Extreme Insulin Resistance of the Central Adipose Depot In Vivo

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Despite the well-described association between obesity and insulin resistance, the physiologic mechanisms that link these two states are poorly understood. The present study was performed to elucidate the role of visceral adipose tissue in whole-body glucose homeostasis. Dogs made abdominally obese with a moderately elevated fat diet had catheters placed into the superior mesenteric artery so that the visceral adipose bed could be insulinized discretely. Omental insulin infusion was extracted at ~27%, such that systemic insulin levels were lower than in control (portal vein) insulin infusions. Omental infusion did not lower systemic free fatty acid levels further than control infusion, likely because of the resistance of the omental adipose tissue to insulin suppression and the confounding lower systemic insulin levels. The arteriovenous difference technique showed that local infusion of insulin did suppress omental lipolysis, but only at extremely high insulin concentrations. The median effective dose for suppression of lipolysis was almost fourfold higher in the visceral adipose bed than for whole-body suppression of lipolysis. Thus, the omental adipose bed represents a highly insulin-resistant depot that drains directly into the portal vein. Increased free fatty acid flux to the liver may account for hepatic insulin resistance in the moderately obese state. *Diabetes* 51:755–761, 2002

Insulin resistance is an important risk factor for type 2 diabetes and plays a role in many other diseases, including polycystic ovarian syndrome, cancer, and atherosclerosis. Several studies have reported an association between abdominal obesity and insulin resistance (1,2), but the physiological causality underlying this linkage remains unclear. We and others have demonstrated a strong correlation between free fatty acid (FFA) levels and hepatic glucose output (3,4) and have hypothesized that omental fat contributes to liver insulin resistance by increasing portal delivery of FFAs (5–7). This could lead to inappropriately elevated hepatic glucose production, hyperinsulinemia, and eventual β-cell failure.

Omental adipose tissue has been shown in vitro to be relatively resistant to insulin suppression of lipolysis (8), and therefore would be expected to contribute disproportionately to hepatic FFA levels, especially in the hyperinsulinemic state. However, insulin sensitivity of visceral adipose tissue assessed in vivo is less well defined. In this regard, Meek et al. (9) recently measured splanchnic FFA release in vivo and reported that overall splanchnic lipolysis is relatively resistant to insulin suppression. However, the explicit in vivo sensitivity of visceral adipocytes remains to be directly measured.

The present study was performed to characterize the dynamic regulation of omental adipocyte lipolysis in vivo under hyperinsulinemic conditions. Male dogs were made abdominally obese with a modest daily supplement of 2 g/kg body wt of cooked bacon grease for 4 weeks. Euglycemic clamp experiments were performed in the conscious state, and insulin was infused either directly into the omentum via the superior mesenteric artery, causing relative omental hyperinsulinemia, or into the portal vein on a separate occasion (control). Portal vein sampling was used to directly measure the net omental lipolysis in response to selective increases in omental insulin.

RESEARCH DESIGN AND METHODS

Animals. Experiments were performed on eight male mongrel dogs (31.9 ± 2.2 kg [mean ± SE] at the time of experiments). For 4 weeks before experiments, the animals were fed a diet supplemented with fat, representing a normal weight-maintaining diet plus a supplement of ~450 kcal from cooked bacon grease. The effects of fat supplementation on the development of insulin resistance and its compensation have been presented; five of these eight dogs were included in the previous study (10). One week before experimentation, with the dogs under general anesthesia, chronic catheters (Fig. 1) were implanted as previously described (11). Catheters were placed in the carotid artery for sampling of arterial blood and in the femoral vein for infusion of tritiated glucose. Two catheters were placed into the portal vein, one 4 cm upstream from the porta hepatis for portal replacement infusions of glucagon and insulin and one ~1 cm upstream from the porta hepatis for sampling of portal vein blood. We have previously shown that infusions given >3 cm upstream from the liver are overall equally distributed among the liver lobes (12). An additional chronic catheter was placed in the superior mesenteric artery (SMA) ~2–3 cm distal to its bifurcation from the abdominal aorta, such that the tip of the catheter was ~7 or 8 cm from the bifurcation. This catheter was used to infuse insulin directly into the omentum, since the SMA perfuses >90% of the omental fat depot (13). On the morning of the experiment, an acute catheter was inserted into the saphenous vein for the variable infusion of labeled glucose.

Euglycemic clamp protocol. Each dog underwent two types of euglycemic clamps in random order. All clamps were performed after a 16-h overnight fast. After a fasting sample was taken at −150 min, infusions of somatostatin (1 $\mu g \cdot kg^{-1} \cdot min^{-1}$) (SRIF; Bachem California, Torrance, CA) and high-performance liquid chromatography–purified [3-^{3}H]D-glucose (25 μCi + 0.25 μCi/min) (NEN Research Products, DuPont, Boston, MA) were started into the femoral vein. Portal replacement infusions of insulin (0.3 $mU \cdot kg^{-1} \cdot min^{-1}$) (porcine insulin; Novo-Nordisk, Copenhagen, Denmark) and glucagon (1.0 ng $\cdot kg^{-1} \cdot min^{-1}$) (porcine glucagon; Sigma Chemical, St. Louis, MO) were

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$AUC_{INSULIN}$, insulin area under the curve; ED_{50}, median effective dose; EGP, endogenous glucose production; FFA, free fatty acid; SMA, superior mesenteric artery.

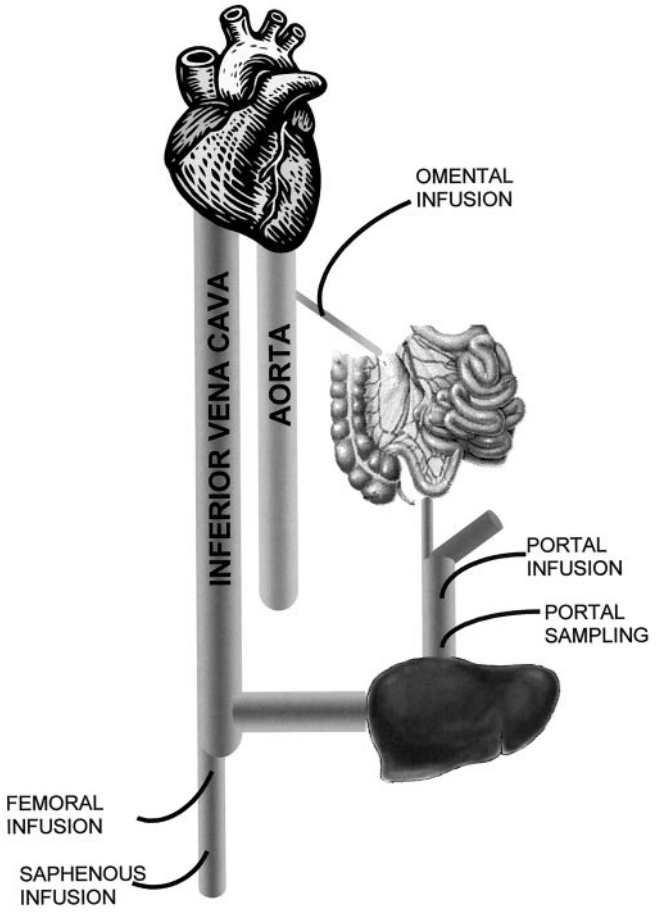

FIG. 1. Chronic indwelling catheters were placed in the portal vein for both sampling and infusions of insulin and glucagon; in the superior mesenteric artery for infusion of insulin; in the femoral vein for infusion of glucose tracer; and in either the left or right carotid artery for sampling of arterial blood (not shown). A saphenous vein catheter was acutely placed the morning of the experiment for variable infusion of labeled glucose.

initiated at the same time. Glucose and glucose specific activity were clamped using a variable infusion of labeled glucose into the saphenous vein ("hot Ginf" method). After an equilibration period, basal samples were taken at −20, −10, and 0 min. At time 0, three successive 90-min insulin infusions were given at 0.3, 0.6, and 1.2 mU · kg^{-1} · min^{-1}. These stepwise insulin infusions were given either directly into the superior mesenteric artery (in omental studies) or into the portal vein (in control studies). Samples were taken at 5, 10, 15, 20, 30, 45, 60, 75, and 90 min after the initiation of each insulin infusion.

Assays. Samples were collected and assayed for glucose, FFAs, glycerol, insulin, glucagon, and tritiated glucose as previously described (10,14).

Calculations. Basal values were defined as the mean of all basal samples. Steady-state values were defined as the average of the last 30 min of each insulin infusion step (SS1, SS2, and SS3). Insulin area under the curve ($AUC_{INSULIN}$) was calculated for each insulin infusion period using the trapezoidal rule and normalizing the basal AUC to a 90-min period. The average slope of the line for systemic $AUC_{INSULIN}$ versus insulin infusion rate reflects the efficiency with which a given insulin infusion raises systemic insulin concentration and was used to calculate extraction of insulin by the omentum before it reached the systemic circulation. Omental insulin extraction was estimated only for paired experiments as 1 minus the ratio of the average slopes of the systemic $AUC_{INSULIN}$ versus insulin infusion rate for each route (control and omental; Fig. 3). Omental insulin levels were estimated as equal to arterial levels during basal periods and throughout the control experiments; during omental insulin infusion, they were estimated using the equation

$$INS_{OMENTAL} = INS_{ARTERIAL} + \frac{infusion_{OMENTAL}}{plasma\ flow_{OMENTAL}}$$

where omental plasma flow is estimated as 10 ml · kg^{-1} · min^{-1} (15). The K_M parameters for insulin suppression of arterial FFA levels were calculated for each experiment by fitting a modification of the Michaelis-Menten equation:

$$FFA = FFA_{BASAL} - \frac{V_{max} \times insulin}{K_M + insulin}$$

where FFA_{BASAL}, V_{max}, and K_M are all fitted parameters.

Portal blood sampling was successful in four omental and four unpaired control experiments; therefore, all values of portal concentrations and omental lipolysis rates report only these eight experiments. Omental lipolysis was estimated by the equation

$$omental\ lipolysis = \frac{(FFA_{PV,smoothed} - FFA_{ART,smoothed}) \times PPF}{weight}$$

where FFA levels were presmoothed using an optimal segments algorithm (16) and PPF is portal plasma flow, estimated to be 16 ml · kg^{-1} · min^{-1} (17,18). This equation calculates net FFA output from the omentum, which is equal to total omental lipolysis minus omental FFA reesterification and oxidation. Whole-body lipolysis was estimated as a percent of its basal value by assuming it to be proportional to the systemic FFA levels (i.e., assuming no significant changes in FFA uptake). All calculations were made using Microsoft Excel 2000 on an IBM-compatible computer. All fits were performed with SlideWrite Plus for Windows (version 5.0; Advanced Graphics Software, Encinitas, CA).

Statistics. All outcome variables were analyzed with general linear-model ANOVA, testing for effects of dog, insulin infusion route, insulin step, and interaction between insulin infusion route and step (Minitab, State College, PA). Individual comparisons between different insulin infusion rates for each infusion route were made by calculating Bonferroni 95% simultaneous confidence intervals, which adjust the P values for multiple comparisons.

RESULTS

Glucose and insulin. Glucose concentration decreased slightly during insulin infusions ($P < 0.001$) but was not different between infusion routes (NS, ANOVA). Although glucose specific activity was not different between infusion routes, it tended to increase during the first insulin infusion period in both groups ($P < 0.05$ for basal vs. each SS period, ANOVA) but then remained stable for the remainder of the experiment (Table 1; Fig. 2). As expected,

TABLE 1
Euglycemic clamp values

Infusion route	Fasting		Basal		SS1 (0.3 mU · kg^{-1} · min^{-1})		SS2 (0.6 mU · kg^{-1} · min^{-1})		SS3 (1.2 mU · kg^{-1} · min^{-1})	
	Omental	Control	Omental	Control	Omental	Control	Omental	Control	Omental	Control
Glucose (mg/dL)	96 ± 2	92 ± 2	123 ± 14	128 ± 18	103 ± 6	106 ± 7	96 ± 2	95 ± 2*	94 ± 3*	89 ± 3†
SA (×10^3 dpm/mg)	—	—	5.6 ± 0.4	5.2 ± 0.6	6.4 ± 0.3*	6.1 ± 0.5	7.0 ± 0.4‡	6.9 ± 0.6†	7.1 ± 0.3‡	6.8 ± 0.4†
Insulin pmol/l	138 ± 20	197 ± 71	59 ± 8	59 ± 11	111 ± 23	123 ± 14	167 ± 38*	192 ± 32†	321 ± 66‡	365 ± 65‡

Data are means ± SE. SS1, SS2, and SS3 represent the average values of the last 15 minutes of the corresponding insulin step infusion. *$P < 0.05$, †$P < 0.01$, and ‡$P < 0.001$ vs. basal period.

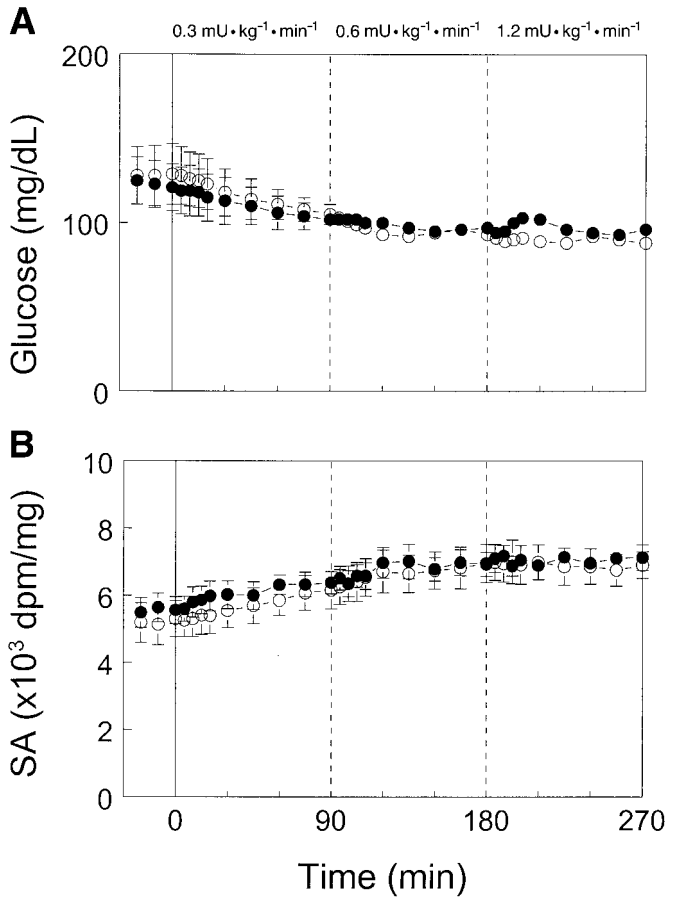

FIG. 2. Plasma glucose (*A*) and glucose specific activity (*B*) during euglycemic clamp experiments. Specific activity was slightly higher throughout the omental infusion experiments (●) than during control experiments (○). This difference did not change during the experiment, however, implying that the difference was independent of omental insulin.

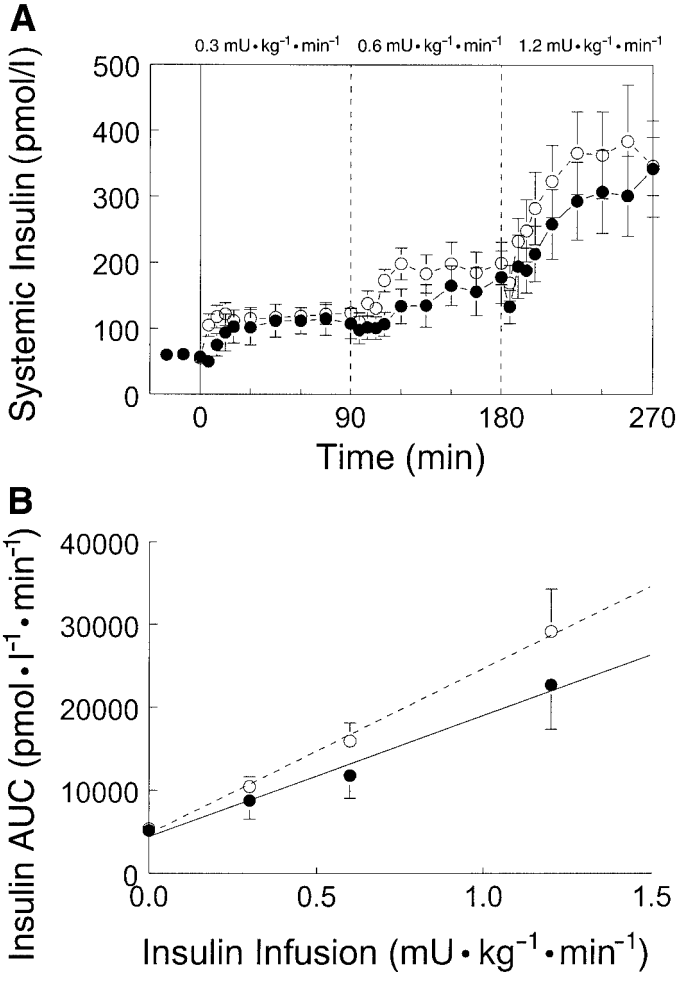

FIG. 3. *A*: Systemic insulin concentrations with omental infusion (●) and control infusion (○). *B*: The total systemic insulin area under the curve for each insulin infusion period (see RESEARCH DESIGN AND METHODS for calculations).

fasting insulin concentrations in these animals were elevated (163 ± 42 pmol/l) compared with the normal range previously observed in our laboratory (11,14,19), due to the high-fat diet and concomitant insulin resistance. Somatostatin and portal insulin replacement at 0.3 mU · kg^{-1} · min^{-1} therefore yielded basal insulin concentrations below the fasting levels in these animals, yet within the range expected for normal animals (59 ± 8 pmol/l) (Table 1). Although there was no significant effect of insulin infusion route on steady-state systemic insulin levels (P = 0.28, ANOVA), the $AUC_{INSULIN}$ was lower with omental infusion than control ($P > 0.05$, ANOVA). The slope of the relationship between $AUC_{INSULIN}$ and insulin infusion rate was decreased with omental infusion (Fig. 3). This was presumably due to first-pass omental extraction of insulin, which was calculated as 27 ± 7% with omental infusion.

FFAs and glycerol. As expected, systemic FFA levels were suppressed with insulin infusion in a dose-response pattern, declining 80% at the highest dose ($P < 0.01$). The route of insulin infusion (SMA/omental versus systemic/control) had no significant effect on arterial FFA concentrations. At first glance, this might be interpreted to indicate that omental insulin was not more effective in overall suppression of lipolysis than intraportal insulin. However, this interpretation is confounded by the apparent degradation of insulin in the omental tissue bed. The effect of omental infusion on overall lipolysis is more correctly assessed from the dose response between systemic insulin concentration and FFA levels. With omental infusion, we observed a tendency for a leftward shift (K_M = 35 ± 9 vs. 58 ± 12 pmol/l, omental versus control; P = 0.08, unpaired t test) (Fig. 4). That this tendency was slight suggested that the omental fat tissue was particularly resistant to direct SMA infusion of insulin. Therefore, net omental lipolysis was calculated from the four experiments in each group in which portal vein sampling was successful (Table 2; Fig. 5). Direct infusion of insulin into the omental adipose tissue bed tended to suppress omental lipolysis (omental, P = 0.12). Systemic insulin infusion had no effect on omental lipolysis (control, P = 0.85). Of course, direct infusion of insulin into the omentum via the SMA causes a marked increase in local insulin concentration far above the systemic increment. Therefore, we compared the relationship between local omental insulin concentration and net lipolysis of the omental tissue bed with suppression of lipolysis for the entire body (Table 3; Fig. 6). When the insulin and lipolysis data in Table 3 were fitted by linear regression, the slope of the line for suppression of omental lipolysis was less than one-third of

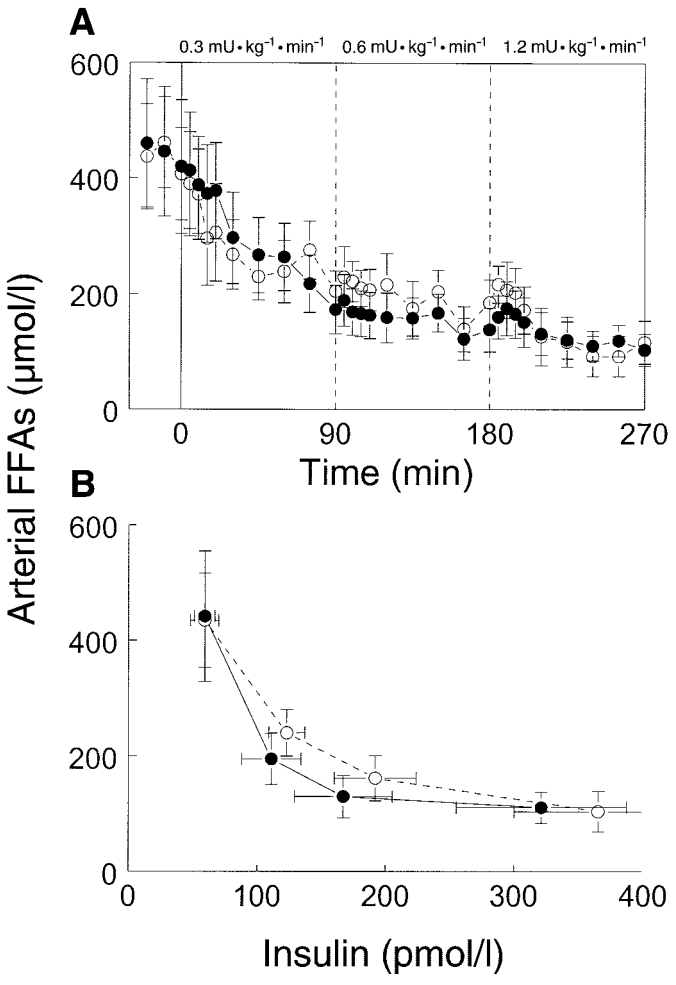

FIG. 4. Arterial FFA levels during euglycemic clamps. *A*: The time course, with no significant difference between omental (●) and control (○) infusions. *B*: The relationship between arterial FFA and systemic insulin levels, demonstrating a slight rightward shift with control infusion, implying greater suppression of arterial FFA levels with omental insulin.

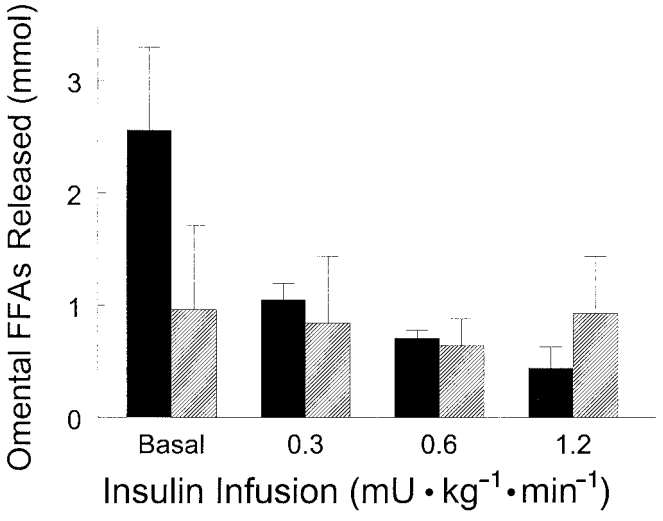

FIG. 5. Omental FFA production, calculated from the integrated lipolysis over the last 30 min of each insulin infusion period. Lipolysis was calculated from the A-V FFA difference in four unmatched animals (see RESEARCH DESIGN AND METHODS). Omental infusion (■) yielded a suppression of FFA production, whereas control infusion (▨) showed no change across all insulin infusion levels.

that for suppression of whole-body lipolysis (control, -0.22 ± 0.04; omental, -0.07 ± 0.01; $P = 0.011$). This shift reflects a profound threefold increase in insulin resistance of the omental adipose bed in vivo relative to lipolysis of the body as a whole.

Glucose fluxes. Glucose fluxes were not different between the two routes of insulin infusion (Fig. 7). However, omental insulin extraction yielded lower systemic insulin levels with omental infusions than with control infusions. Thus, omental insulin infusion suppressed endogenous glucose production (EGP) to the same extent as control infusion despite the lower systemic insulin levels. This implies that omental insulinization contributed to suppression of EGP independent of systemic insulin levels. The relationship between portal vein FFA levels and EGP was not different with route of insulin infusion (Fig. 8).

DISCUSSION

The omental adipose depot is unique in many respects. Unlike subcutaneous fat, effluent from omental fat empties directly into the portal vein. Omental fat has been reported to be relatively resistant to insulin suppression of lipolysis in vitro (8,20), and the tissue is sensitive to β_3-adrenergic stimulation of lipolysis (21,22). Any or all of these characteristics may contribute to the observed strong correlation between abdominal obesity and whole-body insulin resistance. Although portal vein FFAs are strong candidate signals for hepatic insulin resistance, the possible contributions of central subcutaneous fat cannot be discounted (23). In a previous study, we found expansion of both

TABLE 2
FFA levels and lipolysis rates during stepwise insulin infusion in eight paired experiments

Infusion route ($n = 4$)	Basal	SS1 (0.3 mU · kg^{-1} · min)		SS2 (0.6 mU · kg^{-1} · min)		SS3 (1.2 mU · kg^{-1} · min)	
	Pooled ($n = 8$)	Omental	Control	Omental	Control	Omental	Control
Portal vein FFAs (μmol/l)	517 ± 74	275 ± 71	305 ± 72	223 ± 81*	200 ± 46†	102 ± 10‡	167 ± 70†
Arterial FFAs (μmol/l)	403 ± 63	230 ± 81	253 ± 39	117 ± 77	149 ± 33*	80 ± 10‡	103 ± 47†
V-A difference (μmol/l)	114 ± 37	46 ± 17	52 ± 35	46 ± 4	51 ± 20	22 ± 7	64 ± 35
Omental lipolysis (mol · kg^{-1} · min^{-1})	1.7 ± 0.5	0.8 ± 0.1	1.0 ± 0.5	1.0 ± 0.4	0.8 ± 0.2	0.4 ± 0.1	0.9 ± 0.6

Data are means ± SE. *$P < 0.05$, †$P < 0.02$, and ‡$P < 0.01$ vs. basal period.

TABLE 3
Systemic insulin levels and whole-body lipolysis (n = 8) compared with omental insulin levels and omental lipolysis (n = 4)

	Systemic		Omental	
	Insulin (pmol/l)	Lipolysis (% of basal)	Insulin (pmol/l)	Lipolysis (% of basal)
Basal	57.8 ± 6.3	100 ± 0	59.5 ± 8.5	100 ± 0
SS1 (0.3 mU · kg^{-1} · min^{-1})	106.4 ± 11.2	62 ± 12	277.6 ± 20.1	52 ± 25
SS2 (0.6 mU · kg^{-1} · min^{-1})	155.6 ± 19.1	39 ± 9	504.3 ± 37.1	53 ± 21
SS3 (1.2 mU · kg^{-1} · min^{-1})	328.4 ± 44.1	23 ± 5	1043.2 ± 71.6	20 ± 8

Linear regression equations for systemic line fit: $y = -0.22x + 0.92$; for omental line fit: $y = -0.07x + 0.90$. Slopes are different by unpaired t test, $P = 0.011$.

subcutaneous and visceral fat in dogs fed fat for 4 weeks (10). In the present study, we implanted chronic catheters into the SMA of dogs to examine the physiologic role of the omental fat depot in vivo. Stepwise insulin infusions into the SMA during euglycemic clamps were used to generate supraphysiological omental insulin concentrations despite concentration of systemic insulin in the physiological range. Insulin was infused into the portal vein as a control for the elevations in portal and systemic insulin levels. In this manner, we were able to directly show severe insulin resistance of omental fat in vivo.

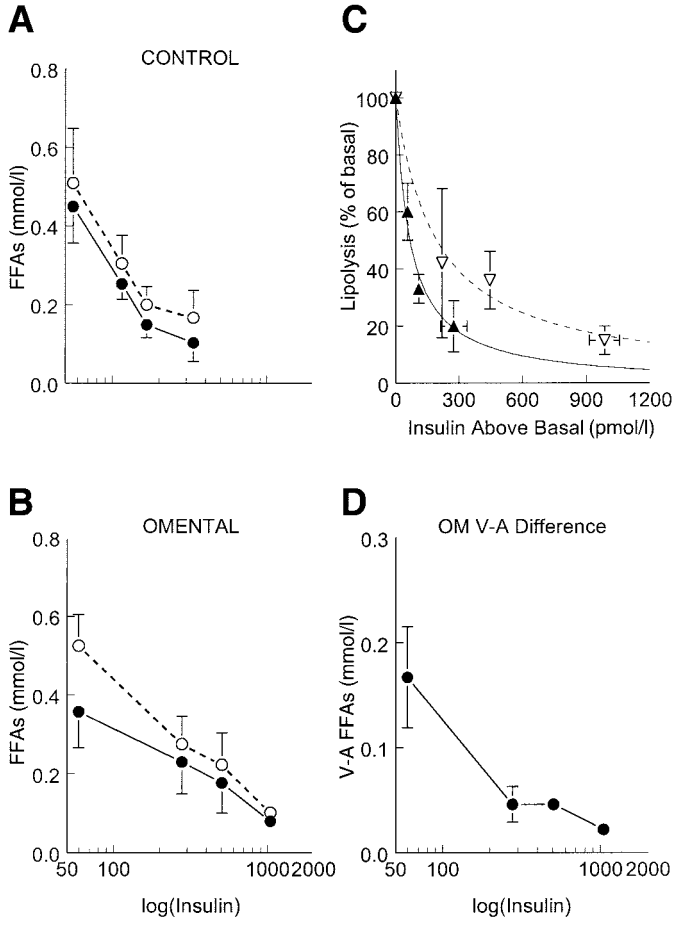

FIG. 6. *A*: Portal venous (●) and carotid arterial (○) FFA levels as a function of systemic insulin concentration during control portal vein insulin infusion. *B*: Portal venous (●) and carotid arterial (○) FFA levels as a function of omental insulin concentration during omental insulin infusion. *C*: Omental (▲) and whole-body (▽) lipolysis as a function of the increase in local insulin levels. There is a rightward shift in omental lipolysis. *D*: V-A FFA concentration difference (●) as a function of omental insulin concentration.

Our data contrast with studies examining insulin sensitivity of omental adipose tissue in vitro in human tissues. Studies have shown a substantial resistance of omental adipocytes to insulin compared with subcutaneous adipocytes in both obese (24) and nonobese (20,25) women. However, although the median effective dose (ED_{50}) of visceral adipose tissue in the present study was approximately three times higher than that of subcutaneous adipose tissue, this parameter was only increased 2- to 2.5-fold in the human in vitro studies. Although this could be caused by sex and/or species differences, it could also reflect differences in the cellular milieu. Circulating hormones such as incretins and catecholamines may affect adipocyte sensitivity in vivo. Concentrations of paracrine and endothelial-derived factors would be altered in vitro. It is also possible that direct neuronal control of the

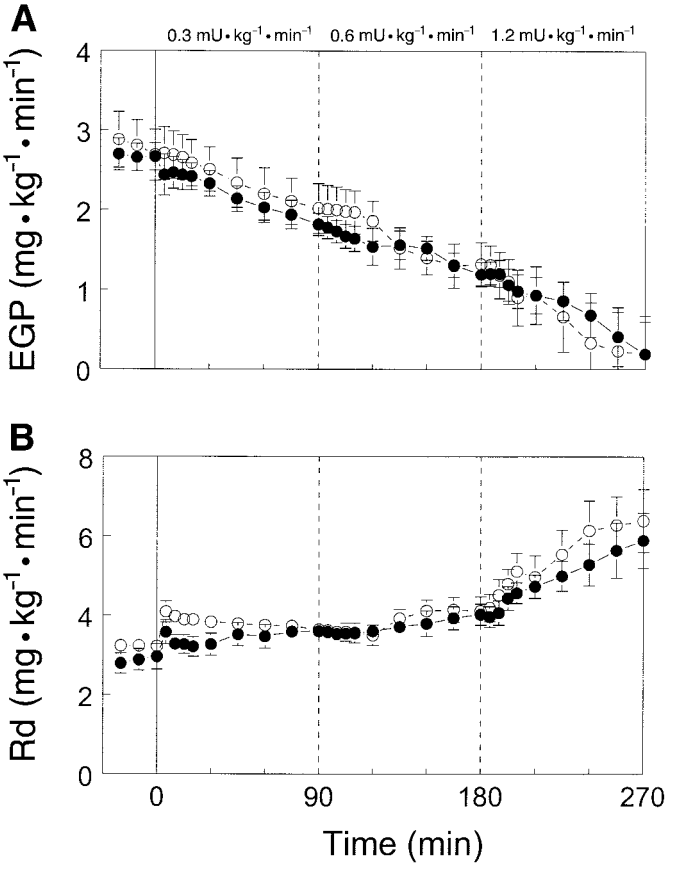

FIG. 7. EGP (*A*) and glucose uptake (Rd) (*B*), during omental (●) and control (○) infusions of insulin. There were no significant effects of route of insulin infusion on either of these fluxes.

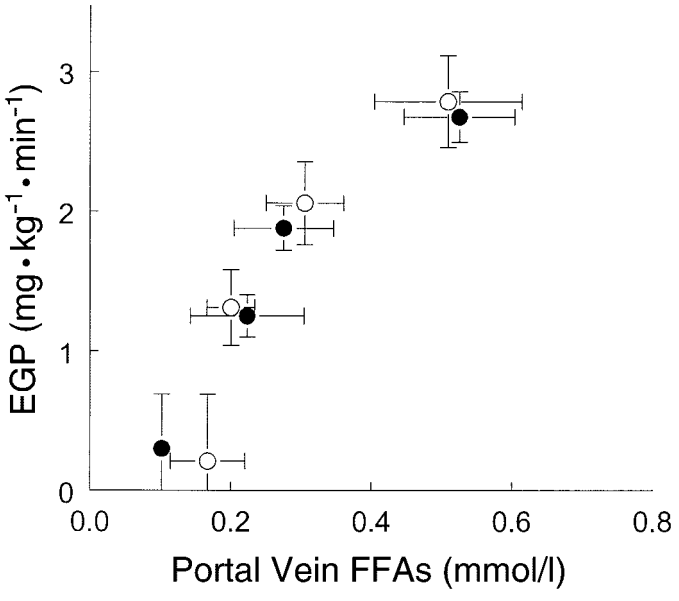

FIG. 8. EGP as a function of portal vein FFA levels during omental (●) and control (○) infusions of insulin. Data are from the four dogs in each group in which portal vein sampling was successful.

omental fat depot by the central nervous system could affect insulin sensitivity, a finding that would remain undetectable in in vitro studies.

The present study confirms the findings of Meek et al. (9), who used hepatic vein sampling to calculate splanchnic lipolysis in humans. However, because hepatic vein plasma FFA levels reflect metabolism of both omental adipose tissue and liver, the Meek study relied on arteriovenous (A-V) difference of both labeled and unlabeled palmitate for the calculation of omental lipolysis. Because of the error inherent to the A-V difference technique, calculations relying on two A-V differences are prone to especially large errors. Also, because the splanchnic FFA uptake was much larger than release in the Meek study, small changes in lipolysis may have been difficult to detect. Finally, the Meek study used different patient groups for each insulin dose, which can increase error. Despite these problems, Meek et al. showed that splanchnic palmitate release was significantly more resistant to insulin suppression than was that from the leg, although ED_{50} values were not reported. Thus, the insulin resistance of lipolysis reported by Meek et al. may well have reflected omental adipose as opposed to lipolysis by the liver itself. An important finding in the present study is that omental lipolysis was not significantly suppressed when systemic insulin levels were raised to 365 pmol/l with portal insulin infusion. In our experience, insulin levels do not exceed 300 pmol/l after a mixed meal in normal dogs. Even in fat-fed dogs, systemic insulin levels only reach 430 pmol/l for 5 min after a mixed meal and decline to <360 pmol/l after that (data not shown). Therefore, postprandial insulin secretion would likely not suppress omental lipolysis. The nonsuppressible omental lipolysis in the postprandial state could help explain why abdominal obesity is strongly associated with hepatic insulin resistance. We and others have shown a strong correlation between FFA levels and hepatic glucose production (11); this could partially explain the inappropriately elevated glucose production in the face of hyperinsulinemia seen in obesity and insulin resistance. Also, increased hepatic delivery of FFAs should increase hepatic triglyceride levels, which also may play a role in insulin resistance.

Although insulin infusion rates were matched between omental and control experiments, systemic insulin levels were significantly lower with omental infusion. This was due to extraction of insulin by the omentum, which we calculated to be ~27%. This is in contrast to the study of Pye et al. (26), which found no evidence for omental extraction of insulin in lean dogs. This may indicate that the abdominally obese dog exhibits a substantial increase in omental insulin extraction. Livingston et al. (27) reported no effect of obesity on insulin receptor affinity or number per cell in omental adipocytes. However, it is reasonable to assume that an increase in the total number of omental adipocytes would lead to a proportional increase in the number of insulin receptors, thereby increasing total omental insulin extraction.

Despite the suppression of omental lipolysis with omental infusion of insulin, we did not detect differences between the two protocols in systemic FFA and glycerol levels. There are several possible explanations for this apparent paradox. It may have been due to the substantial insulin extraction by the omentum, which confounds comparisons between omental and control infusion. Lower systemic insulin levels with omental infusion would be expected to yield less suppression of subcutaneous lipolysis, which could partially mask the effect of omental insulin to suppress omental lipolysis. In fact, there was an apparent leftward shift in the systemic FFAs versus systemic insulin relationship with omental infusion, such that at the same systemic insulin levels, omental infusion was able to suppress systemic FFAs further than control infusion.

Alternatively, it is possible that omental lipolysis did not contribute greatly to systemic FFA and glycerol levels in these experiments, and so its suppression by omental insulin did not yield observable effects in the systemic levels of these metabolites. However, Guo et al. (28) have shown the splanchnic bed to be a significant source (~15–20%) of basal FFA release in obese women. It is possible that even the extremely high local insulin levels achieved with omental infusion of insulin were unable to appreciably suppress omental lipolysis. However, this latter possibility is disputed by the A-V difference results.

Omental lipolysis was difficult to quantify due to the inconsistency of portal sampling and the noise inherent in A-V difference measurements. However, portal sampling was successful with four dogs in each protocol. FFA release from the omentum was suppressed in a dose-dependent manner with omental insulin and not affected by portal insulin infusion. As a function of local insulin levels, there is a profound rightward shift of omental lipolysis compared with whole-body lipolysis. This demonstrates that omental adipose is very insulin resistant in vivo, even though it extracts one-fourth of the insulin that passes through it.

Additionally, the calculation of omental lipolysis depends on the assumption that portal blood flow remains constant throughout the euglycemic clamp procedure. Although portal blood flow was not measured in the

present study, previous studies have shown no significant change in portal blood flow with portal insulin infusion (29).

Endogenous glucose production was similar with omental versus control insulin infusion. This was not surprising given the fact that neither arterial nor portal vein FFA levels were altered by route of insulin infusion. Because omental adipose is severely resistant to suppression of lipolysis by insulin, one would expect that it would tend to bolster hepatic glucose output during hyperinsulinemic states, such as after a meal. In fact, omental lipolysis was not suppressed at even the highest rate of portal insulin infusion (1.2 $mU \cdot min^{-1} \cdot kg^{-1}$). Therefore, the omental release of FFAs is likely not suppressed by physiological insulin secretion. Over time, this might be expected to lead to increased hepatic triglyceride levels. When omental adiposity increases, this insulin resistant depot could exert even greater effects on the liver, perhaps representing an early step in the development of whole-body insulin resistance.

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REFERENCES

1. Evans DJ, Hoffmann RG, Kalkhoff RK, Kissebah AH: Relationship of body fat topography to insulin sensitivity and metabolic profiles in premenopausal women. *Metabolism* 33:68–75, 1984
2. Golay A, Chen N, Chen YD, Hollenbeck C, Reaven GM: Effect of central obesity on regulation of carbohydrate metabolism in obese patients with varying degrees of glucose tolerance. *J Clin Endocrinol Metab* 71:1299–1304, 1990
3. Bergman RN, Bradley DC, Ader M: On insulin action in vivo: the single gateway hypothesis. *Adv Exp Med Biol* 334:181–198, 1993
4. Mittelman SD, Fu YY: Sympathetic response uncouples free fatty acids from endogenous glucose output (Abstract). *Diabetes* 47 (Suppl. 1): A28, 1998
5. Björntorp P: "Portal" adipose tissue as a generator of risk factors for cardiovascular disease and diabetes. *Arteriosclerosis* 10:493–496, 1990
6. Peiris AN, Mueller RA, Smith GA, Struve MF, Kissebah AH: Splanchnic insulin metabolism in obesity: influence of body fat distribution. *J Clin Invest* 78:1648–1657, 1986
7. Bergman RN, Van Citters GW, Mittelman SD, Dea MK, Hamilton-Wessler M, Kim SP, Ellmerer M: Central role of the adipocyte in the metabolic syndrome. *J Invest Med* 49:119–126, 2001
8. Zierath JR, Livingston JN, Thorne A, Bolinder J, Reynisdottir S: Regional difference in insulin inhibition of non-esterified fatty acid release from human adipocytes: relation to insulin receptor phosphorylation and intracellular signalling through the insulin receptor substrate-1 pathway. *Diabetologia* 41:1343–1354, 1998
9. Meek SE, Nair KS, Jensen MD: Insulin regulation of regional free fatty acid metabolism. *Diabetes* 48:10–14, 1999
10. Mittelman SD, Van Citters GW, Kim SP, Davis DA, Dea MK, Hamilton-Wessler M, Bergman RN: Longitudinal compensation for fat-induced insulin resistance includes reduced insulin clearance and enhanced B-cell response. *Diabetes* 49:2116–2125, 2000
11. Rebrin K, Steil GM, Getty L, Bergman RN: Free fatty acid as a link in the regulation of hepatic glucose output by peripheral insulin. *Diabetes* 44:1038–1045, 1995
12. Bergman RN, Ader M, Finegood DT, Pacini G: Extrapancreatic effect of somatostatin infusion to increase glucose clearance. *Am J Physiol* 247: E370–E379, 1984
13. Little AF, Baron RL, Peterson MS, Confer SR, Dodd GD III, Chambers TP, Federle MP, Oliver JH, Orons PD, Sammon JK: Optimizing CT portography: a prospective comparison of injection into the splenic versus superior mesenteric artery. *Radiology* 193:651–655, 1994
14. Rebrin K, Steil GM, Mittelman SD, Bergman RN: Causal linkage between insulin suppression of lipolysis and suppression of liver glucose output in dogs. *J Clin Invest* 98:741–749, 1996
15. Grandison AS, Yates J, Shields R: Capillary blood flow in the canine colon and other organs at normal and raised portal pressure. *Gut* 22:223–227, 1981
16. Bradley DC, Steil GM, Bergman RN: OOPSEG: a data smoothing program for quantitation and isolation of random measurement error. *Comput Methods Programs Biomed* 46:67–77, 1995
17. Skerjanec A, O'Brien DW, Tam TK: Hepatic blood flow measurements and indocyanine green kinetics in a chronic dog model. *Pharm Res* 11:1511–1515, 1994
18. Dauzat M, Layrargues GP: Portal vein blood flow measurements using pulsed Doppler and electromagnetic flowmetry in dogs: a comparative study. *Gastroenterology* 96:913–919, 1989
19. Ader M, Bergman RN: Peripheral effects of insulin dominate suppression of fasting hepatic glucose production. *Am J Physiol* 258:E1020–E1032, 1990
20. Richelsen B, Pedersen SB, Moller-Pedersen T, Bak JF: Regional differences in triglyceride breakdown in human adipose tissue: effects of catecholamines, insulin, and prostaglandin E2. *Metabolism* 40:990–996, 1991
21. Galitzky J, Reverte M, Carpene C, Lafontan M, Berlan M: Beta 3-adrenoceptors in dog adipose tissue: studies on their involvement in the lipomobilizing effect of catecholamines. *J Pharmacol Exp Ther* 266:358–366, 1993
22. Lonnqvist F, Krief S, Strosberg AD, Nyberg S, Emorine LJ, Arner P: Evidence for a functional beta 3-adrenoceptor in man. *Br J Pharmacol* 110:929–936, 1993
23. Frayn KN: Visceral fat and insulin resistance—causative or correlative? *Br J Nutr* 83 (Suppl. 1):S71–S77, 2000
24. Mauriege P, Brochu M, Prud'homme D, Tremblay A, Nadeau A, Lemieux S: Is visceral adiposity a significant correlate of subcutaneous adipose cell lipolysis in men? *J Clin Endocrinol Metab* 84:736–742, 1999
25. Bolinder J, Kager L, Ostman J, Arner P: Differences at the receptor and postreceptor levels between human omental and subcutaneous adipose tissue in the action of insulin on lipolysis. *Diabetes* 32:117–123, 1983
26. Pye S, Watarai T, Davies G, Radziuk J: Comparison of the continuously calculated fractional splanchnic extraction of insulin with its fractional disappearance using a new double-tracer technique. *Metabolism* 42:145–153, 1993
27. Livingston JN, Lerea KM, Bolinder J, Kager L, Backman L, Arner P: Binding and molecular weight properties of the insulin receptor from omental and subcutaneous adipocytes in human obesity. *Diabetologia* 27:447–453, 1984
28. Guo Z, Hensrud DD, Johnson CM, Jensen MD: Regional postprandial fatty acid metabolism in different obesity phenotypes. *Diabetes* 48:1586–1592, 1999
29. Pagliassotti MJ, Holste LC, Moore MC, Neal DW, Cherrington AD: Comparison of the time courses of insulin and the portal signal on hepatic glucose and glycogen metabolism in the conscious dog. *J Clin Invest* 97:81–91, 1996