Free fatty acid-induced peripheral insulin resistance augments splanchnic glucose uptake in healthy humans

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Free fatty acid-induced peripheral insulin resistance augments splanchnic glucose uptake in healthy humans. Am J Physiol Endocrinol Metab 283: E346–E352, 2002. First published April 9, 2002; 10.1152/ajpendo.00329.2001.—To investigate the effect of elevated plasma free fatty acid (FFA) concentrations on splanchnic glucose uptake (SGU), we measured SGU in nine healthy subjects (age, 44 ± 4 yr; body mass index, 27.4 ± 1.2 kg/m²; fasting plasma glucose, 5.2 ± 0.1 mmol/l) during an Intra-lipid-heparin (LIP) infusion and during a saline (Sal) infusion. SGU was estimated by the oral glucose load (OGL)–insulin clamp method: subjects received a 7-h euglycemic insulin (100 mU·min⁻¹·m²⁻¹) clamp, and a 75-g OGL was ingested 3 h after the insulin clamp was started. During glucose ingestion, the steady-state glucose infusion rate (GIR) during the insulin clamp was decreased to maintain euglycemia. SGU was calculated by subtracting the integrated decrease in GIR during the period after glucose ingestion from the ingested glucose load. [3-3H]glucose was infused during the initial 3 h of the insulin clamp to determine rates of endogenous glucose production (EGP) and glucose disappearance (Rg). During the 3-h euglycemic insulin clamp before glucose ingestion, Rg was decreased (8.8 ± 0.5 vs. 7.6 ± 0.5 mg·kg⁻¹·min⁻¹, P < 0.01), and suppression of EGP was impaired (0.2 ± 0.04 vs. 0.07 ± 0.03 mg·kg⁻¹·min⁻¹, P < 0.01). During the 4-h period after glucose ingestion, SGU was significantly increased during the LIP vs. Sal infusion study (30 ± 2 vs. 20 ± 2%, P < 0.005). In conclusion, an elevation in plasma FFA concentration impairs whole body glucose Rg and insulin-mediated suppression of EGP in healthy subjects but augments SGU.

Splanchnic glucose uptake (SGU) accounts for the disposal of approximately one-third of an oral glucose load (18, 24, 26). Hyperglycemia per se enhances SGU in proportion to the increase in plasma glucose concentration, and this mass-action effect of hyperglycemia to augment SGU is dependent upon maintained portal liver; oral glucose; skeletal muscle

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(3, 4, 15, 33, 34, 36, 43), by augmenting basal hepatic gluconeogenesis (37), and by impairing the suppression of hepatic glucose production by insulin (4, 7, 16). In contrast to their action on muscle glucose uptake and hepatic glucose production/gluconeogenesis, very little is known about the effect of elevated plasma FFA levels on splanchnic (hepatic) glucose uptake in humans in vivo.

The current study was designed to determine the effect of an elevation in plasma FFA concentration on SGU after glucose ingestion in healthy nondiabetic human subjects. To quantitate SGU, we employed a combined euglycemic insulin clamp-oral glucose load technique (OGL-clamp) developed in our laboratory (12, 18) and subsequently modified by Ludvik et al. (26).

**METHODS**

**Subjects.** Nine healthy nondiabetic subjects with a mean body weight of 74.5 ± 4 kg participated in the study. There were 4 males and 5 females with a mean age of 44 ± 4 yr and body mass index of 27.4 ± 12 kg/m². The fasting plasma glucose concentration and HbA₁c were 5.2 ± 0.1 mmol/l and 4.9 ± 0.1%, respectively. All subjects had a normal 2-h oral glucose (75 g) tolerance test. The plasma lipid levels were as follows: total cholesterol = 192 ± 10 mg/dl, low-density lipoprotein cholesterol = 119 ± 11 mg/dl, high-density lipoprotein cholesterol = 55 ± 2 mg/dl, and triglycerides 121 ± 12 mg/dl. None of the subjects had any significant medical problems, and their weight was stable for at least 3 mo before the study. None of the subjects was taking any medications 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. At 0600 on the next day, a catheter was placed in an antecubital vein, and subjects received anything except water. At 0600 on the evening before the study, subjects reported to the Clinical Research Center at San Antonio.

**Participation.** The protocol was approved by the Institutional Review Board of the General Clinical Research Center at 1800 on the evening before the study. At 0600 on the next day, a catheter was placed in an antecubital vein, and subjects received anything except water. At 0600 on the evening before the study, subjects reported to the Clinical Research Center at San Antonio.

**Euglycemic insulin clamp.** At 1100, subjects ingested 75 g of 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. 2). Within 3–3.5 h after glucose ingestion, the glucose infusion rate returned to or exceeded the rate at 180 min, indicating that the absorption of the oral glucose load was complete (Fig. 2).

**Analytical determinations.** Plasma glucose concentration was measured by the glucose oxidase method (Beckman Instruments, Fullerton, CA). Plasma insulin (Diagnostic Products, Los Angeles, CA) and C-peptide (Diagnostic Systems, Webster, TX) concentrations were measured by RIA. Tritiated glucose specific activity was determined on deproteinized barium/zinc plasma samples, as previously described (20). Plasma FFA concentration was determined by an enzymatic colorimetric quantification method (Wako Chemicals, Nuss, Germany). Although blood samples were immediately placed on ice and spun in a refrigerated centrifuge within 15–20 min, it is possible that in vitro lipolysis resulted in an overestimation of the plasma FFA concentration in vivo.

**Calculations.** During the euglycemic hyperinsulinemic clamp before the ingestion of glucose (0–180 min), the rate of total body glucose appearance (Ra) was calculated using Steele’s equation (42) and a distribution volume of 250 ml/kg. EGP was calculated by subtracting the exogenous glucose infusion rate from Ra. The rate of insulin-mediated total body glucose disposal (Ra) 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.

The amount of glucose that escaped splanchnic uptake was calculated as follows: the average glucose infusion rate between 180 and 380 min was subtracted from the reference glucose infusion rate to obtain the integrated decrement in the glucose infusion rate. The integrated decrement in the exogenous glucose infusion rate after glucose ingestion was multiplied by the subject’s body weight and time to obtain the amount of glucose escaping the splanchnic bed. The reference}

**Fig. 1.** Specific activity ([disintegrations·min⁻¹·dpm·mg⁻¹] of [³H]glucose during 150–180 min of the oral glucose load (OGL)-insulin clamp studies performed with Intralipid/heparin (●) and saline (●) infusions.
glucose infusion rate was calculated as the mean glucose infusion rate obtained by drawing a line between the 180- and 380-min time points. The amount of glucose escaping the splanchnic bed was subtracted from the oral glucose load (75 g) to calculate the SGU. Previous studies (25, 26) have shown that glucose absorption from the gastrointestinal tract after glucose ingestion is complete within 3–3.5 h, and this 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 380 min. The calculation of SGU assumes that residual EGP during the combined OGL-100 mU·m−2·min−1 insulin clamp is negligible. Because EGP was almost completely suppressed during the 150- to 180-min period of the insulin clamp before glucose ingestion and because portal hyperinsulinemia and hyperglycemia (as occurred after glucose ingestion) are potent inhibitors of hepatic glucose production (10–12), we believe that this assumption is quite reasonable.

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. During the initial 3 h of the euglycemic insulin clamp, the plasma glucose concentrations were similar during the Intralipid and saline studies (5.5 ± 0.1 vs. 5.5 ± 0.1 mmol/l). After glucose ingestion, there was a very small increase in the plasma glucose concentration (5.7 ± 0.2 vs. 5.7 ± 0.2 mmol/l, P = not significant) in both studies during the 180- to 300-min time period (Fig. 3). From 300 to 420 min the plasma glucose concentration remained constant in both the Intralipid and saline studies (5.4 ± 0.1 vs. 5.4 ± 0.1 mmol/l; Fig. 3).

The plasma insulin concentrations (Fig. 3) did not differ significantly between the Intralipid and saline studies during the 180-min euglycemic insulin clamp (1,062 ± 72 vs. 1,102 ± 60 pmol/l) or during the OGL-insulin clamp (180–420 min; 1,094 ± 66 vs. 1,120 ± 60). 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 before the start of the insulin clamp (time 0) were similar in the Intralipid and saline studies (0.20 ± 0.03 and 0.22 ± 0.03 nmol/l, respectively) and declined to 0.10 ± 0.03 and 0.13 ± 0.04 nmol/l, respectively, during the 180 min of the euglycemic insulin clamp. After glucose ingestion, the plasma C-peptide concentrations were similar in the Intralipid and saline studies (0.15 ± 0.06 and 0.20 ± 0.07 nmol/l, respectively).

Plasma FFA levels (Fig. 3) before the start of the Intralipid and saline infusion were similar (0.58 ± 0.06 vs. 0.56 ± 0.05 mmol/l, respectively). The plasma FFA concentration increased to 3.2 ± 0.5 mmol/l during the 2-h period after the start of the Intralipid infusion and were significantly higher than during the saline infu-

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**Fig. 2.** Glucose infusion rate during the OGL-insulin clamp studies performed with saline (■) and Intralipid/heparin (●) infusion.

**Fig. 3.** Plasma glucose, insulin, and free fatty acid (FFA) concentrations during the OGL-insulin clamp. Subjects ingested 75 g of glucose at 180 min. Intralipid/heparin (●) or saline (■) infusion was started 2 h before the insulin clamp.
sion, 0.51 ± 0.06 mmol/l (P < 0.001). During the 180-min euglycemic insulin clamp performed with Intralipid, the mean plasma FFA levels remained significantly higher than during the saline study (2.78 ± 0.4 vs. 0.21 ± 0.02 mmol/l, P < 0.001; Fig. 3). During the 240 min after glucose ingestion, the plasma FFA concentration during the Intralipid study remained elevated compared with the same time period during the saline study (2.28 ± 0.3 vs. 0.11 ± 0.01 mmol/l, P < 0.001; Fig. 3).

Glucose infusion rate. The time course of the exogenous intravenous glucose infusion rate is shown in Fig. 2. 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 required to maintain euglycemia was significantly reduced from 150 to 180 min in the Intralipid vs. saline infusion (7.4 ± 0.5 vs. 8.7 ± 0.5 mg·kg⁻¹·min⁻¹, respectively, P < 0.01). After glucose ingestion, there was an abrupt decline in the glucose infusion rate required to maintain euglycemia in both the Intralipid and saline studies (Fig. 2). By 380 min the glucose infusion rate returned to the pre-OGL value in all subjects, indicating that the absorption of the oral glucose load was complete. EGP, determined during the 150- to 180-min period of the euglycemic insulin clamp, was suppressed by >90% during both the lipid and saline infusion studies but was significantly higher in the Intralipid vs. saline infusion study (0.2 ± 0.04 vs. 0.07 ± 0.03 mg·kg⁻¹·min⁻¹, respectively, P < 0.01). The whole body Rₐ was significantly reduced from 150 to 180 min in the Intralipid vs. saline infusion (7.6 ± 0.5 vs. 8.8 ± 0.5 mg·kg⁻¹·min⁻¹, P < 0.01).

SGU. SGU during the OGL-insulin clamp performed with Intralipid (22.5 ± 1.5 g) was significantly higher (P < 0.005) compared with the saline study (15.3 ± 1.5 g). The percentage of the oral glucose taken up by the splanchnic tissues also was significantly higher (Fig. 4) during the Intralipid vs. saline study (30 ± 2 vs. 20 ± 2%, P < 0.005). The Intralipid-induced decrement in whole body Rₐ during the insulin clamp study correlated positively with the increase in SGU (r² = 0.55, P = 0.02), i.e., subjects with the greatest decrease in Rₐ during the Intralipid infusion study demonstrated the greatest increase in SGU (Fig. 5).

DISCUSSION

In the present study we have employed the OGL-insulin clamp technique (12, 18, 26) to examine the effect of an increase in the plasma FFA concentration on net SGU in healthy nondiabetic human subjects with normal glucose tolerance. Our results demonstrate that elevation of the plasma FFA concentration by an Intralipid infusion resulted in a decrease in whole body glucose uptake, which primarily reflects muscle glucose uptake (10), and a reciprocal increase (Fig. 5) in SGU by 47% (15.3 to 22.5 g) after glucose ingestion. As previously shown by us and others (4, 16, 43), the elevation in plasma FFA concentration during Intralipid infusion impaired the suppression of EGP by insulin, although the magnitude of the impairment was small because the high insulin infusion rate caused a >90% suppression of EGP in both groups.

Although we did not measure SGU before glucose ingestion, previous studies from our laboratory have demonstrated that SGU under basal conditions is small and varies little from person to person (11–13, 18). During the 3.5-h period after glucose ingestion, the mean rate of glucose uptake by the splanchnic tissues was 1.01 ± 0.10 mg·kg⁻¹·min⁻¹ during the saline infusion. This rate of SGU was two times that observed with comparable levels of hyperglycemia plus hyperinsulinemia created by intravenous glucose/insulin administration (12, 18). These results are consistent with our previous observations in humans (12, 18) and with those of Adkins et al. (1) and Cherrington (8) in dogs and demonstrate that the oral route of glucose administration has a specific effect to enhance SGU. Pagliassotti et al. (31) have demonstrated in dogs that a portal signal, generated by the glucose gradient between the portal vein and hepatic artery, is responsible for the greater increase in splanchnic (hepatic) glucose uptake after oral compared with intravenous glucose administration. The partitioning of an oral glucose load among the tissues is also regulated by the portal signal. Thus Moore et al. (29) in conscious dogs have shown that the
increase in hepatic glucose uptake after glucose ingestion is associated with a reciprocal decrease in peripheral (primarily muscle) glucose uptake. Furthermore, Galassetti et al. (19) demonstrated that a negative arterial-portal gradient decreases skeletal muscle glucose uptake while enhancing hepatic glucose uptake in dogs. The results of the current study demonstrate, for the first time in humans, the existence of a similar mechanism in which cross talk between the liver and the peripheral tissues works in a coordinated manner to maintain normal glucose tolerance after glucose ingestion. During the OGL-insulin clamp with saline infusion, 15.3 g (20%) of the 75-g oral glucose load were taken up by the splanchnic tissues (primarily liver), and the remaining 59.7 g of glucose were taken up by extra splanchnic tissues, primarily muscle. During the last 30 min of the euglycemic insulin clamp performed with Intralipid, the increase in plasma FFA concentrations induced peripheral insulin resistance and decreased glucose $R_d$ by $\sim$13%. Because the plasma FFA, glucose, and insulin concentrations during the 4-h period (180–420 min) of the OGL-insulin clamp were virtually identical to those during the 150- to 180-min period of the euglycemic insulin clamp, one can assume that a similar defect in peripheral glucose disposal would prevail after glucose ingestion and that 19% of 59.7 g, or $\sim$8 g less of the oral glucose, would be taken up by peripheral tissues compared with the saline infusion OGL-insulin clamp study. SGU during the OGL-insulin clamp with Intralipid was 22.5 ± 1.5 g, which represents an increment of 7.2 ± 1.5 g compared with the saline OGL-insulin clamp study. This amount agrees quite closely with the expected decrease ($\sim$8 g) in peripheral glucose uptake. Thus, in healthy humans, as in dogs (1, 8), after glucose ingestion there appears to be a reciprocal relationship between SGU and peripheral glucose uptake. In a recently published study (38) using a different experimental design (hyperinsulinenic hyperglycemic clamp combined with hepatic vein catheterization), Shah et al. demonstrated that elevated plasma FFA levels (Intralipid/heparin infusion) significantly impaired leg (muscle) glucose uptake in healthy nondiabetic human subjects, and there was a tendency, although not statistically significant, for SGU to increase. These observations are consistent with those in the present study. Thus, when peripheral insulin resistance is induced by Intralipid infusion, the splanchnic (liver) tissues compensate by augmenting glucose uptake in an attempt to maintain normal oral glucose tolerance. A similar mechanism can be inferred from the results of Ludvik et al. (26). These investigators reported that SGU was increased in obese vs. lean nondiabetic subjects. Because the obese subjects in this study (26) were resistant to insulin, one would predict that an intact portal signal would redirect the disposition of the ingested glucose load from peripheral to splanchnic tissues, and this is precisely what was observed. Whether the portal signal remains intact in type 2 diabetic subjects remains to be determined, but studies by Ferrannini and colleagues (17, 18) suggest that the normal cross talk between peripheral and hepatic tissues may be disrupted.

The precise mechanisms responsible for the generation of the portal signal have yet to be defined, but neural and humoral mechanisms have been implicated. A role for humoral agents, including the intestinal incretins, has been suggested (14), but definite proof has yet to be forthcoming. Studies performed in animals provide evidence for the role of the autonomic nervous system and hypothalamus in mediating the portal signal. The liver, as the sensor organ, detects the increased glucose concentration gradient between portal vein and the hepatic artery via intrahepatic sensory effector nerves and generates a cholinergic signal that augments hepatic glucose uptake, thus coupling glucose absorption and hepatic glucose uptake (2, 28, 39–41). Adkins-Marshall et al. (2) demonstrated in dogs that the enhancement in hepatic glucose uptake after intraportal glucose infusion was abolished by hepatic denervation. Consistent with these observations, Shiota et al. (41) demonstrated in conscious dogs that portal vein acetylcholine infusion enhanced hepatic glucose uptake of intravenously administered glucose, although adrenergic blockade had no effect on hepatic glucose uptake. These results suggest a role for the parasympathetic nervous system in the generation of the portal signal after portal glucose delivery. Studies by Shimazu et al. (39, 40) and Minokoshi et al. (28) in rats have shown that the lateral and ventromedial hypothalamic nuclei play important roles in the central integration of the parasympathetic stimuli generated by the portal signal and relay the efferent response to the liver and peripheral tissues via sympathetic nerves (28). Matsuhisa et al. (27) have demonstrated in dogs that a reduction in glucose gradient between the brain and the portal vein blunts the enhancement in hepatic glucose uptake (despite the presence of a negative portal-arterial gradient in the liver after intraportal glucose delivery) and completely abolishes the effect of the intraportal glucose infusion to decrease peripheral (muscle) glucose uptake. Thus the hypothalamus and the autonomic (both parasympathetic and sympathetic) nervous system both participate in the regulation of peripheral (muscle) and hepatic glucose uptake, leading to the coordinated disposal of an oral glucose load. However, recent studies by Hsieh and colleagues (22, 23) suggest that the signal that brings about the suppressive effect of portal glucose delivery on peripheral glucose uptake may originate in the liver itself.

The OGL-hyperglycemic clamp technique originally was developed in our laboratory to quantitate SGU (12, 18). More recently, Ludvik et al. (26) modified the OGL-hyperglycemic clamp technique by administering the oral glucose load during a euglycemic insulin clamp study. This modification has the advantage of providing more reproducible and constant steady-state plasma insulin concentrations (Fig. 3), since the arterial plasma glucose concentration is maintained at euglycemic levels. Thus, after glucose ingestion at 180 min, the plasma insulin concentrations did not change significantly from their values during the last 30 min of.

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the euglycemic insulin clamp (150- to 180-min time period). Furthermore, the insulin infusion rate (100 mU·m−2·min−1) produced pharmacological plasma insulin concentrations that caused a nearly complete suppression (>90%) of EGP during 180 min of both the saline and Intralipid infusion studies. Moreover, the resultant portal hyperglycemia (after glucose ingestion) further serves to ensure the complete suppression of hepatic glucose production. The OGL-insulin clamp technique has the additional advantages that it is noninvasive (hepatic vein catheterization not required), 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 (12, 18, 26).

The OGL-insulin clamp technique assumes that the absorption of the oral glucose load is complete (75 g) within 4 h and that EGP is completely suppressed. With respect to the first assumption, previous studies have demonstrated that an oral glucose load, comparable to that employed in the present study, is absorbed within 3–3.5 h (25, 26). This was confirmed in the present study by return of the exogenous glucose infusion rate during the 380- 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 during the saline infusion studies 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 EGP remained suppressed during the 4 h after glucose ingestion. Elevated plasma FFA concentrations have been shown to impair insulin-mediated suppression of EGP in prior studies (4, 7, 16), and we observed a similar impairment during the last hour of the euglycemic insulin clamp performed with the Intralipid infusion. However, the slightly higher rate of EGP would only serve to underestimate the magnitude of the increase in SGU that was observed during the Intralipid infusion.

As reported by other investigators (3, 4, 15, 16, 35, 36, 43), we also observed a reduction in whole body glucose R4 by ~13% during the last 30 min of the euglycemic insulin clamp (before glucose ingestion) during Intralipid infusion (compared with saline infusion). Randle and colleagues (32, 33) proposed more than 30 years ago that elevated blood FFA levels contribute to the development of insulin resistance in obesity and type 2 diabetes by decreasing glucose uptake and oxidation in muscle. 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 (3, 6). Two distinct mechanisms account for this late inhibition of glucose disposal: 1) an inhibition of glucose transport and/or phosphorylation (36) and 2) a decrease in muscle glycogen synthase activity (5).

In summary, an acute elevation in the plasma FFA concentration in healthy subjects impairs whole body glucose disposal and insulin-mediated suppression of EGP, but it augments SGU. These results provide evidence for cross talk (that is independent of hyperglycemia) between peripheral tissues (muscle) and liver in the maintenance of normal glucose homeostasis. Thus the development of insulin resistance in muscle is associated with a “compensatory” increase in SGU, and oral glucose tolerance remains normal. The precise mechanisms responsible for this cross talk remain to be defined. It also is unclear whether this cross talk is disrupted during the diabetic state.

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