Free Fatty Acids Reduce Splanchnic and Peripheral Glucose Uptake in Patients With Type 2 Diabetes

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Splanchnic glucose uptake (SGU) plays a major role in the disposal of an oral glucose load (OGL). To investigate the effect of an elevated plasma free fatty acid (FFA) concentration on SGU in patients with type 2 diabetes, we measured SGU in eight diabetic patients (mean age 51 ± 4 years, BMI 29.3 ± 1.4 kg/m^2, fasting plasma glucose 9.3 ± 0.7 mmol/l) during an intravenous Intralipid/heparin infusion and 7–10 days later during a saline infusion. SGU was estimated by the OGL insulin clamp method: subjects received a 7-h euglycemic-hyperinsulinemic clamp (insulin infusion rate = 100 mU · m^{-2} · min^{-1}), and a 75-g OGL was ingested 3 h after starting the insulin clamp. After glucose ingestion, the steady-state glucose infusion rate during the insulin clamp was decreased appropriately to maintain euglycemia. SGU was calculated by subtracting the integrated decrease in glucose infusion rate during the 4-h period after glucose ingestion from the ingested glucose load (75 g). 3-[^{3}H]glucose was infused during the 3-h insulin clamp before glucose ingestion to determine the rates of endogenous glucose production and glucose disappearance (R_d). Intralipid/heparin or saline infusion was initiated 2 h before the start of the OGL clamp. Plasma FFA concentrations were significantly higher during the OGL clamp with the intralipid/heparin infusion than with the saline infusion (2.5 ± 0.3 vs. 0.11 ± 0.02 mmol/l, $P < 0.001$). During the 3-h insulin clamp period before glucose ingestion, Intralipid/heparin infusion reduced R_d (4.4 ± 0.3 vs. 5.3 ± 0.3 mg · kg^{-1} · min^{-1}, $P < 0.01$). During the 4-h period after glucose ingestion, SGU was significantly decreased during the intralipid/heparin versus saline infusion (30 ± 2 vs. 37 ± 2%, $P < 0.01$). In conclusion, an elevation in plasma FFA concentration impairs both peripheral and SGU in patients with type 2 diabetes. *Diabetes* 51:3043–3048, 2002

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EGP, endogenous glucose production; FFA, free fatty acid; OGL, oral glucose load; R_a, glucose appearance; R_d, glucose disappearance; SGU, splanchnic glucose uptake.

Splanchnic glucose uptake (SGU) plays a major role in the disposal of an oral glucose load (OGL) (1–6). Hyperglycemia per se enhances SGU in proportion to the increase in plasma glucose concentration, such that the splanchnic glucose clearance remains unchanged (2). This mass action effect of hyperglycemia to augment SGU depends on maintained portal insulin levels (2). Insulin per se, in the absence of hyperglycemia, does not increase SGU (2,7). Studies by DeFronzo et al. (3) and Adkins et al. (5) have shown that the gastrointestinal route of glucose administration has a specific enhancing effect on SGU (3) and that after glucose ingestion, the fractional, as well as absolute uptake of glucose by the splanchnic tissues is significantly greater than the combined effects of hyperinsulinemia plus hyperglycemia created by intravenous glucose/insulin administration (2,5).

Disturbances in free fatty acid (FFA) metabolism are characteristic in type 2 diabetic individuals (8–11), who manifest day-long increased plasma FFA levels (11) and increased rates of lipolysis (8–11). Elevated plasma FFA concentrations have been shown to impair glucose metabolism by competing with glucose as an oxidative fuel in the muscle and by inhibiting the more proximal steps of insulin action in muscle (12–18), as well by augmenting hepatic gluconeogenesis and impairing the suppression of hepatic glucose production by insulin (14,19,20). In contrast to their action on muscle glucose uptake and hepatic glucose production/gluconeogenesis, little is known about the effect of elevated plasma FFA levels on splanchnic/hepatic glucose uptake in humans in vivo. It has been shown that elevated plasma FFA concentrations inhibit glucokinase in the liver in vitro (21) and that glucokinase activity is decreased in the liver of type 2 diabetic subjects (22). Some (23–25), but not all (4,26), studies have demonstrated an impairment in SGU in type 2 diabetic subjects. Thus, it is possible that elevated plasma FFA levels are responsible for or contribute to the defect in SGU that has been observed in some type 2 diabetic individuals.

The current study was designed to determine the effect of an elevation in plasma FFA concentration on SGU after glucose ingestion in patients with type 2 diabetes. To quantitate SGU, we used a combined euglycemic insulin clamp OGL technique developed in our laboratory (3,27) and subsequently modified by Ludvik et al. (28).

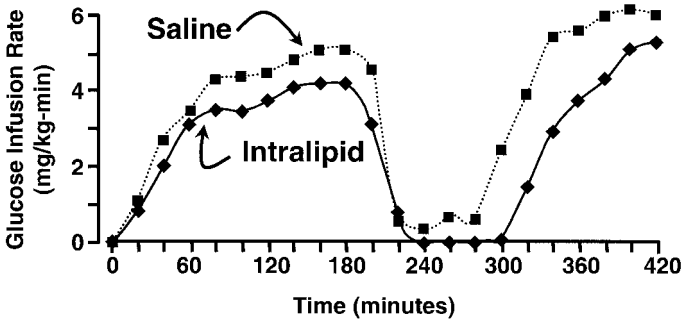

FIG. 1. Glucose infusion rate during the combined OGL 100 mU · m^{-2} · min^{-1} insulin clamp.

RESEARCH DESIGN AND METHODS

Subjects. Eight patients with type 2 diabetes participated in the study. There were six men and two women with a mean age of 51 ± 4 years and a diabetes duration of 6 ± 2 years. The BMI was 29.3 ± 1.4 kg/m^2. The fasting plasma glucose concentration and HbA_{1c} were 9.3 ± 0.7 mmol/l and 7.4 ± 0.4%, respectively. The plasma lipids were as follows: total cholesterol 201 ± 12 mg/dl, LDL cholesterol 135 ± 11 mg/dl, HDL cholesterol 38 ± 2 mg/dl, and triglycerides 157 ± 17 mg/dl. Other than diabetes, the subjects had no significant medical problems, and their weight was stable for at least 3 months before the study. Four of the eight diabetic subjects were being treated with diet alone, and four were treated with sulfonylureas. The sulfonylureas were discontinued 48 h before the study. No diabetic subject had ever received insulin, metformin, or a thiazolidinedione. No subject was taking any medication other than sulfonylureas known to affect glucose metabolism. Subjects were instructed to maintain their usual diet and not to engage in vigorous exercise for at least 3 days before the study. The purpose, nature, and potential risks of the study were explained to all subjects, and written consent was obtained before their participation. The protocol was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio.

Study design. Subjects were admitted to the General Clinical Research Center at 1800 on the evening before the study, and a standard weight-maintaining meal (55% carbohydrate, 30% fat, and 15% protein) was ingested between 1830 and 1900. After 2000, subjects refrained from eating or drinking anything except water. At 2200, a catheter was placed in the antecubital vein, and a variable low-dose insulin infusion (8–12 mU · m^{-2} · min^{-1}) was initiated to reduce and maintain the plasma glucose concentration to ~5.6 mmol/l.

At 0600 the following day, subjects received in random order an infusion of *1*) 20% Intralipid (0.2 ml · m^{-2} · min^{-1}) with heparin (0.2 units · kg^{-1} · min^{-1}) or *2*) normal saline (0.2 ml · m^{-2} · min^{-1}). The Intralipid and saline studies were performed within a 7- to 10-day interval. At 0600, a second catheter was inserted retrogradely into a vein on the dorsum of the hand for blood sampling, and the hand was placed in a heated box (60°C) for the duration of the study. Two hours after the start of Intralipid/heparin infusion (0800), a euglycemic insulin (100 mU · m^{-2} · min^{-1}) clamp was begun and continued for 7 h. Arterialized blood samples were collected every 5 min for plasma glucose determination, and a 20% glucose infusion was adjusted to maintain the plasma glucose concentration at ~5.6 mmol/l (29). During the first 180 min of the euglycemic insulin clamp, a primed (25 μCi)-continuous (0.25 μCi/min) infusion of 3-[^{3}H]glucose was infused to measure endogenous glucose production (EGP). The tritiated glucose infusion was discontinued after 180 min, when the glucose load was ingested. Insulin, glucose, 3-[^{3}H]glucose, and Intralipid/heparin were infused via the antecubital vein. Plasma samples for determination of plasma insulin and FFA concentration were obtained every 15–30 min throughout the study. Plasma samples for the determination of 3-[^{3}H]glucose specific activity were obtained every 5–10 min during the 150- to 180-min period of the euglycemic insulin clamp. During the 150- to 180-min time period of the insulin clamp, the exogenous glucose infusion rate required to maintain euglycemia was constant (Fig. 1). Three hours after starting the insulin clamp (1100), subjects ingested 75 g glucose over a 5-min period. As the oral glucose was absorbed, the exogenous intravenous glucose infusion rate was reduced appropriately to maintain euglycemia (Fig. 1). Within 3–3.5 h after glucose ingestion, the glucose infusion rate returned to or exceeded the rate at 180 min, indicating complete absorption of the OGL (Fig. 1).

Analytical determinations. Plasma glucose was measured by the glucose oxidase method (Beckman Instruments, Fullerton, CA). Plasma insulin (Diagnostic Products, Los Angeles, CA) and C-peptide (Diagnostic Systems Laboratories, Webster, TX) concentrations were measured by radioimmunoassay. Tritiated glucose specific activity was determined on deproteinized bariun/zinc plasma samples as previously described (9). Plasma FFA concentrations were determined by an enzymatic colorimetric quantification method (Wako Chemicals, Nuess, Germany).

Calculations. During the euglycemic insulin clamp before the ingestion of glucose (0–180 min), the rate of total body glucose appearance (R_a) was calculated using Steele's equation (30) and a distribution volume of 250 ml/kg. EGP was calculated by subtracting the exogenous glucose infusion rate from R_a. The rate of insulin-mediated total body glucose disappearance (R_d) was determined by adding the rate of EGP to the exogenous glucose infusion rate. The tritiated glucose infusion was discontinued at 180 min, and EGP was not determined during the 180- to 420-min time period after the ingestion of glucose during this study.

SGU was calculated as follows: the glucose infusion rate after oral glucose ingestion was subtracted from the reference glucose infusion rate to obtain the decrement in the exogenous glucose infusion rate. The reference glucose infusion rate was calculated as the mean of the glucose infusion rate during the 150- to 180-min time period (before glucose ingestion) and the 390- to 420-min time period. The integrated decrement in the exogenous glucose infusion rate after glucose ingestion was multiplied by the subject's body weight and by the time interval to return to the reference glucose infusion rate to calculate the amount of glucose escaping the splanchnic bed. The amount of glucose escaping the splanchnic bed was subtracted from the OGL (75 g) to calculate the SGU. Previous studies (23,28,31) have shown that glucose absorption from the gastrointestinal tract after glucose ingestion is complete within 3–3.5 h, and this result was confirmed in the present study by the sharp rise in the exogenous glucose infusion rate in all subjects to or above the pre-OGL rate (150–180 min) by 390 min. This calculation assumes that residual EGP during the combined OGL–100 mU · m^{-2} · min^{-1} insulin clamp is negligible.

Statistical analysis. Statistical calculations were performed with StatView for Windows, version 5.0 (SAS Institute, Cary, NC). Changes from baseline within a group were evaluated using the paired Student's t test. Differences between the Intralipid and saline infusion studies were evaluated by ANOVA. Significant differences between the two studies were confirmed by the Bonferroni test. Pearson correlation coefficients were used for correlation analysis. Data are presented as means ± SE. A P value <0.05 was considered to be statistically significant.

RESULTS

Plasma glucose, insulin, C-peptide, and FFA concentrations. The plasma glucose concentrations after the overnight insulin infusion were similar during the saline and Intralipid infusion studies (6.1 ± 0.2 vs. 6.0 ± 0.1 mmol/l). During the initial 3 h of the euglycemic insulin clamp, the plasma glucose concentrations were similar during the Intralipid and saline studies (5.6 ± 0.1 vs. 5.5 ± 0.1 mmol/l). After glucose ingestion, the plasma glucose concentration increased slightly in both studies during the 180- to 300-min time period (Fig. 2), even though the exogenous glucose infusion rate was reduced to zero. The increment in plasma glucose concentration (180–300 min) was slightly greater in the Intralipid study than in the saline study (Δ = 1.6 ± 0.2 vs. Δ = 0.8 ± 0.2 mmol/l, $P <$ 0.05). The plasma glucose concentration returned to 5.5 mmol/l at 300 min and remained constant at this level in both the Intralipid and saline studies (5.5 ± 0.2 vs. 5.4 ± 0.1 mmol/l) between 300 and 420 min (Fig. 2).

The plasma insulin concentrations (Fig. 2) did not differ significantly between the Intralipid and saline studies during the 180-min euglycemic insulin clamp (1,069 ± 100 vs. 1,130 ± 105 pmol/l) or during the OGL insulin clamp (180–420 min) (Fig. 2). During the 180- to 300-min time period, when the plasma glucose concentration rose slightly, there was no increase in the plasma insulin concentration. The plasma C-peptide concentrations after the overnight insulin infusion (time 0) were similar in the Intralipid and saline studies (0.17 ± 0.05 and 0.16 ± 0.05 nmol/l, respectively) and declined to 0.06 ± 0.02 and 0.06 ± 0.03 nmol/l, respectively, during the initial 180 min

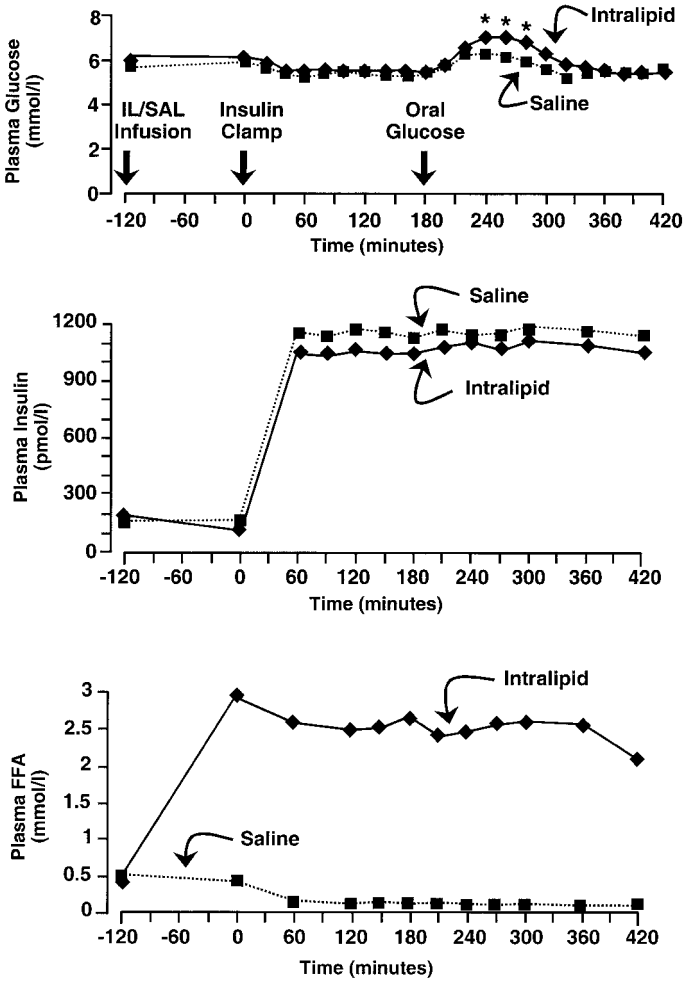

FIG. 2. Plasma glucose, insulin, and FFA concentrations during the OGL insulin clamp. *P < 0.05. IL, Intralipid/heparin infusion; SAL, saline infusion.

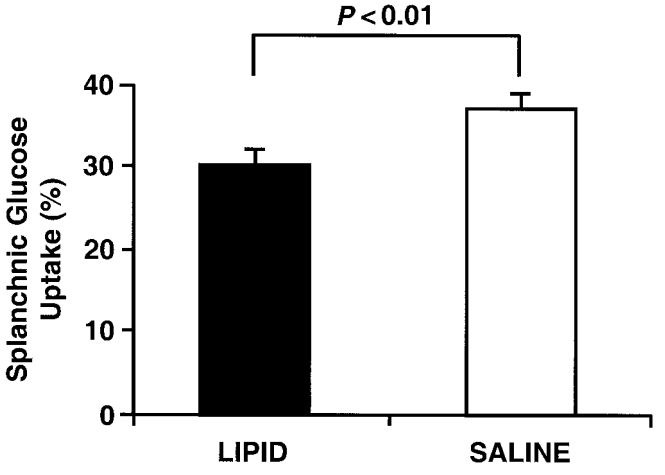

FIG. 3. SGU during the OGL insulin clamp studies performed with saline and Intralipid infusion.

of the euglycemic insulin clamp. After glucose ingestion, the plasma C-peptide concentrations were similar in the Intralipid and saline studies (0.10 ± 0.04 and 0.10 ± 0.03 nmol/l, respectively).

Plasma FFA levels (Fig. 2) after the overnight insulin infusion were similar in the Intralipid and saline studies (0.44 ± 0.06 vs. 0.41 ± 0.03 mmol/l). Plasma FFA levels increased to 2.9 ± 0.4 mmol/l during the 2-h period after the start of the Intralipid infusion and were significantly higher than levels during the saline infusion (0.38 ± 0.05 mmol/l, $P < 0.001$). During lipid infusion compared with saline infusion, the mean plasma FFA levels remained significantly higher during the 180-min euglycemic insulin clamp and during the 420-min OGL insulin clamp (0.11 ± 0.02 vs. 2.52 ± 0.3 mmol/l, $P < 0.001$) (Fig. 2).

Glucose infusion rate. The time course of the exogenous intravenous glucose infusion rate is shown in Fig. 1. The mean glucose infusion rate increased steadily during the initial 150 min of the insulin clamp with both saline and Intralipid infusion and reached a plateau from 150 to 180 min in both studies. The glucose infusion rate was significantly reduced during 150–180 min in the Intralipid versus saline infusion (4.2 ± 0.3 vs. 5.1 ± 0.3 mg · kg^{-1} · min^{-1}, $P < 0.01$). After glucose ingestion, there was an abrupt decline in the glucose infusion rate required to maintain euglycemia in both groups (Fig. 1). By 390 min, the glucose infusion rate returned to the pre-OGL value in all subjects, indicating complete absorption of the OGL. During the 180- to 420-min period of the OGL insulin clamp, the glucose infusion rate was significantly reduced in the Intralipid versus saline group (2.3 ± 0.4 vs. 3.5 ± 0.3 mg · kg^{-1} · min^{-1}) ($P < 0.01$). EGP, determined during the 150- to 180-min period of the euglycemic insulin clamp, was suppressed similarly during the lipid and saline infusion studies (0.18 ± 0.04 vs. 0.15 ± 0.05 mg · kg^{-1} · min^{-1}, respectively; $P > 0.05$). The whole-body R_d rate was significantly reduced during 150–180 min in the Intralipid versus saline infusion (4.4 ± 0.3 vs. 5.3 ± 0.3 mg · kg^{-1} · min^{-1}, $P < 0.01$).

SGU. SGU during the OGL insulin clamp performed with Intralipid (23.1 ± 2.0 g) was significantly reduced ($P < 0.01$) compared with the saline study (27.9 ± 2.1 g). The percentage of the oral glucose taken up by the splanchnic tissues also was significantly lower (Fig. 3) during the Intralipid versus saline study (30.8 ± 2.7 vs. 37.2 ± 2.7%, $P < 0.01$). The decrement in SGU during the OGL insulin clamp study correlated well with the HbA_{1c} ($r = 0.83$, $P = 0.01$) of the study subjects, i.e., type 2 diabetic subjects with poorest glycemic control demonstrated the greatest decrease in SGU during the Intralipid infusion study.

DISCUSSION

In the present study, we used the OGL insulin clamp technique (27,28) to examine the effect of an elevated plasma FFA concentration on SGU in subjects with type 2 diabetes. To the best of our knowledge, this is the first study to examine this effect in diabetic subjects, and our results demonstrate that SGU after glucose ingestion is significantly impaired by an elevated plasma FFA concentration in patients with type 2 diabetes. Previous studies have examined the effect of an elevated plasma FFA concentration on insulin-mediated suppression of endogenous (primarily hepatic) glucose production and stimulation of peripheral (primarily muscle) glucose uptake in type 2 diabetic subjects (14,15), but they did not measure SGU. In the present study, elevation of the plasma FFA concentration by Intralipid infusion reduced SGU by 17%, and the decrease in SGU was positively correlated with the

HbA_{1c}, indicating that poor glycemic control is an important factor that determines the magnitude of the inhibitory effect of increased plasma FFA levels on the splanchnic uptake of an ingested glucose load.

In the liver, glucose transport and phosphorylation are mediated via the GLUT2 transporter and glucokinase, respectively. In the only previous study that examined GLUT2 in liver of type 2 diabetic subjects, GLUT2 transporter protein was found to be increased approximately twofold compared with lean nondiabetic control subjects (32). Because hyperglycemia has been found to upregulate GLUT2 in animal models of diabetes (33), one could argue that the magnitude of the increase in GLUT2 protein described by Burguera et al. (32) was inappropriate. Nonetheless, when viewed in absolute terms, in the only study in which it was measured, hepatic GLUT2 protein was found to be increased in type 2 diabetic patients compared with lean nondiabetic control subjects. Abnormalities in the regulation of glucokinase have been demonstrated in both animal and human models of diabetes. Caro et al. (22) demonstrated a 50% decrease in hepatic glucokinase activity in morbidly obese type 2 diabetic subjects who underwent liver biopsies. Impaired glucose uptake and diminished conversion of glucose to glycogen have been demonstrated in individuals with maturity-onset diabetes of the young, who are heterozygous for mutations in the glucokinase gene (34), as well as in glucokinase-deficient rats (35) and mice (36). One previous in vitro study has shown that FFAs inhibit hepatic glucokinase activity (21), and this in vitro observation (21) may explain the FFA-induced inhibition of SGU (from 37 to 30%, $P < 0.01$) in type 2 diabetic patients in the present study. In a recent publication, Rigalleau et al. (37) examined the effect of an elevation in plasma FFA concentration in nondiabetic subjects and failed to demonstrate any inhibitory effect on SGU. This observation (37) suggests that the FFA-induced inhibition of SGU in type 2 diabetic patients may be an acquired defect. Consistent with this scenario, we found a positive correlation between decreased SGU and the HbA_{1c}, suggesting that poor glycemic control may sensitize the liver to the inhibitory effect of elevated plasma FFA levels. With regard to this, animal studies have shown that hyperglycemia impairs hepatic glucokinase activity (33,38). Furthermore, it is possible that if the present study was performed at each subject's elevated fasting plasma glucose concentration (i.e., without an overnight insulin infusion), an even greater FFA-induced inhibition of SGU may have been observed.

The OGL hyperglycemic clamp technique originally was developed in our laboratory to quantitate SGU (3,27). Using this technique, we demonstrated that the oral route of glucose administration had a specific effect to enhance SGU beyond that observed with comparable levels of hyperglycemia plus hyperinsulinemia created by intravenous glucose/insulin administration. These results subsequently were confirmed by Cherrington and associates (5,39). More recently, Ludvik et al. (28) modified the OGL hyperglycemic clamp technique by administering the OGL during a euglycemic insulin clamp study. This modification has the advantage of providing more reproducible state plasma insulin concentrations (Fig. 2) because the arterial plasma glucose concentration is maintained at euglycemic levels. Nonetheless, even though we decreased the exogenous glucose infusion rate to zero after administration of the oral glucose, we observed a very small rise in plasma glucose concentration during the 180- to 300-min time period after glucose ingestion. Importantly, however, the plasma insulin concentrations did not increase from their pre-OGL values in response to this small increase in plasma glucose concentration. The OGL insulin clamp technique has the additional advantages that it is noninvasive, can be performed repetitively to follow changes in SGU, and circumvents the problems of tracer cycling and non–steady-state conditions that exist with the double tracer technique. Both the OGL hyperglycemic clamp and OGL insulin clamp techniques have been validated by direct comparison with the hepatic vein catheter technique (3,27,28). As noted above, during the OGL insulin clamp study, the plasma glucose concentration increased slightly after glucose ingestion and was 0.8 mmol/l higher during the Intralipid study than during the saline study for the 180- to 300-min time interval. This slightly higher plasma glucose concentration during the Intralipid study was accounted for by *1*) an increased escape of glucose from the splanchnic tissues and *2*) a decreased R_d rate by peripheral tissues. It should be noted that hyperglycemia enhances SGU in direct proportion to the increase in plasma glucose concentration, such that the splanchnic glucose clearance remains unchanged (2). Thus, if anything, one would have expected a mild stimulation of SGU by the higher plasma glucose concentrations in the Intralipid study, not a decrease. A better reflection of the true effect of elevated plasma FFA levels on SGU can be obtained by calculating the splanchnic glucose clearance, which declined from 1.54 ± 0.1 to 1.18 ± 0.1 ml $\cdot$ kg^{-1} $\cdot$ min^{-1}, or by 24% ($P < 0.005$).

The OGL insulin clamp technique assumes that there is complete absorption of the OGL (75 g) within 4 h and that EGP is completely or near completely suppressed. With respect to the first assumption, several studies have demonstrated that an OGL, comparable to that used in the present study, is completely absorbed within 3–3.5 h (23,28,31). This was confirmed in the present study by return of the exogenous glucose infusion rate during the 390- to 420-min time period to values that were equal to or greater than the glucose infusion rate at 180 min, i.e., immediately before ingestion of the glucose load. Because EGP was nearly completely suppressed during the last hour of the euglycemic insulin clamp and the plasma insulin concentration remained constant after glucose ingestion, one can reasonably assume that it remained suppressed during the 4 h after glucose ingestion.

As reported by other investigators (12–18,20), we also observed a reduction in the whole-body R_d rate by ~16% during the last 30 min of the euglycemic insulin clamp (before glucose ingestion) during Intralipid infusion (compared with saline infusion). Randle et al. (18,40) proposed more than 30 years ago that elevated blood FFA levels play a key role in the development of insulin resistance in obesity and type 2 diabetes, based on their demonstration that increased FFA availability decreased glucose uptake and oxidation in isolated perfused rat hearts and hemidiaphragm. Consistent with the operation of the Randle cycle, elevated plasma FFA levels in healthy humans have

been shown to inhibit insulin-stimulated glucose oxidation within 1–2 h, followed by an inhibition of glucose uptake and glycogen synthesis within 3–4 h (15,41). Two mechanisms have been shown to account for this late inhibition of glucose disposal: *1*) an inhibition of glucose transport and/or phosphorylation (17,42) and *2*) a decrease in muscle glycogen synthase activity (43). Elevated plasma FFA levels also have been demonstrated to inhibit insulin-stimulated glucose uptake in type 2 diabetic individuals (14). The present results demonstrate that in type 2 diabetic patients, the inhibitory effect of elevated plasma FFA concentrations persists for upwards of 7 h.

Lastly, it could be argued that the elevated plasma glycerol concentrations that arise from the infusion of Intralipid/heparin could have some influence on peripheral glucose uptake and/or SGU. Previous studies have shown that glycerol infusion, to mimic the plasma glycerol concentrations observed during the Intralipid/heparin infusion, had no effect on peripheral glucose uptake (14,17), SGU (37,44), or EGP (37,44). Therefore, we believe that the impairment in SGU in type 2 diabetic subjects in the present study can reasonably be attributed to the elevation in plasma FFA concentration.

In summary, an acute elevation in the plasma FFA concentration in type 2 diabetic patients impairs both splanchnic (primarily hepatic) and peripheral (primary muscle) glucose uptake after the ingestion of a glucose load. Because the splanchnic area takes up ~30–40% of an ingested glucose load (1–6,23,24), inhibition of SGU by elevated plasma FFA levels represents an important potential site of impaired glucose homeostasis in type 2 diabetic individuals, especially in those who are in poor glycemic control. As a corollary, our observations also suggest that drugs, which enhance SGU and/or lower plasma FFA levels (45), may be beneficial in improving glycemic control in type 2 diabetic patients.

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