg/m^2)
Glycerol	9	36 ± 4	70 ± 5	38 ± 3	26 ± 2
IL/hep	7	33 ± 5	76 ± 8	41 ± 4	28 ± 2

Data are means ± SE.

RESEARCH DESIGN AND METHODS

Subjects. After approval from the Mayo Institutional Review Board, 16 nondiabetic women gave written consent to participate in the study. All subjects were in good health and were at a stable weight. None of the first-degree relatives of the volunteers had a history of diabetes. None of the volunteers regularly engaged in vigorous exercise. Subjects were on no medications other than oral contraceptive pills, estrogen, or thyroxin replacement. One woman in the Intralipid/heparin (IL/hep) group was taking continuous conjugated equine estrogen and one each of ethinyl estradiol plus norgestrel and ethinyl estradiol plus desogestrel. One woman in the glycerol group was taking conjugated equine estrogen plus medroxyprogesterone acetate and one each of ethinyl estradiol plus norethindrone and ethinyl estradiol plus norgestimate per day. 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 study. Subject characteristics are given in Table 1. Age, weight, and BMI did not differ between the IL/hep and glycerol groups.

Experimental design. The experimental design has been reported in detail elsewhere (11). In brief, subjects were admitted to the Mayo Clinic General Clinical Research Center at ~1700 on the evening before the study and fed a standard meal between 1730 and 1800. They then fasted (with the exception of an occasional sip of water). At 0600 on the following morning, an 18-gauge catheter was inserted into the left forearm vein and an infusion of either Intralipid (Baxter Healthcare, Deerfield, IL; 20%, 0.013 ml · kg^{-1} · min^{-1}) and heparin (200 units prime, 0.2 unit · kg^{-1} · min^{-1} continuous) or glycerol (5 μmol · kg^{-1} · min^{-1} in four subjects and 20 μmol · kg^{-1} · min^{-1} in five subjects) was started.

Volunteers were taken to an intervention radiology suite at ~0800, where femoral arterial, femoral venous, and hepatic venous catheters were placed as previously described (3). Subjects were then returned to the general clinic research center for the remainder of the study. At 1000 (time 0), infusions of [3-^{3}H]glucose (12 μCi prime and 0.12 μCi/min continuous; New England Nuclear, Boston, MA), insulin (in 1.25% albumin minus 1.0 mU · kg^{-1} · min^{-1}), somatostatin (72 ng · kg^{-1} · min^{-1}; Bachem California, Torrance, CA), glucagon (0.65 ng · kg^{-1} · min^{-1}; Eli Lilly , Indianapolis, IN), and growth hormone (3.0 ng · kg^{-1} · min^{-1}; Genentech , San Francisco, CA) were started and continued until study end at 1400. Indocyanine green (0.25 mg/min; Akorn, Buffalo Grove, IL) was infused into the femoral artery beginning at 1300. A glucose infusion also was begun at 1000, and the rate was adjusted to maintain plasma glucose concentrations at ~8.3 mmol/l over the next 4 h. To minimize the change in plasma glucose specific activity, all infused glucose contained [3-^{3}H]glucose. In addition, the "basal" [3-^{3}H]glucose infusion was reduced in a pattern designed to mimic the anticipated pattern of change in glucose production (3). This resulted in plasma [3-^{3}H]glucose specific activity remaining essentially constant with the coefficient of variation averaging 3.9 ± 0.9% from 60 min onward and 1.3 ± 0.1% during the final 30 min of the study, when glucose turnover was measured. Palmitate and Amino acid tracers were also infused as part of another experiment.

Blood was collected at 0600, 1000, 1100, 1200, 1300, 1330, 1340, 1350, and 1400. Samples for FFAs were placed in tubes containing 50 μl Paraoxon (diethyl-*p*-nitrophenyl-phosphate) (Sigma Chemicals, St. Louis, MO) diluted to 0.04% in diethyl ether to prevent ex vivo lipolysis (30). All blood samples were collected in prechilled syringes and dispensed into prechilled tubes. Samples were centrifuged immediately at 4°C, and the plasma obtained from these tubes was stored at −20°C until analysis.

Analytical techniques. Plasma glucose, insulin, C-peptide, and growth hormone concentrations and [^{3}H]glucose specific activity were measured as previously described (11). Plasma Indocyanine green concentrations were determined by high-performance liquid chromatography using the method of Awni et al. (31). Plasma glycerol and FFA concentrations were measured by a microfluorometric enzymatic method (32). Body composition (including fat-free mass [FFM] and total fat mass) was measured by dual-energy X-ray absorptiometry (DPX-IQ scanner, SmartScan TM Version 4.6; Hologic, Walthan, MA) (33).

Calculations. Whole-body glucose disappearance was calculated using Steele's steady-state equations (34). Leg and splanchnic glucose balances were calculated as previously described (3,11). In brief, leg and splanchnic glucose balances were calculated by multiplying the arterial venous glucose differences by blood flow. Splanchnic and leg glucose extraction ratios were calculated by dividing the arterial-venous difference in tracer concentration by the arterial tracer concentration. Splanchnic glucose uptake was calculated as the product of splanchnic tracer extraction ratio, arterial glucose concentration, and splanchnic blood flow. Splanchnic glucose production was calculated by subtracting splanchnic glucose uptake from splanchnic glucose balance. Although splanchnic glucose production is technically a negative number, for the sake of clarity it is presented as a positive value.

Statistical analysis. Data in the figures and text are expressed as means ± SE. Rates are expressed as micromole per kilogram of whole body FFM per minute with the exception of leg uptake, which is expressed as micromole per kilogram of leg FFM per minute. The means of the reponses during the final 30 min of the study were used for statistical analysis. Since there were no differences among whole-body glucose disposal, leg glucose uptake, splanchnic glucose uptake, or splanchnic glucose production, the results of the two glycerol infusions were combined for analysis (glycerol group) and compared with the results observed during the IL/hep infusion. Student's two-tailed nonpaired t test was used to test for differences between the IL/hep and glycerol groups. A P value of <0.05 was considered statistically significant.

RESULTS

Plasma FFA, glucose, and glycerol concentrations. Plasma FFA concentrations before IL/hep or glycerol infusion did not differ between groups (Fig. 1). IL/hep infusion (begun at –240 min) increased FFAs, and insulin (begun at 0 min) decreased FFAs. FFA concentrations during the insulin infusion were higher ($P < 0.001$) on the IL/hep than glycerol study days (0.60 ± 0.08 vs. 0.05 ± 0.00 mmol/l). Plasma glucose concentrations did not differ between IL/hep and glycerol study groups before or during the IL/hep and glycerol infusions (Fig. 1). Glucose concentrations also did not differ between the two groups during

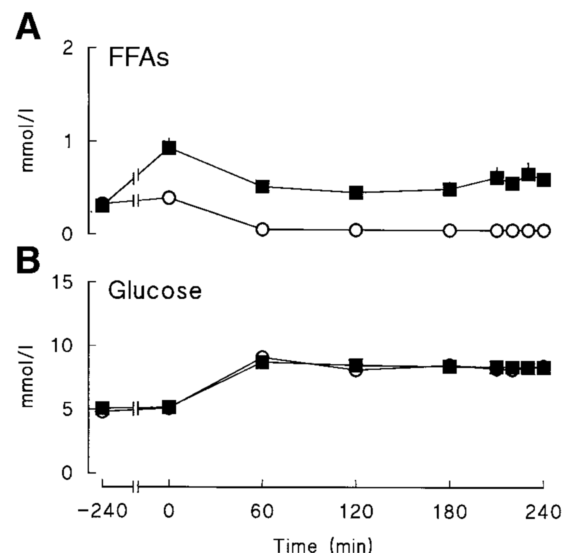

FIG. 1. Plasma FFAs and glucose concentrations during IL/hep (■) and glycerol (○) infusions. The IL/hep and glycerol infusions were started at –240 min and exogenous glucose, insulin, and somatostatin infusions at time 0.

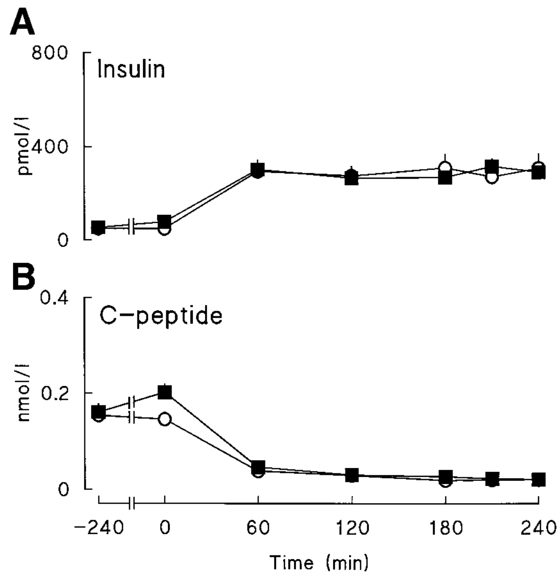

FIG. 2. Plasma insulin and C-peptide concentrations during IL/hep (■) and glycerol (○) infusions. The IL/hep and glycerol infusions were started at –240 min and exogenous glucose, insulin, and somatostatin infusions at time 0.

the final 30 min of the hyperglycemic-hyperinsulinemic clamps (8.4 ± 0.2 vs. 8.3 ± 0.1 mmol/l).

Plasma glycerol concentrations were comparable between the two groups (126 ± 18 vs. 120 ± 23 μmol/l) before the IL/hep and glycerol infusions. Although plasma glycerol concentrations were slightly lower during the final 30 min of the IL/hep than glycerol infusions (322 ± 86 vs. 437 ± 93 μmol/l), the values were not statistically different between the two groups.

Plasma insulin, C-peptide, glucagon, and growth hormone concentrations. Plasma insulin and C-peptide concentrations before the IL/hep or glycerol infusions were comparable in both study groups (Fig. 2). The exogenous insulin and somatostatin infusions (begun at time 0) resulted in a prompt increase in insulin and a prompt decrease in C-peptide concentrations. Plasma insulin (309 ± 114 vs. 379 ± 33 pmol/l) and C-peptide (0.02 ± 0.0 vs. 0.02 ± 0.0 nmol/l) concentrations during the final 30 min of the IL/hep and glycerol infusions did not differ between the two study groups. Plasma glucagon and growth hormone concentrations also did not differ between the two groups (data not shown).

Plasma [3-^{3}H]glucose specific activity, glucose infusion rates, and rates of glucose disappearance. Plasma [3-^{3}H]glucose specific activity was constant from 60 min onwards in both study groups, enabling accurate measurement of glucose turnover (Fig. 3). The glucose infusion rates required to maintain the target plasma glucose concentration during the final 30 min of study were lower (Fig. 4) in the IL/hep than glycerol group (30.8 ± 2.6 vs. 65.0 ± 7.9 μmol · kg^{-1} · min^{-1}; $P < 0.005$). Similarly, whole-body glucose disappearance rates were lower ($P < 0.001$) during the final 30 min of the IL/hep than glycerol infusion (37.0 ± 2.2 vs. 70.9 ± 8.7 μmol · kg^{-1} · min^{-1}).

Leg and splanchnic glucose uptake and splanchnic glucose production. Whereas leg glucose uptake (24.3 ± 4.2 vs. 59.6 ± 10.0 μmol · kg^{-1} · min^{-1}) was lower ($P < 0.02$) during the final 30 min of the IL/hep than glycerol infusions, splanchnic glucose uptake (12.2 ± 1.8 vs. 9.6 ± 1.6 μmol · kg^{-1} · min^{-1}) did not differ between groups (Fig. 5). Similarly, leg tracer extraction (4.3 ± 0.4 vs. 12.3 ± 1.9%) was lower ($P < 0.01$) in the IL/hep than glycerol group, whereas splanchnic glucose extraction did not differ between groups (5.3 ± 0.5 vs. 4.5 ± 0.8%). On the other hand, splanchnic glucose production (Fig. 6) was higher ($P < 0.01$) in the IL/hep than glycerol group (8.2 ± 0.8 vs. 4.3 ± 0.7 μmol · kg^{-1} · min^{-1}).

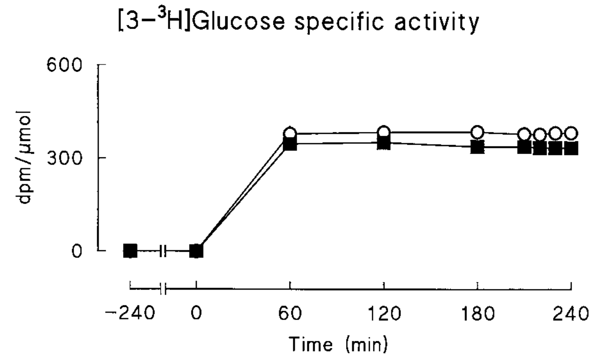

FIG. 3. Plasma [3-^{3}H]glucose specific activity during IL/hep (■) and glycerol (○) infusions. The IL/hep and glycerol infusions were started at –240 min. The [3-^{3}H]glucose, exogenous insulin, and somatostatin infusions were started at time 0.

DISCUSSION

Numerous studies have established that elevated FFAs can cause insulin resistance (5–10). However, as pointed out by Frias et al. (14), the effects of gender on the response to FFAs has been less well defined. The current experiments indicate that in the presence of hyperglycemia and hyperinsulinemia, elevated FFAs impair glucose metabolism in women. Glucose disposal was lower and splanchnic glucose production was higher in women during IL/hep than

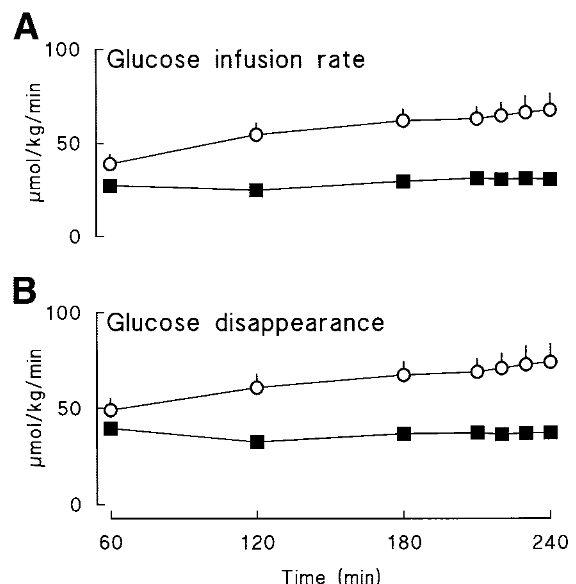

FIG. 4. *A*: The glucose infusion rates required to maintain plasma glucose concentrations at target levels. *B*: The whole rates of glucose disappearance. The IL/hep (■) and glycerol (○) infusions were started at –240 min and exogenous insulin and somatostatin infusions at time 0.

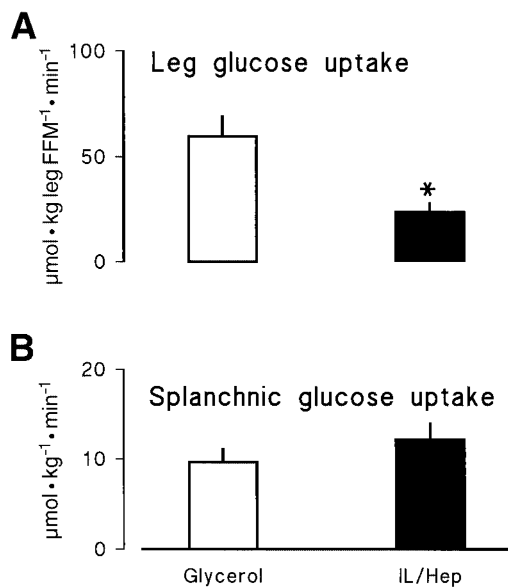

FIG. 5. Leg (*A*) and splanchnic (*B*) glucose uptake observed during the final 30 min of the IL/hep and glycerol infusions. *P < 0.02.

glycerol infusion. The lower rates of glucose disposal appear to be due to a decrease in muscle glucose uptake, since leg but not splanchnic glucose uptake was lower on the IL/hep than glycerol study days. These data indicate that alterations in fat metabolism potentially can contribute to alterations in carbohydrate metabolism in women.

The results of the current study and those of Frias et al. (14) differ in that the latter concluded that in the presence of hyperinsulinemia and euglycemia, elevated FFAs decreased whole-body glucose disposal in men but not women. In contrast, the present data indicate that in the presence of combined hyperinsulinemia and hyperglycemia, elevated FFAs can impair glucose disposal in women. These discordant findings are intriguing, since with the exception of the glucose concentrations present during the clamps, the experimental conditions of these two studies were similar in many regards. The age and BMI of the participants were comparable in both studies. Both used essentially the same insulin infusion rates, and similar plasma insulin concentrations were achieved. Although the plasma FFA concentrations were somewhat higher in the study of Frias et al. (14), this probably was due to the fact that lipase inhibitors were not added to the blood samples to prevent ex vivo lipolysis. However, since the duration and infusion rates of Intralipid and heparin were comparable in the two studies, it is likely that plasma FFA concentrations were comparable as well.

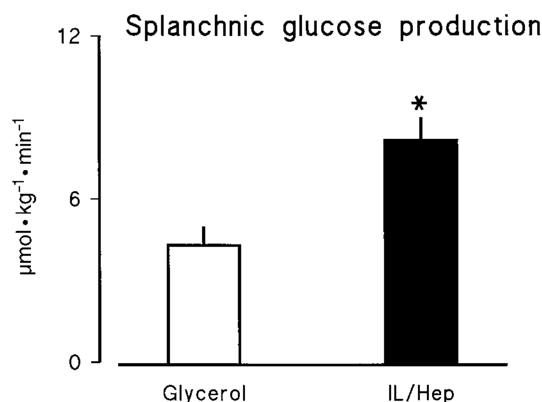

FIG. 6. Splanchnic glucose production observed during the final 30 min of the IL/hep and glycerol infusions. *P < 0.01.

The concentration at which glucose was clamped is the major difference between the two studies. Frias et al. (14) clamped glucose at ~5 mmol/l, whereas glucose was clamped at ~8 mmol/l in the present study. Glucose and insulin both stimulate translocation of GLUT4 transporters to the plasma membrane. However, they do so via different mechanisms. Insulin stimulates glucose transport, at least in part, by increasing the activity of phosphatidylinositol 3-kinase (35). Conversely, FFAs decrease glucose transport and inhibit the activity of this enzyme (10). In contrast to insulin, hyperglycemia stimulates glucose transport via a calcium-sensitive pathway (36). To the best of our knowledge, the effects of elevated FFAs on this pathway are not known. It therefore is possible that elevated FFAs inhibit insulin action only in men but inhibit the effects of glucose per se or the interaction of glucose and insulin on glucose uptake in both women and men.

Elevated FFAs impair suppression of glucose production (6,11,13). The present study shows that this also occurs in women. Splanchnic glucose production was higher ($P < 0.01$) on the IL/hep than glycerol study days. Men characteristically have more visceral fat than women (22). However, obese women have more visceral fat than lean women (37). Increased visceral fat alone or in combination with increased rates of visceral lipolysis presumably increases the FFA concentrations to which the liver is exposed. The present data, taken together with previous studies showing the elevated FFAs can stimulate gluconeogenesis (13), glucose-6-phosphatase activity (38), and endogenous glucose production (6,13), suggest that high plasma FFA potentially can lead to inappropriately elevated rates of glucose production.

In summary, the current studies show that in the presence of hyperglycemia and hyperinsulinemia, elevated FFAs decrease whole-body glucose disposal and increase splanchnic glucose production in women. The decrease in glucose disposal appears to be primarily due to lower muscle glucose uptake, since elevated FFAs impaired leg but not splanchnic glucose extraction. Taken together, these data indicate that conditions that chronically elevate FFAs (e.g., estrogen deficiency, obesity, and diabetes), which likely contribute to alterations in glucose metabolism in women. Additional studies will be required to determine whether the magnitude of FFA effects on glucose metabolism in women are comparable with those observed in men studied under the same conditions.

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