Effects of Free Fatty Acids and Glucose on Splanchnic Insulin Dynamics

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The mechanism of hyperinsulinemia that accompanies insulin resistance in some abdominally obese and diabetic individuals is poorly understood. Both increased secretion of insulin and decreased clearance have been demonstrated. The present study was undertaken to examine the role of free fatty acids (FFAs) and glucose in regulating splanchnic insulin dynamics in vivo. Plasma FFA levels were raised approximately twofold via an intralipid/heparin infusion in eight lean women. Insulin dynamics were assessed using the individual's C-peptide kinetic coefficients. Studies were performed in the basal state and during two levels of glycemia, 7 and 11 mmol/l. Studies were repeated using saline, and thus each subject served as her own control. Under basal conditions, raising FFA flux resulted in a modest increase in plasma insulin concentration (PIC) secondary to an increase in insulin secretion rate (ISR); however, endogenous insulin clearance (EIC) was not influenced. During the 7 mmol/l hyperglycemic clamp, maintaining a high FFA flux resulted in a 30% increase in PIC above the effect produced by glucose alone. This represents the cumulative effects of stimulation of ISR and inhibition of EIC. Clamping plasma glucose at 11 mmol/l while maintaining a high FFA flux increased PIC twofold above that produced by glucose alone. This increase in PIC was mainly due to a significant reduction in EIC without an accompanying increase in ISR (392 ± 159 and 787 ± 187 ml/min with and without intralipid infusion, respectively). Analysis of variance indicated that the suppressive effect of FFA on EIC was independent of the effect of glucose. The effect of the two substrates seems to be additive. *Diabetes* 46:57–62, 1997

Abdominally obese subjects, particularly those with glucose intolerance and/or NIDDM, exhibit several metabolic disorders including dyslipidemia, hypertension, and atherogenesis (1). Hyperinsulinemia is also a characteristic feature of this obesity phenotype (2,3) and appears to play a causative role in the pathogenesis of associated atherogenic risks (4–6).

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BHCP, biosynthetic human C-peptide; EIC, endogenous insulin clearance; FFA, free fatty acid; ISR, insulin secretion rate; PIC, plasma insulin concentration.

Peripheral plasma insulin level is a function of both pancreatic production and metabolic clearance. Increased insulin secretion has been demonstrated in human obesity (7). The mechanism of insulin hypersecretion is manifold. Direct stimulation of pancreatic islets by glucose and indirect response to peripheral insulin resistance have been suggested (8). There is also some evidence suggesting that free fatty acids (FFAs) might act as secretagogues (9). Insulin clearance is also a function of body weight (10). While obese individuals show decreased clearance (11,12), weight loss is associated with an increase in clearance (13,14). Furthermore, individuals with abdominal obesity are distinguished from those with lower-body obesity by approximately threefold reduction in insulin clearance in the face of an increased insulin production (8). Caloric restriction results in reduction of visceral adipose mass, which correlates with improvement in insulin clearance (15).

The liver is a main site for insulin clearance, removing approximately 50–60% during the first portal passage (16). In abdominally obese individuals, increased size and lipolytic activity of the intra-abdominal/visceral fat depot could result in high portal vein and systemic FFA flux (17). We have, therefore, hypothesized that increased FFA flux in abdominal obesity might play a role in regulating splanchnic insulin dynamics. Work from our group (18) and others (19) has demonstrated that exposure of isolated rat hepatocytes to increasing concentrations of albumin-bound fatty acids could result in decreased insulin receptor binding, as well as post-receptor processing and degradation. Increasing FFA levels in the portal vein of isolated rat livers resulted in reduced insulin clearance (20). On the other hand, glucose intolerance and raised fasting plasma glucose levels are also characteristic features of some abdominally obese subjects (21) and might also influence hepatic removal of the hormone.

The present study was undertaken to determine the effects of increased supply of FFA and glucose on pancreatic insulin secretion, metabolic clearance, and consequently overall peripheral insulin levels. Plasma FFA flux was elevated in healthy lean women via an infusion of intralipid and heparin under basal conditions and at two levels of glycemia (7 and 11 mmol/l). Insulin secretion and endogenous clearance rates were then assessed, and the interactions between FFA and glucose under these conditions were examined.

RESEARCH DESIGN AND METHODS

Human subjects. Volunteers were recruited by advertisement. Eight lean premenopausal (28 ± 2 years of age) Caucasian women were studied. All were in good health, did not smoke, and were not on any medication. Their weight averaged 59 ± 2 kg, body mass index 22 ± 0.6 kg/m^2, and their waist-to-hip circumference ratio 0.71 ± 0.01. Subjects who qualified for the study had an oral

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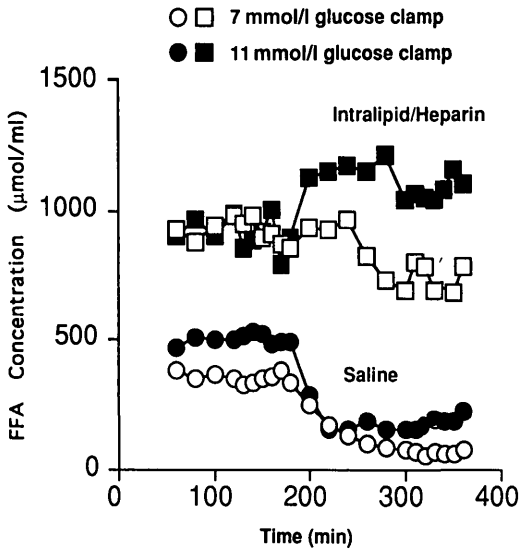

FIG. 1. Representative profile of FFA concentrations during basal state and two levels of clamped hyperglycemia. Experiments were done with either saline or intralipid/heparin infusion.

glucose tolerance test after an overnight fast to exclude individuals with glucose intolerance and diabetes (22). Fasting plasma glucose levels were 5.1 ± 0.1 mmol/l, and fasting plasma insulin levels averaged 26 ± 2 pmol/l. Study participants signed a written consent approved by the Human Research Review Committee and the General Clinical Research Center Advisory Committee. Studies were performed at the Clinical Research Center of the Medical College of Wisconsin in Milwaukee, WI. Subjects were prescribed a weight-maintenance diet 2 weeks prior to the studies. Studies were done in the follicular phase of their menstrual cycle, and a negative βHCG pregnancy test was obtained before the study.

Study protocol

Hyperglycemic clamp studies. Studies were conducted on two separate days, a control day and an intralipid day. Each person served as her own control. Subjects were divided into two groups, four subjects each, that underwent either the 7 or 11 mmol glucose clamp. Basal conditions, however, were identical in both groups. After a 14-h overnight fast, an 18-gauge polyethylene catheter was inserted into the antecubital vein of one arm for all infusions; a 20-gauge catheter was inserted retrogradely into a wrist vein of the contralateral arm, and the hand was kept in a heated box to obtain arterialized blood samples. ^{3}H-oleate (New England Nuclear, Boston, MA) was prepared for infusion as previously described (23) and was infused continuously at a rate of 0.5 μCi/min to monitor FFA flux on both study days for the duration of the study. During the control day, subjects were infused with normal saline at 39 ml/h for 360 min. At 180 min, a glucose clamp was started using a variable glucose infusion to maintain steady-state glycemia (7 or 11 mmol/l) (24). On the intralipid day, plasma FFA concentration was raised and maintained at (1 mmol/l by simultaneous infusion of 20% intralipid (Kabivitrum, Franklin, OH) at 30 ml/h and 9 ml/h heparin at a concentration of 100 units/ml for a total of 360 min. In addition, boluses of 200 units heparin were given at 0, 90, 180, and 240 min. Blood samples were drawn every 20 min for the first 120 min and every 10 min thereafter up to 180 min. Similarly, during the 3-h clamp period, samples were obtained every 20 min for the first 2 h, followed by 10-min samples for the last hour of the clamp. Samples for blood glucose were drawn every 5 min during the clamp and analyzed on site, and a variable glucose infusion was adjusted accordingly. Blood was collected in EDTA tubes for FFA determination. All samples were kept on ice until the plasma was separated and stored at −70°C for later assay of plasma insulin, C-peptide, and FFA concentration and specific activity.

Intravenous biosynthetic human C-peptide (BHCP) bolus study. Subjects returned on a separate day for a BHCP bolus study. After an overnight fast, each subject received an intravenous bolus injection of BHCP (50 nmol) (Lilly, Indianapolis, IN) as described previously (25). Timed blood samples were obtained over 2 h to determine the plasma disappearance of C-peptide.

Analytical procedures. Plasma glucose was measured by the glucose oxidase method with a Beckman Analyzer (Brea, CA). Plasma insulin and C-peptide were measured in triplicate using a commercial solid phase ^{125}I-radioimmunoassay (LINCO Research, Inc., St. Charles, MO). Plasma FFA concentrations and specific radioactivity were determined using a Model 1050 Hewlett-Packard HPLC (Brookfield, WI), employing the procedure described by Miles et al. (26). Briefly, phenacyl derivatives of the FFAs were prepared and resuspended in a solution of acetonitrile:water (83:17). Samples were injected into a 5-μm (4.5 × 250 mm) reverse-phase octadecyl silica column and eluted with acetonitrile:water (83:17) at a rate of 2 ml/min. External standards containing stearic, elaidic, oleic, palmitic, linoleic, arachidonic, palmitoleic, myristic, and linolenic acid were included with each run to calculate individual FFA concentrations. An internal standard ($^{2}H_{31}$-palmitate) was added to the samples and standards to assess recovery. $^{2}H_{31}$-palmitate elutes as a separate identifiable peak ~2 min prior to natural palmitate. The effluent was passed through a variable wavelength detector (254 nm) and then to a fraction collector containing scintillation vials. The fraction containing the radioactive oleate tracer was collected, dried under nitrogen, and resuspended in scintillation fluid (Biosafe II, Research Products International, Mt. Prospect, IL). Radioactivity was quantified in a Packard Tri-Carb Scintillation Counter. The data were collected, integrated, and stored using a Hewlett-Packard Chemstation software program. Total FFA was defined as the sum of stearic, elaidic, oleic, palmitic, linoleic, arachidonic, palmitoleic, myristic, and linolenic acid concentrations.

Calculations

FFA kinetics. Data were analyzed using steady-state equations (26). Physiological steady state was maintained during the basal period, as there was no exogenous glucose infusion. Isotopic equilibrium was reached 30 min after starting the isotope infusion. Although the physiological and isotopic steady states were disrupted with the start of each clamp, they were reestablished within 45–60 min. The means of seven samples obtained during the last 60 min of each 3-h study phase were used to calculate oleate specific radioactivity and total FFA concentration and flux rate. Total FFA flux rates were calculated as (Radioactive oleate infusion rate/Oleate specific radioactivity) × (total FFA concentration/Oleate concentration).

Insulin secretion rates (ISRs) and endogenous insulin clearance (EIC). C-peptide decay curves were analyzed using the SAAM program, utilizing a mathematical two-compartment model representing the plasma and extracellular pools, and the kinetic coefficients were determined for each individual as previously described (25). ISR was estimated by deconvoluting the plasma C-peptide concentrations during the basal period and during clamped hyperglycemia, using the individual's own kinetic coefficients. EIC was calculated as the ratio of the total area under the ISR curve to the total area under the plasma insulin concentration (PIC) curve for both the basal period and during clamped hyperglycemia.

To define the relationship between the pancreatic ISR and the simultaneously measured peripheral insulin concentration during the clamped hyperglycemia, ISR and PIC were expressed as percentages of their respective mean basal values. Similar increases in the ISR and the peripheral insulin concentration indicated that no change in EIC had occurred. A relatively greater increase in the peripheral insulin concentration than in the ISR indicated a reduction in the clearance rate of endogenously secreted insulin.

Statistical analysis. Results are expressed as means ± SE. Two-way analysis of variance was used to assess the independent, additive, or interactive effects of glucose and FFA on ISR, PIC, and EIC during the two levels of hyperglycemia with or without intralipid/heparin infusion. The paired two-tailed Student's *t* test was used to examine the effect of raising FFA levels on the above parameters during the basal state or the hyperglycemic clamps by comparing the means with or without intralipid. Statistical analyses were performed using the StatView 4.0 statistical analysis program for the Macintosh. *P* values ≤ 0.05 were accepted as statistically significant.

RESULTS

Figure 1 shows a representative profile of plasma FFA concentrations achieved during saline or intralipid/heparin infusion, both in the basal state and during clamped hyperglycemia. Calculated steady-state FFA flux during the basal period increased from 503 ± 48 in the control study to 907 ± 134 μmol/min with the infusion of intralipid and heparin. Clamping plasma glucose at either 7 or 11 mmol/l significantly suppressed the flux of FFA during the control day (102 ± 40 and 96 ± 18 μmol/min, respectively). FFA flux, however, remained significantly high throughout both clamp levels with the continuous infusion of intralipid/heparin (1,063 ± 125 and 1,079 ± 163 μmol/min during the 7 and 11 mmol/l glucose clamps, respectively).

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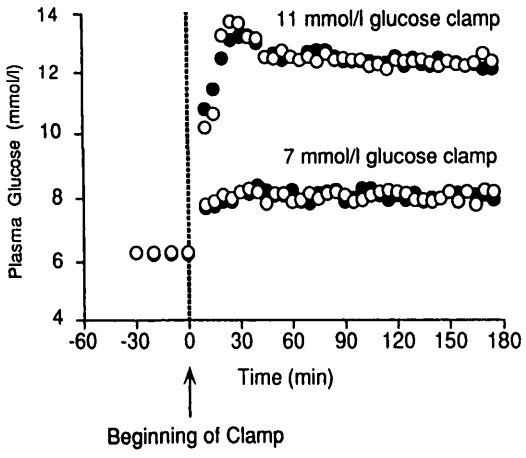

FIG. 2. Plasma glucose levels during basal state and two levels of clamped hyperglycemia. ○, saline day; ●, intralipid day.

TABLE 1
Effects of intralipid and glucose on splanchnic insulin dynamics in normal healthy females

	ISR (pmol/min)	PIC (pmol/l)	MCR (ml/min)
Basal			
Saline day	30.4 ± 3.3	40.4 ± 8.9	1,159 ± 203
Intralipid day	35.4 ± 3.7*	46.9 ± 7.7*	1,046 ± 166
Clamp-I			
Saline day	51.0 ± 3.4†	69.2 ± 9.1†	968 ± 78
Intralipid day	60.4 ± 4.1*†	92.8 ± 8.3*†	765 ± 34*†
Clamp-II			
Saline day	136.2 ± 20.4†	280 ± 73.6†	787 ± 187†
Intralipid day	114.1 ± 25.4†	420 ± 78*†	392 ± 159*†

MCR, metabolic clearance rate; clamp-I, plasma glucose concentration of 7 mmol/l; clamp-II, plasma glucose concentration of 11 mmol/l. *Significantly different from saline day ($P < 0.05$). †Significantly different from basal value ($P < 0.05$).

The mean concentration of glucose in the basal state was not affected by intralipid infusion and averaged 4.61 ± 0.28 and 4.77 ± 0.5 mmol/l on the control and intralipid days, respectively. Figure 2 shows the levels of glycemia reached and maintained during both clamp levels. The mean coefficient of variation of plasma glucose during clamp studies was <5% on both study days.

Effects of increased FFA flux on splanchnic insulin dynamics under basal conditions. Since basal study conditions were identical, basal data are calculated as the means ± SE of all eight study subjects. As shown in Table 1, raising FFA flux under basal conditions significantly increased ISR from 30.4 ± 3.3 pmol/min during saline to 35.4 ± 3.7 pmol/min with intralipid infusion. Basal PIC also increased significantly from 40.4 ± 8.9 to 46.9 ± 7.7 pmol/l in the control day and the intralipid day, respectively. The calculated basal EIC rates were comparable on both study days, averaging 1,159 ± 203 and 1,046 ± 166 ml/min with normal and raised FFA flux, respectively. The increase in PIC in the basal state thus reflected the increase in ISR.

Effects of increased FFA flux on splanchnic insulin dynamics during the hyperglycemic clamps. During the control day, when the plasma glucose was clamped at 7 mmol/l, ISR increased to 51 ± 3.4 from a basal rate of 30.4 ± 3.3 pmol/min. The PIC correspondingly rose by ~70%, from a basal value of 40.4 ± 8.9 to 69.2 ± 9.1 pmol/l. This increase mainly reflects the increase in ISR, since the calculated EIC did not change significantly. Upon raising the FFA flux during the intralipid day, ISR was further increased to 60.4 ± 4.1 pmol/min during the glucose clamp from a basal value of 35.4 ± 3.7, which was significantly higher ($P < 0.05$) than that of the control day (Table 1 and Fig. 3). Unlike the control day, however, PIC was nearly doubled and was significantly higher than the increase seen in the absence of intralipid and heparin infusion. In addition, raising FFA flux at that level of glycemia significantly decreased EIC to 765 ± 34 ml/min from 968 ± 78 ml/min during saline infusion. The increased PIC at that level of glycemia, therefore, results from both increased ISR and a concomitant decline in EIC.

When the plasma glucose was clamped at 11 mmol/l, the ISR rose to 136.2 ± 20.4 pmol/min during the control day. However, the ISR did not rise further in the presence of elevated FFA at that level of glycemia (intralipid day), remaining at 114.1 ± 25.4 pmol/min (Table 1 and Fig. 3). During the high-glucose clamp, PIC rose nearly sixfold when saline was infused (from 40.4 ± 8.9 to 280 ± 73.6 pmol/l). When FFA flux was raised, however, PIC increased ninefold and was significantly higher ($P < 0.05$) during the clamp than on the control day (420 ± 78 vs. 280 ± 73.6 pmol/l). EIC fell to 787 ± 187 ml/min during the control day and to 392 ± 159 ml/min during the intralipid day. As shown in Table 1, the decline in EIC during the high-glucose clamp was significant relative to that of the basal state, with or without intralipid infusion. In addition, the effect of intralipid was significant compared with that

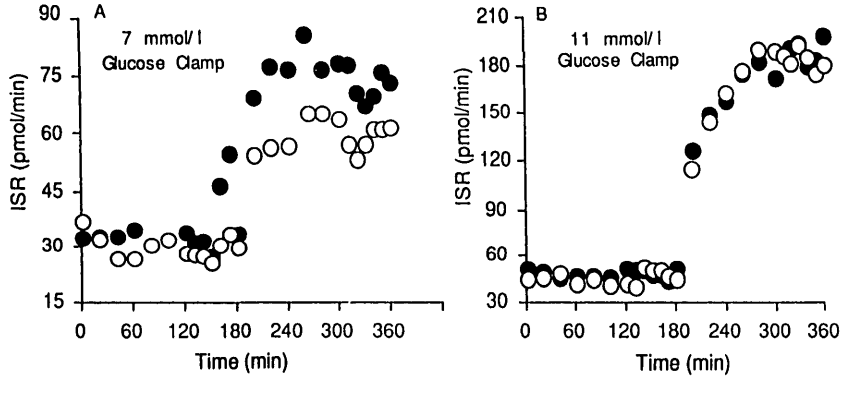

FIG. 3. Insulin secretion rate during basal state and two levels of clamped hyperglycemia, 7 mmol/l (*A*) and 11 mmol/l (*B*). ○, saline day; ●, intralipid day.

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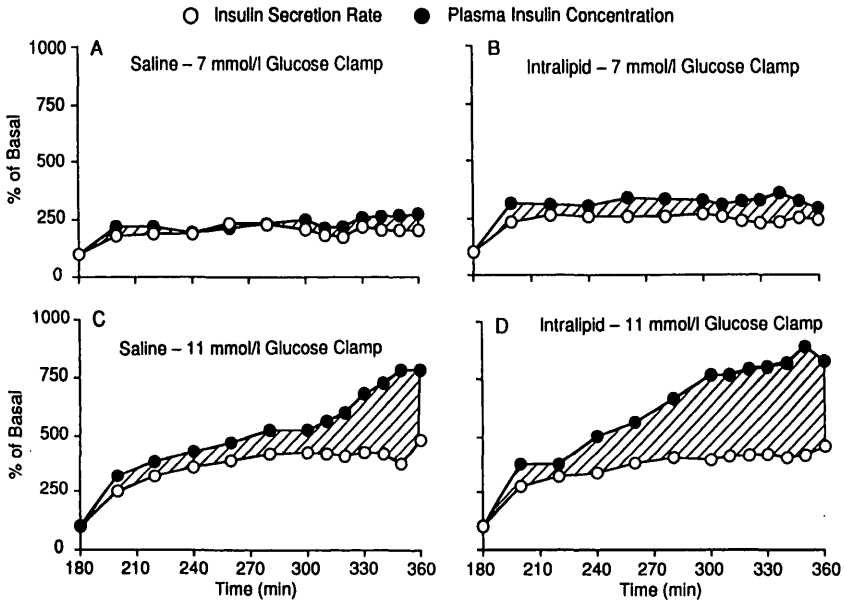

FIG. 4. ISR (○) and PICs (●) expressed as percentages of their basal value. *A*: level-I glucose clamp (7 mmol/l) during saline infusion. *B*: level-I glucose clamp during intralipid infusion. *C*: level-II glucose clamp (11 mmol/l) during saline infusion. *D*: level-II glucose clamp during intralipid infusion.

of the saline day. The increase in PIC at that level of glycemia results primarily from diminished EIC.

Synergistic effects of glucose and FFA on EIC. In Fig. 4, ISR and PIC are expressed as percentages of their mean basal values, taken as 100%. During the 7 mmol/l glucose clamp, while saline was infused (Fig. 4*A*) both ISR and PIC increased by ~200% of basal, which suggests no change in EIC. Raising FFA flux at the same level of glycemia (Fig. 4*B*) resulted in a slightly larger increase in both ISR and PIC, particularly during the first 2 h of the clamp. A trend toward a reduction in clearance is evidenced by an increase in the shaded area between the two curves. The effect of glucose on endogenous clearance can be seen by comparing Fig. 4*A* (7 mmol/l glucose clamp, saline day) to Fig. 4*C* (11 mmol/l glucose clamp, saline day). Both ISR and PIC increased during the 11 mmol/l glucose clamp. However, the relative increase in PIC was greater than that of the ISR, indicating a decline in EIC. Raising FFA flux at that level of glycemia further decreased the EIC, as evidenced by a significantly greater area between the two curves (Fig. 4*D*). To compare the effects of glucose and FFA and their possible interactivity, analysis of variance was applied as shown in Table 2. Glucose alone not only increased ISR and PIC, but also significantly decreased EIC. FFA, on the other hand, marginally increased PIC while significantly decreasing EIC. The effects of glucose and FFA seem to be independent and additive.

Effect of intralipid infusion on peripheral glucose utilization. The absolute amount of glucose metabolized did not differ during either saline or intralipid/heparin infusion with either level of glycemia (3.7 ± 0.1 and 3.1 ± 0.2 mmol · m^{-2} · min^{-1} during the 7 mmol/l glucose clamp, and 9.6 ± 1.9 and 8.4 ± 0.7 mmol · m^{-2} · min^{-1} during the 11 mmol/l glucose clamp with saline or intralipid/heparin infusion, respectively). The relative amount of glucose metabolized over the prevailing insulin concentration (M/I), however, was significantly blunted during the intralipid compared with the saline infusion. Raising plasma FFA flux during either level of glycemia resulted in significant reduction ($P = 0.03$) in the M/I ratio from 35.5 ± 5 to 23.6 ± 3 (μmol · m^{-2} · min^{-1})/(pmol · l^{-1}).

DISCUSSION

The present study demonstrated that raising plasma FFA flux in normal lean women via a continuous infusion of intralipid and heparin alters splanchnic insulin dynamics. Under basal conditions, raising FFA flux resulted in higher PIC consequent to increased ISR. As expected, raising plasma glucose to physiological postprandial levels (7 mmol/l) significantly increased ISR and PIC. Maintaining a high FFA flux during the glucose clamp resulted in significantly higher PIC due to the additive effects of an increase in pancreatic secretion and a decrease in EIC. Clamping plasma glucose at a higher level (11 mmol/l), as might be seen in glucose-intolerant and/or diabetic abdominally obese individuals, further increased PIC, as ISR increased and EIC decreased. At that level of glycemia, infusion of intralipid did not further increase ISR but synergistically decreased EIC, exceeding the effect of glucose alone. The suppressive effects of glucose and FFA on EIC were independent and additive.

While the role of glucose in stimulating insulin secretion is well established, the insulinotropic effects of fatty acids are not certain. In the present study, intralipid infusion modestly increased basal ISR. During the 7 mmol/l glucose clamp, an additive stimulatory effect of FFA on pancreatic secretion was also observed. This effect, however, was not seen when plasma glucose was clamped at 11 mmol/l. The mechanisms by which raising plasma FFA flux could influence insulin secretion are not clear. On the one hand, it is possible that the

TABLE 2
Effects of intralipid and glucose on splanchnic insulin dynamics by two-way analysis of variance

Parameter measured	Intralipid		Glucose		Interactivity	
	F	*P*	*F*	*P*	*F*	*P*
ISR	0.15	0.70	17.6	0.001	0.91	0.36
PIC	4.05	0.05	46.4	< 0.001	2.18	0.13
EIC	5.33	0.04	4.55	0.05	0.55	0.47

ISR, insulin secretion rate; PIC, plasma insulin concentration; EIC, endogenous insulin clearance.

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enhanced insulin secretion could be due to anticipated increase in hepatic glucose production secondary to increased supply of gluconeogenic precursors during intralipid infusion. On the other hand, it is also possible that FFA might exert direct effects on the secretory process. Previous studies have shown that the pancreatic response to FFAs is influenced by the duration of exposure (27,28), structure of the fatty acid (29), and level of glycemia (30). While acute exposure of pancreatic islets to fatty acids results in a stimulatory effect (31–33), long-term incubation impairs the glucose-induced insulin release (34). Similarly, intralipid infusion in rats enhanced insulin secretion after 3 h, while extending the infusion to 24 or 48 h resulted in a time-dependent inhibition of glucose-induced insulin secretion (28). Polyunsaturated and medium-chain fatty acids have been shown to stimulate insulin secretion in vitro and in vivo. Intralipid contains a higher concentration of polyunsaturated fatty acids, mainly linoleic acid. Incubating isolated pancreatic islets with albumin-bound palmitate did not have an effect on insulin release in the absence of glucose but increased insulin release when glucose was added to the incubation medium. In the present study, excessive glucose stimulation at the higher clamp level may have masked the changes occurring by raising FFA flux. The mechanism by which FFA could influence insulin secretion differs from that of glucose. Several mechanisms have been suggested, including increased Ca^{2+} influx, formation of long-chain acyl CoA esters (35), generation of metabolic signals (36), and β-cell hyperplasia (37).

In the present study, both glucose and FFA synergistically and independently suppressed EIC. The liver is by far the major organ responsible for insulin metabolism. This involves a series of steps, which include binding to a specific membrane receptor, internalization and intracellular compartmentalization of the insulin-receptor complex, and finally proteolytic degradation by a specific insulin-degrading enzyme (38). Conditions that might alter any of these events will eventually affect insulin degradation and endogenous clearance. We have previously hypothesized that in abdominally obese subjects, the enhanced lipolysis and increased flux of FFA in the portal vein from the expanded lipolytically active visceral adipose tissue—and hence the increased exposure of the liver to elevated FFA levels—might contribute to the altered hepatic insulin dynamics. Indeed, exposure of isolated rat hepatocytes to increasing concentrations of FFA has profound effects on hepatocyte insulin-receptor binding, internalization, and receptor-mediated degradation. The effect on the binding process is energy dependent and is closely linked to enhanced lipid oxidation (39,40). The post-binding events, however, appear to involve other mechanisms, possibly protein acylation. Further, raising portal vein FFA levels in isolated rat livers results in a decrease in insulin clearance (20). The present study shows that in humans, increased FFA flux while glucose is maintained at physiological postprandial levels alters endogenous insulin clearance. That insulin clearance was further decreased at the 11 mmol/l glucose level when intralipid was infused without an accompanying increase in ISR suggests that the mechanism by which FFA suppresses hepatic insulin clearance is independent of the level of insulinemia. In addition to direct effects of FFA on splanchnic insulin metabolism, indirect mechanisms may be involved. Our results show that the insulin sensitivity index (M/I) was lower during the intralipid infusion, suggesting impairment of insulin-mediated glucose utilization. This may result in a compensatory stimulation of insulin secretion and/or reduction of clearance.

Our results show a dose-dependent reduction in the clearance of endogenously secreted insulin in response to the two levels of glycemia, irrespective of the effect of intralipid. Glucose has been shown to affect splanchnic insulin clearance (41). Different experimental conditions, including the route of administration (42–44) and the prevailing glucose concentration (41,44), yielded different results, and the mechanism is uncertain.

In conclusion, increased FFA flux together with higher levels of glycemia, as might be seen in abdominally obese individuals, particularly those who are glucose intolerant or overtly diabetic, might be partially responsible for the state of hyperinsulinemia observed in those individuals. At basal and physiological glucose concentrations, the increase in peripheral insulin level is mainly due to increased insulin secretion rates. At higher glucose levels, decreased endogenous clearance is largely responsible for the hyperinsulinemia.

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