Original Articles

Insulin Regulation of Regional Free Fatty Acid Metabolism

Shon E. Meek, K. Sreekumaran Nair, and Michael D. Jensen

Studies were conducted to determine whether regional free fatty acid (FFA) release is differentially regulated by insulin. Systemic, leg, and splanchnic palmitate rate of appearance ([9,10-^{3}H]palmitate) was measured in 26 healthy adults using the euglycemic-hyperinsulinemic clamp technique to achieve a physiological range of plasma insulin concentrations. We found that insulin inhibited systemic, leg, and splanchnic palmitate release in a dose-dependent fashion over the range of insulin infused (0–1.0 mU · kg^{-1} · min^{-1}). Progressive hyperinsulinemia changed the leg from a net producer to a net FFA consumer, whereas the splanchnic bed converted from a net FFA consumer to a net producer. At the 0.5 mU · kg^{-1} · min^{-1} insulin infusion rate, leg FFA release was almost completely suppressed, whereas even with the 1.0 mU · kg^{-1} · min^{-1} insulin infusion rate, splanchnic FFA release decreased by only ~65% (P < 0.05 leg vs. splanchnic). These results demonstrate the regional heterogeneity of insulin-regulated FFA release in vivo, and indicate that visceral adipose tissue lipolysis is more resistant to insulin suppression than is leg lipolysis in humans. *Diabetes* 48:10–14, 1999

Insulin is one of the most important hormones regulating adipose tissue free fatty acid (FFA) release because of its potent antilipolytic effects (1,2). Dose-dependent effects of insulin on whole body (systemic) FFA metabolism have been demonstrated (1,2). Insulin dose-response characteristics for systemic lipolysis could represent a uniform adipose tissue response to this hormone or the net effects of variable responses from different fat depots. In vitro studies have suggested that regional differences in the antilipolytic effects of insulin may be present (3); however, whether these differences are present in vivo remains unknown.

The importance of understanding whether there are regional differences in insulin regulation of FFA release relates primarily to visceral adipose tissue. FFAs originating from visceral adipose tissue lipolysis enter directly into the portal vein, exposing the liver to more FFAs than would be predicted from systemic FFA availability data (4,5). FFAs influence several important hepatic functions, including glucose production (6), VLDL secretion (7), and insulin clearance (4,5). If visceral lipolysis is inhibited by insulin to a greater or lesser degree than systemic lipolysis, measures of systemic FFA availability will not accurately reflect hepatic FFA exposure. We therefore measured the dose-response characteristics of systemic, splanchnic, and leg FFA release in normal humans to determine whether the upper body and splanchnic adipose tissue respond differently to insulin than does leg adipose tissue.

RESEARCH DESIGN AND METHODS

Subjects. A total of 26 healthy adults (13 women, 13 men) between the ages of 21 and 38 years participated in the study, with 3 men and 3 women in each insulin dose group. Table 1 provides the characteristics of the volunteers. Each participant had a BMI between 18 and 27 and had normal hepatic and renal function. Participants taking medications known to affect FFA metabolism were excluded. The purpose and potential risks of the study were explained, and informed written consent was obtained from each subject. Volunteers were simultaneously infused with amino acid tracers as part of a study of insulin regulation of regional protein metabolism (20).

Materials. [9,10-^{3}H]palmitate was obtained from American Radiolabeled Chemicals (St. Louis, MO) and prepared for intravenous infusion as a 0.3% albumin in 0.9% NaCl solution. Regular Humulin insulin (Lilly, Indianapolis, IN) was prepared with 0.05% albumin in 0.9% NaCl for intravenous infusion. Indocyanine green (CardioGreen; Becton Dickinson, Cockeysville, MD) was used in these studies.

Assays. Plasma glucose was measured using a glucose analyzer (Beckman, Fullerton, CA). Plasma insulin was measured by radioimmunoassay (8). Epinephrine and norepinephrine were measured by high-performance liquid chromatography (HPLC) with electrochemical detection (9). Indocyanine green was measured by spectrophotometry on the day of the study. Plasma palmitate concentration and specific activity, as well as the isotopic purity of the [^{3}H]palmitate, were determined using HPLC (10).

Protocol. Subjects received a weight-maintaining diet from the Mayo Clinic General Clinical Research Center (GCRC) for 3 days before the study. The diet consisted of 20% protein, 50% carbohydrate, and 30% fat. All subjects maintained their usual level of physical activity. Total body and regional fat and fat-free mass (FFM) were measured within 3 days of the study using dual-energy X-ray absorptiometry (model DPX; Lunar Radiation, Madison, WI) (11). Each subject was scanned for 20 min, and whole body and regional analysis was performed using software version 3.6y.

Each participant was admitted to the GCRC the evening before the study. An 18-gauge catheter was placed in a forearm vein, and 0.9% NaCl was infused at a controlled rate. Each subject received one 325-mg aspirin tablet with the evening meal the day before the study to reduce the possibility of developing catheter-related platelet microthrombi.

Studies were conducted after a 12-h overnight fast in the GCRC. The morning of the study, each subject was transferred to the Mayo Clinic Vascular Radiology Department, where catheters were placed under sterile conditions into the right femoral artery, right femoral vein, and hepatic vein using standard percutaneous techniques, as previously described (12). Infusions of 0.9% NaCl were used to keep the catheters patent between blood samplings.

Blood samples from the femoral artery, femoral vein, and hepatic vein were collected during the baseline and insulin or saline infusion time intervals. Baseline samples were obtained 30, 40, 50, and 60 min after starting the [^{3}H]palmi-

From the Endocrine Research Unit, Mayo Clinic, Rochester, Minnesota.

Address correspondence and reprint requests to Michael D. Jensen, MD, Endocrine Research Unit, 5-164 W. Joseph, Mayo Clinic, Rochester, MN 55905. E-mail: jensen.michael@mayo.edu.

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FFA, free fatty acid; FFM, fat-free mass; GCRC, General Clinical Research Center; HPLC, high-performance liquid chromatography.

tate infusion (0.3 μCi/min), which was then discontinued after the last sample. Each participant was randomly assigned to receive an intravenous infusion of saline (no insulin) or a single dose of insulin (0.25, 0.5, or 1.0 mU · kg^{-1} · min^{-1}) through a peripheral vein for 150 min after the baseline blood samples had been collected. A 50% dextrose solution was infused through a peripheral vein to maintain euglycemia during the insulin infusion interval (150–300 min) along with 0.9% NaCl with 20 mEq KCl/l at 50 ml/h. A second series of blood samples was obtained at 10-min intervals over the last 30 min of the insulin or saline infusion. The [^{3}H]palmitate tracer was restarted 30 min before the second sampling period. After completion of the study, the catheters were removed and hemostasis obtained.

A primed continuous (~220 μg/min) infusion of indocyanine green was administered into the femoral artery sheath to allow measurement of leg and splanchnic plasma flow (13,14). The infusion was begun 30 min before blood sampling for kinetic measurements began.

Each volunteer received a single insulin dose because it was considered impractical to leave the sampling catheters in place for a sufficient period of time to examine the entire insulin dose curve for each volunteer or to study the same individual on four occasions.

Calculations and statistics. Steady-state plasma palmitate concentrations and specific activity were used together with leg (13) and splanchnic (14) plasma flow to measure regional FFA uptake and release (15,16) for each sampling period. Upper body nonsplanchnic palmitate release was calculated as previously described (17). Net splanchnic FFA release, as assessed using the combination of isotope dilution and arteriovenous balance techniques, does not measure total visceral adipose tissue FFA release. Instead, it is a minimum estimate of visceral adipose tissue FFA release because the liver takes up a significant proportion of FFAs, both those being delivered from the systemic circulation and those originating from visceral adipose tissue lipolysis. Unfortunately, because extrahepatic splanchnic FFA uptake also occurs (18), it is not possible to determine the fraction of splanchnic FFA uptake that occurs in the liver without access to the portal vein. Because the liver does not itself release FFAs (18), however, the combination of isotope dilution and arteriovenous balance techniques does allow the measurement of net appearance of new FFAs in the hepatic vein that have originated from visceral adipose tissue lipolysis. Thus, splanchnic FFA release as presented here represents the contribution of visceral adipose tissue lipolysis to systemic FFA availability.

The insulin dose-response characteristics of regional palmitate release were linearized by converting the appearance rate values to their natural logarithm. Insulin's effect on regional palmitate release was analyzed by regressing the natural logarithm of leg and splanchnic palmitate release versus the plasma insulin concentration to determine whether the slopes of the lines describing the relationships differed.

Results are expressed as means ± SE. Statistical comparisons between groups at baseline and during the insulin dose interval were made using analysis of variance and subsequent nonpaired Student's *t* tests with Bonferroni correction if a significant difference was detected among variables that were not part of the primary hypotheses to be tested.

RESULTS

Subject characteristics. Table 1 provides characteristics of subjects categorized by the insulin dose groups to which they were assigned. Age, weight, height, and percent body fat were not significantly different between the groups. Likewise, fasting plasma glucose concentrations were not significantly different between groups.

The hepatic vein catheter became dislodged in one man and one woman in the 0.5 mU · kg^{-1} · min^{-1} insulin dose group. Two additional volunteers were recruited to ensure adequate splanchnic data; however, the leg and systemic FFA values from the two subjects without splanchnic data were included in portions of the final analysis.

TABLE 1
Subject characteristics

Insulin group (mU · kg^{-1} · min^{-1})	*n*	Age (years)	Weight (kg)	Height (cm)	Body fat (%)
0	6	29 ± 2	69.7 ± 7.2	175 ± 5	20 ± 3
0.25	6	27 ± 2	72.4 ± 6.7	178 ± 5	23 ± 3
0.5	8	27 ± 1	73 ± 5.0	174 ± 4	28 ± 2
1.0	6	27 ± 2	71.1 ± 6.7	173 ± 5	27 ± 2

Data are means ± SE.

TABLE 2
Plasma insulin and catecholamine concentrations

Insulin group (mU · kg^{-1} · min^{-1})	Insulin (pmol/l)	Epinephrine (pmol/l)	Norepinephrine (nmol/l)
0			
Baseline	28 ± 2	229 ± 53	0.57 ± 0.10
Insulin	25 ± 2	269 ± 62	0.57 ± 0.08
0.25			
Baseline	48 ± 6	229 ± 53	0.61 ± 0.10
Insulin	145 ± 21*	426 ± 144	0.67 ± 1.02
0.5			
Baseline	47 ± 5	233 ± 70	0.53 ± 0.07
Insulin	194 ± 7*	446 ± 98	0.62 ± 0.10
1.0			
Baseline	41 ± 5	183 ± 44	0.65 ± 0.07
Insulin	404 ± 26*	204 ± 60	0.81 ± 0.08

Data are means ± SE. *P < 0.05 compared with baseline.

Regional plasma flow. Baseline leg and splanchnic plasma flow for all subjects averaged 283 ± 16 and 875 ± 38 ml/min, respectively. Baseline leg and splanchnic plasma flow in the insulin-treated groups did not differ significantly from the control group that was not treated with insulin.

Plasma insulin and catecholamine concentrations. Baseline plasma insulin and catecholamine concentrations (Table 2) were similar in all insulin dose groups, although the baseline insulin concentrations tended (P = 0.14) to be different in the zero insulin dose group compared with the other groups. Plasma insulin concentrations increased as expected in those groups receiving insulin infusions. Plasma epinephrine and norepinephrine concentrations tended to increase with time in all groups, but the changes were not statistically significant. Plasma epinephrine and norepinephrine concentrations were not different between the groups during the baseline or insulin infusion time intervals.

Net palmitate balance. For all subjects, baseline plasma palmitate concentrations in the femoral artery, femoral vein, and hepatic vein were 133 ± 6, 147 ± 7, and 106 ± 6 μmol/l, respectively. Therefore, in the postprandial state, leg was a net FFA producer, and the splanchnic bed was a net FFA consumer. Figure 1 shows the net leg and splanchnic palmitate balance as a function of the insulin dose infused. At the highest insulin dose, the leg became a net FFA consumer, whereas the splanchnic region became a net FFA producer.

Systemic palmitate kinetics. The [^{3}H]palmitate specific activity achieved plateau values during the sampling periods for all studies. Baseline palmitate flux for all participants was 104 ± 7 μmol/min (2.2 ± 0.2 μmol · kg^{-1} FFM · min^{-1}) and did not differ significantly between the groups. Palmitate flux decreased in a dose-dependent fashion with increasing insulin infusion rates, being 87% suppressed at the highest insulin dose (Table 3). Palmitate flux expressed as a function of systemic insulin concentration also showed a similar dose-dependent decrease with insulin (Fig. 2*A*).

Regional palmitate release. For all subjects, baseline palmitate release was 14.1 ± 1.4, 15.9 ± 2.1, and 58 ± 6 μmol/min,

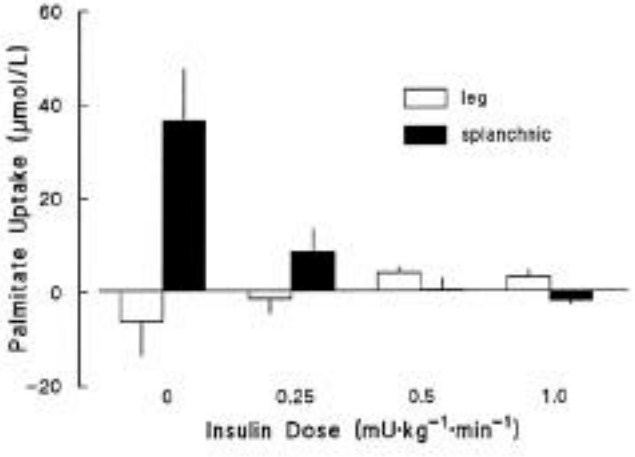

FIG. 1. Leg and splanchnic palmitate balance as a function of insulin dose infused.

respectively, for single leg, splanchnic, and nonsplanchnic upper body regions. Baseline upper body palmitate release relative to upper body fat mass was greater than lower body palmitate release relative to lower body fat mass (8.5 ± 1.0 vs. 4.7 ± 0.4 µmol · kg fat^{-1} · min^{-1}, respectively). Insulin suppressed leg palmitate release in a dose-dependent fashion as compared with the saline control, achieving 77% suppression at the lowest dose infused and almost complete (93%) suppression at the highest dose. Although splanchnic palmitate release was also inhibited in a dose-dependent fashion, the lowest insulin dose did not significantly suppress splanchnic palmitate release below baseline values, and it was only 65% suppressed at the highest insulin infusion rate.

Figure 2*B* and *C* depicts the leg and splanchnic palmitate release for each participant as a function of their plasma insulin concentration. Mild hyperinsulinemia appears to suppress FFA release from both the leg and splanchnic regions. At higher plasma insulin concentrations, leg palmitate release is almost undetectable, whereas splanchnic palmitate release approaches a plateau and is not completely suppressed. Figure 3 depicts a logarithmic plot of leg and splanchnic palmitate release as a function of the systemic insulin concentration. The slopes of the leg and splanchnic palmitate release regression lines are significantly different (P < 0.01), indicating a different insulin dose-response relationship between the two adipose tissue beds.

Leg (both legs) and splanchnic palmitate release as a percentage of total palmitate release is plotted as a function of insulin dose in Fig. 4. At higher plasma insulin concentrations, leg palmitate release relative to systemic palmitate release decreases, whereas splanchnic palmitate release relative to systemic palmitate release increases.

Table 3 provides the nonsplanchnic upper body palmitate release data for each insulin dose group. The same dose-dependent effects are evident, with palmitate release being ~90% suppressed at the highest insulin infusion rate.

Regional palmitate uptake. Table 3 also provides leg and splanchnic palmitate uptake data for each of the insulin dose groups. Leg palmitate uptake was significantly reduced by insulin even at the 0.25 mU insulin dose. Splanchnic palmitate uptake was suppressed by insulin; however, the suppression was not statistically significant at the lowest insulin dose.

DISCUSSION

These studies are the first to examine insulin's dose-dependent effects on leg and splanchnic FFA kinetics in humans. The

TABLE 3
Systemic and regional palmitate kinetics

	Insulin (mU · kg^{-1} · min^{-1})			
	0	0.25	0.5	1.0
Arterial concentration (µmol/l)				
Baseline	122 ± 9	135 ± 11	143 ± 11	135 ± 15
Insulin	148 ± 19	44 ± 17*	19 ± 4*	9 ± 1*
Systemic flux (µmol/min)				
Baseline	100 ± 17	96 ± 7	118 ± 13	99 ± 15
Insulin	115 ± 21	38 ± 12*	21 ± 4*	12 ± 2*
Leg release (µmol/min)				
Baseline	14 ± 3	17 ± 4	14 ± 3	12 ± 2
Insulin	13 ± 1	4 ± 1*	2 ± 1*	2 ± 1*
Splanchnic release (µmol/min)				
Baseline	15 ± 4	15 ± 2	18 ± 7	15 ± 4
Insulin	18 ± 4	10 ± 4	6 ± 2*	5 ± 1*
Nonsplanchnic upper body release (µmol/min)				
Baseline	57 ± 17	46 ± 6	68 ± 11	60 ± 13
Insulin	72 ± 17	21 ± 6*	12 ± 5*	5 ± 2*
Leg uptake (µmol/min)				
Baseline	9.2 ± 1.1	9.5 ± 2.0	12.0 ± 2.0	8.4 ± 2.1
Insulin	9.3 ± 1.3	3.4 ± 0.9*	3.4 ± 0.8*	1.9 ± 0.5*
Splanchnic uptake (µmol/min)				
Baseline	34.5 ± 4.8	45.7 ± 7.1	34.4 ± 5.2	42.7 ± 5.0
Insulin	43.5 ± 7.2	20.9 ± 10.8	6.9 ± 1.6*	4.2 ± 1.0*

Data are means ± SE. Leg values refer to single leg. *P < 0.05 insulin vs. baseline.

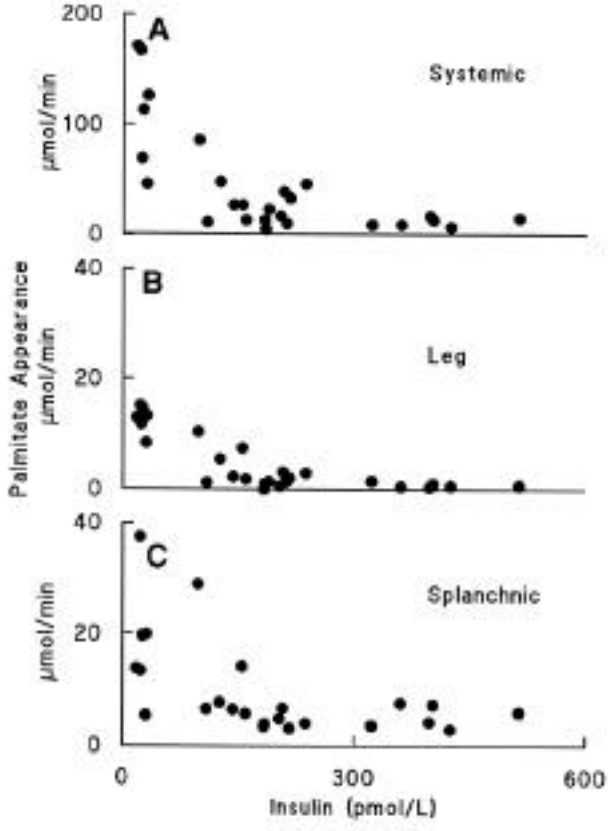

FIG. 2. Systemic (*A*), leg (*B*), and splanchnic (*C*) palmitate release for each volunteer is plotted as a function of the plasma insulin concentration observed during the insulin infusion time interval.

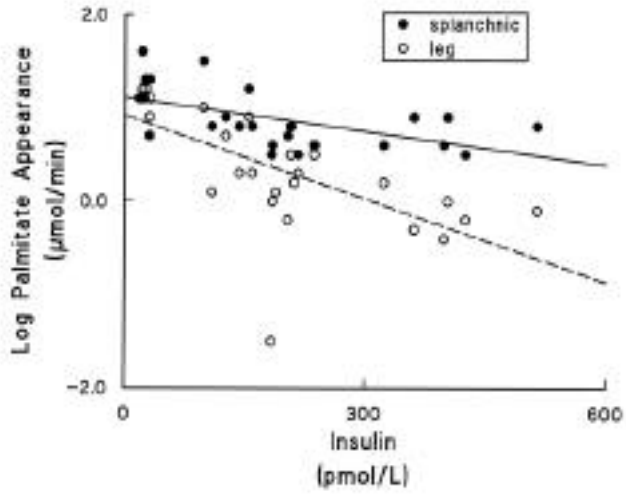

FIG. 3. Natural logarithm of leg and splanchnic palmitate release during the insulin infusion interval is plotted for each volunteer as a function of plasma insulin concentration. ——, leg palmitate release data; — —, splanchnic palmitate release data.

euglycemic-hyperinsulinemic clamp method was combined with regional vascular catheterization and isotope dilution techniques to investigate insulin's ability to suppress adipose tissue lipolysis in different tissue beds. Leg and nonsplanchnic upper body adipose tissue FFA releases were considerably suppressed by each insulin dose tested, and they were almost completely inhibited at the highest dose. Splanchnic FFA release, a minimum estimate of visceral adipose tissue lipolysis, contributed progressively greater proportions of systemic FFA flux at higher plasma insulin concentrations. These results provide strong evidence that visceral adipose tissue in humans is more resistant to insulin's antilipolytic effects than is leg and nonsplanchnic upper body fat. We conclude that measures of systemic FFA availability during hyperinsulinemia will substantially underestimate the amount of FFA to which the liver is exposed.

Although several investigators have examined the effect of insulin on subcutaneous adipocyte lipolysis in vitro, few studies have compared visceral with subcutaneous adipocytes in this regard. The available data suggest that omental adipocytes are less sensitive, and lower body adipocytes are more sensitive, to insulin than are abdominal subcutaneous adipocytes (3). Unfortunately, it is not easy to mimic the complex in vivo hormonal (growth hormone, catecholamine, cortisol) and substrate milieu in which adipocytes normally reside. This makes it difficult to extrapolate in vitro findings to the in vivo situation. We have reported that meal consumption suppresses splanchnic FFA release to a lesser extent, and leg FFA release to a greater extent, than does systemic lipolysis (12,19). However, a mixed meal results in many changes in hormone, catecholamine, and substrate levels that could potentially influence the observed antilipolytic responses. Thus, it was not possible to conclude that regional differences in insulin inhibition of FFA release were present in humans without conducting dose-response studies. The present study isolated the effects of insulin on regional FFA kinetics, and the results imply that the postprandial changes we observed were mediated primarily by insulin.

Although splanchnic FFA release is not a direct measure of visceral adipose tissue lipolysis, the present insulin dose-response studies offer a unique opportunity to compare the inhibition of visceral, leg, and nonsplanchnic upper body adi-

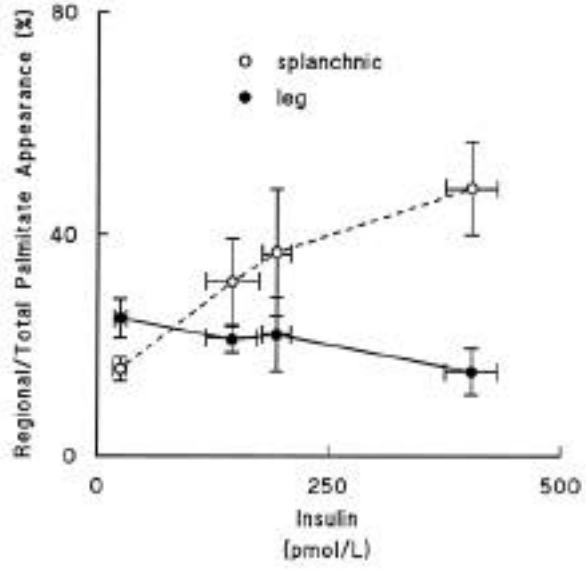

FIG. 4. Percentage of systemic palmitate release derived from leg (both legs) and the splanchnic bed is plotted versus the plasma insulin concentration achieved for each of the insulin dose groups.

pose tissue lipolysis by physiological insulin concentrations. Leg FFA release was almost completely suppressed at plasma insulin concentrations of ~200 pmol/l, whereas splanchnic FFA release, which underestimates visceral lipolysis, was not completely suppressed even at plasma insulin concentrations of ~400 pmol/l. This would suggest that the proportion of FFAs delivered to the liver directly from omental and mesenteric adipose tissue lipolysis increased substantially with progressive hyperinsulinemia.

The relatively greater rates of visceral lipolysis during hyperinsulinemia are an important and intriguing phenomena. This difference in the physiological regulation of lipolysis may ensure a basal level of FFA (and glycerol) availability to the liver after carbohydrate ingestion. A continuing source of energy as FFAs may spare glucose oxidation by the liver, while more dramatic suppression of peripheral FFA availability should facilitate peripheral glucose disposal. Viscerally derived FFAs could become especially critical during insulin-induced hypoglycemia in diabetic patients.

These results have significant implications for the understanding of hepatic FFA delivery in relation to systemic FFA flux. Our findings demonstrate that the suppression of systemic FFA flux by insulin is not an ideal indicator of the decrease in hepatic FFA delivery. Although systemic FFA release decreased by 90% at plasma insulin concentrations of ~400 pmol/l, a much greater percentage of systemic FFA originated from the splanchnic bed under these conditions (Fig. 4). Thus, the insulin-induced reductions in systemic FFA are not entirely representative of the amount of FFA entering the liver. Considering the potential importance of FFAs in modulating hepatic glucose, triglyceride, and insulin metabolism, future studies of these interrelationships will need to take this finding into account. It is possible that these relationships would be exaggerated in conditions such as visceral obesity, which is strongly associated with hepatic insulin resistance, hypertriglyceridemia, and hyperinsulinemia. Large visceral fat stores could be even more resistant to insulin's antilipolytic effects and could contribute in a direct way to increased hepatic FFA delivery after meal ingestion. Further studies are needed to address this possibility.

By chance, the volunteers in the zero insulin dose group were more lean and had lower baseline insulin concentrations than the other three groups. Although the percent body fat of each volunteer in this group was within the range we usually observe for nonobese men and women, two of the men had 10% body fat (the lower range of normal for men), and all three women had <30% body fat, the average value we find in nonobese women. Perhaps because of their tendency toward leanness, this group may have been more insulin-sensitive, accounting for their lower fasting plasma insulin concentrations. Note that the baseline palmitate concentrations and flux in this group were comparable to the other groups, and because they did not receive an insulin infusion during the second experimental interval, any enhanced insulin sensitivity that might have been present could not affect the analysis. We suspect that the baseline differences in body fat and plasma insulin concentrations among the four groups have not affected the conclusions drawn from these studies, because the effect of insulin in suppressing lipolysis appears to be profound in humans within the range of body fat included in this study.

In summary, we examined the dose-response relationship between insulin and regional FFA release. Leg fat was found to be more sensitive to inhibition of lipolysis than was systemic lipolysis, whereas visceral fat is less responsive to insulin. Moderate hyperinsulinemia appears to increase the proportion of FFAs reaching the liver from visceral, as opposed to systemic, sources. Our results have significant implications for understanding the effects of insulin on glucose/fatty acid interrelationships in humans.

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