Primacy of Hepatic Insulin Resistance in the Development of the Metabolic Syndrome Induced by an Isocaloric Moderate-Fat Diet in the Dog

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Obesity is highly correlated with insulin resistance and the development of type 2 diabetes. Insulin resistance will result in a decrease in insulin's ability to stimulate glucose uptake into peripheral tissue and will suppress glucose production by the liver. However, the development of peripheral and hepatic insulin resistance relative to one another in the context of obesity-associated insulin resistance is not well understood. To examine this phenomena, we used the moderate fat–fed dog model, which has been shown to develop both subcutaneous and visceral adiposity and severe insulin resistance. Six normal dogs were fed an isocaloric diet with a modest increase in fat content for 12 weeks, and they were assessed at weeks 0, 6, and 12 for changes in insulin sensitivity and glucose turnover. By week 12 of the diet, there was a more than twofold increase in trunk adiposity as assessed by magnetic resonance imaging because of an accumulation in both subcutaneous and visceral fat depots with very little change in body weight. Fasting plasma insulin had increased by week 6 (150% of week 0) and remained increased up to week 12 of the study (170% of week 0). Surprisingly, there appeared to be no change in the rates of insulin-stimulated glucose uptake as measured by euglycemic-hyperinsulinemic clamps throughout the course of fat feeding. However, there was an increase in steady-state plasma insulin levels at weeks 6 and 12, indicating a moderate degree of peripheral insulin resistance. In contrast to the moderate defect seen in the periphery, there was a marked impairment in insulin's ability to suppress endogenous glucose production during the clamp such that by week 12 of the study, there was a complete inability of insulin to suppress glucose production. Our results indicate that a diet enriched with a moderate amount of fat results in the development of both subcutaneous and visceral adiposity, hyperinsulinemia, and a modest degree of peripheral insulin resistance. However, there is a complete inability of insulin to suppress hepatic glucose production during the clamp, suggesting that insulin resistance of the liver may be the primary defect in the development of insulin resistance associated with obesity. *Diabetes* 52:2453–2460, 2003

It has been well established that obesity is closely associated with insulin resistance, type 2 diabetes, and other chronic diseases such as hypertension, hyperlipidemia, and cardiovascular disease, all of which characterize the metabolic syndrome (1,2). It has recently become clear that there is a large population of normal-weight individuals with significant fat accumulation who may also be at high risk for metabolic disease (3). Numerous studies have found that individuals with relatively low BMI can have a high degree of latent adiposity, particularly with respect to central (or visceral) fat (4,5). In addition, there is accumulating evidence that central obesity represents a higher risk factor for metabolic dysfunction than peripheral fat, signifying an elevated risk even for those considered nonobese based solely on body weight or BMI (1). Latent adiposity and insulin resistance may be particularly prevalent in certain ethnic groups, such as Asian-Americans (6).

Studies examining the relationship between insulin resistance and central versus subcutaneous fat accumulation have shown that there is a higher incidence of insulin resistance in individuals with central obesity than those with subcutaneous obesity (1,7). Therefore, it is presumably the insulin resistance per se that links visceral adiposity with metabolic disease. Despite powerful evidence linking visceral adiposity, insulin resistance, and metabolic disease, it remains unclear what the mechanisms are that specifically link visceral fat and insulin resistance. Although the portal flux of lipid moieties to the liver have been implicated (7), it is also possible that so-called "adipokines," such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, leptin, adiponectin, and/or resistin may be involved (8–10). Clearly, understanding the mechanistic relationship between visceral fat and insulin resistance might lead to therapeutic approaches to sever this relationship and possibly reduce the risks of central obesity.

Our approach to understanding the mechanistic link between visceral adiposity and insulin resistance has been to examine, longitudinally in time, the development of insulin resistance per se and compensatory mechanisms, including hyperinsulinemia. These changes proceed after visceral fat deposition is induced with very modestly increased dietary fat intake. We have performed these studies in the dog model because this large animal model
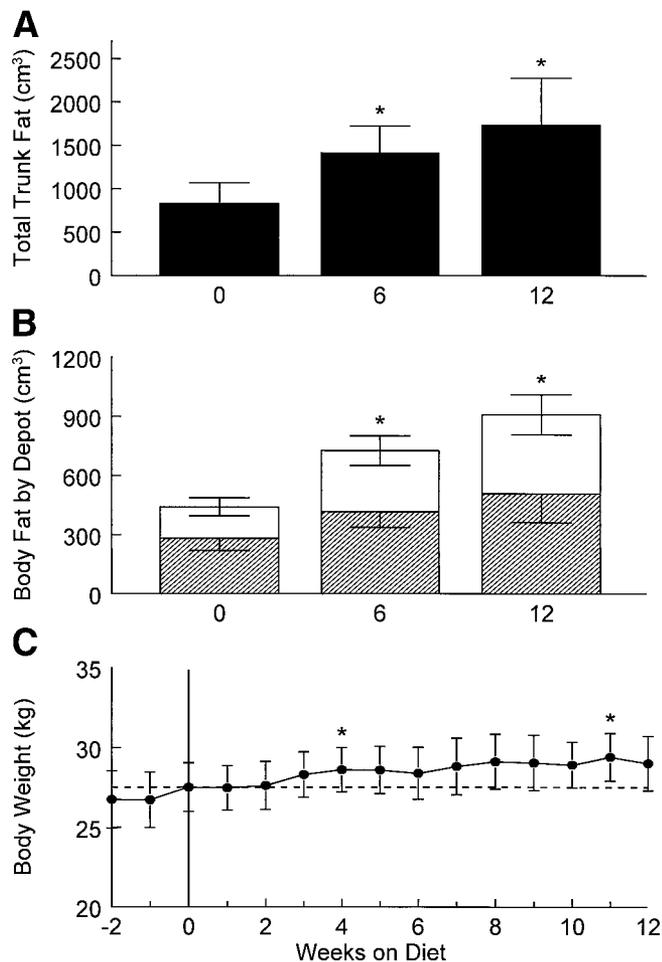
FIG. 1. *A*: Total trunk body fat volume (calculated as cm³ from the sum of all axial slices). *$P < 0.05$ vs. week 0, ANOVA. *B*: Omental (▨) and subcutaneous (☐) fat (calculated as cm³ from the sum of 11 axial slices, the midpoint of which is shown in Fig. 2). *$P < 0.05$ vs. week 0, ANOVA. *C*: Body weight. Although body weight did increase at weeks 4 and 11 during fat feeding, on average body weight did not increase by >1.9 kg throughout the entire course of the study (week 0 value is represented by a dashed line for reference). *$P < 0.05$ vs. week 0, paired *t* test.

allows for repetitive measures of insulin action and other metabolic variables. To date, we have reported the temporal development of insulin resistance as well as the later compensatory hyperinsulinemia and subsequent changes in both insulin secretion and insulin clearance (11).

Although the slowly developing insulin resistance resulting from an isocaloric diet with elevated fat intake (and visceral adiposity) is documented, it is unclear whether the liver or peripheral tissues, including skeletal muscle, are primary in the development of resistance. It has been suggested that deposition of fat as triglycerides in skeletal muscle explains insulin resistance in obesity (12). Alternatively, it is possible that increased flux of free fatty acids (FFAs; or altered adipokines) to liver are primarily responsible. To examine the relative importance of liver versus periphery in a model of the metabolic syndrome, we have chosen to dissect hepatic versus peripheral insulin resistance in animals with a latent visceral adiposity induced by an isocaloric diet with increased fat intake. Rather than using a large increase in fat intake (13), we used this more moderate dietary regimen with the intention to clarify the

primary events in the development of the metabolic syndrome.

RESEARCH DESIGN AND METHODS

Animals. Six male mongrel dogs (27.5 ± 1.5 kg) were housed in the University of Southern California (USC) Keck School of Medicine vivarium under controlled kennel conditions (12-h light/dark cycle). Animals were accepted into the study after physical examination and a comprehensive blood panel. Chronic catheters were surgically implanted 7 to 10 days before the beginning of the study: one was inserted in the jugular vein and advanced to the right atrium for sampling of central venous blood; a second catheter was inserted in the femoral vein and advanced to the vena cava for tracer, insulin, and somatostatin infusion; and a third catheter used for infusion only was placed in the portal vein for a series of experiments to be reported separately. All catheters were led to the neck subcutaneously and exteriorized. Catheters were flushed with heparinized saline (100 units/ml) at least twice a week, and the exteriorization site was cleaned with hydrogen peroxide (4%). Dogs were accustomed to laboratory procedures and were used for experiments only if judged to be in good health as determined by visual observation, body temperature, and hematocrit. On the morning of each experiment, 17-gauge angiocatheters (Allegiance Healthcare, McGaw Park, IL) were inserted percutaneously into the saphenous vein for glucose infusion. The experimental protocol was approved by the USC institutional animal care and use committee.

Diet. Dogs were fed a weight-maintaining standard diet of one can of Hill's Prescription Diet (10% carbohydrate, 9% protein, 8% fat, 0.3% fiber, and 73% moisture [Hill's Pet Nutrition, Topeka, KS]) and 825 g dry diet (36.6% carbohydrate, 26.4% protein, 14.7% fat, and 2.9% fiber [Wayne Dog Food, Alfred Mills, Chicago]) for a period of 2–3 weeks to ensure weight stabilization before conducting any experiments. This standard diet consisted of 3,885 kcal/day: 37.9% from carbohydrates, 26.3% from protein, and 35.8% from fat. After weight stabilization (week 0), dogs were maintained on an isocaloric moderate-fat diet for a period of 12 weeks. This moderate-fat diet was comprised of one can of Hill's Prescription Diet, 715 g dry diet, and 2 g/kg prediet body weight of cooked bacon grease supplied by the USC Keck School of Medicine cafeteria. Thus, the isocaloric moderate fat diet consisted of 3,945 kcal/day, with 32.9% carbohydrate, 22.9% protein, and 44.1 ± 0.4% fat, constituting a total increase of ~8% in fat calories with no significant change in total caloric content.

Magnetic resonance imaging. During weeks 0, 6, and 12 of the moderate-fat diet, magnetic resonance imaging (MRI) scans were performed on the dogs as previously described (11). A total of 30 1-cm axial abdominal images (T1 slices; TR 500 TE:14) were obtained using a General Electric 1.5 Tesla Horizon (version 5.7 software) magnet. Of the 30 images obtained, ~20 of these images were used for analysis of total trunk body fat, depending on the relative torso length of the animal. Images were analyzed using Scion Image (Windows 2000 version Beta 4.0.2; Scion, Frederick, MD), which quantifies fat tissue (pixel value 121–254) and other tissue (20–120) in each slice. Fat volume was calculated by dividing the number of pixels counted as fat by the ratio of the total number of pixels (256 × 256) and known area (34.9 × 34.9 cm) for a 1-cm image. Total trunk fat and tissue were estimated as the integrated fat or tissue across all 20 slices. Percent fat was calculated as the total trunk fat divided by the total trunk tissue. Omental fat was defined as fat within the peritoneal cavity in a 11-cm region of the thorax, using the slice at the level where the left renal artery branches from the abdominal aorta as a midpoint landmark. Percent omental fat was calculated as the omental fat divided by the total tissue area in these same slices.

Euglycemic-hyperinsulinemic clamps. The euglycemic-hyperinsulinemic clamps were performed as previously described (14,15) at weeks 0, 6, and 12 of the moderate-fat diet. Animals were familiarized with the Pavlov sling at least 1 week before the first experiment. At ~7:00 A.M. on the day of the clamp, animals were brought to the laboratory and placed in the Pavlov sling. A 19-gauge angiocatheter was placed in a saphenous vein and secured; ~30 min later ($t = -120$ min), a primed continuous infusion of high-performance liquid chromatography–purified [3-³H]glucose (25 μCi + 0.25 μCi/min infusion; DuPont-NEN, Boston, MA) was started. After tracer equilibration, basal samples were taken at −30, −20, −10, and −1 min. At time $t = 0$ min, a somatostatin infusion (1.0 μg · min⁻¹ · kg⁻¹; Bachem California, Torrance, CA) was started to suppress endogenous insulin and glucagon secretion, and it was continued for the duration of the experiment. Porcine insulin was infused (1.5 mU · kg⁻¹ · min⁻¹; Novo Nordisk) into the femoral vein to attain hyperinsulinemia. Glucose was clamped at basal levels by a variable glucose infusion labeled with [3-³H]D-glucose (2.2 μCi/g) to minimize fluctuations in plasma specific activity. Blood samples were drawn from the jugular catheter every 10 min from −30 to 60 min, every 15 min from 60 to 120 min, and then every 10
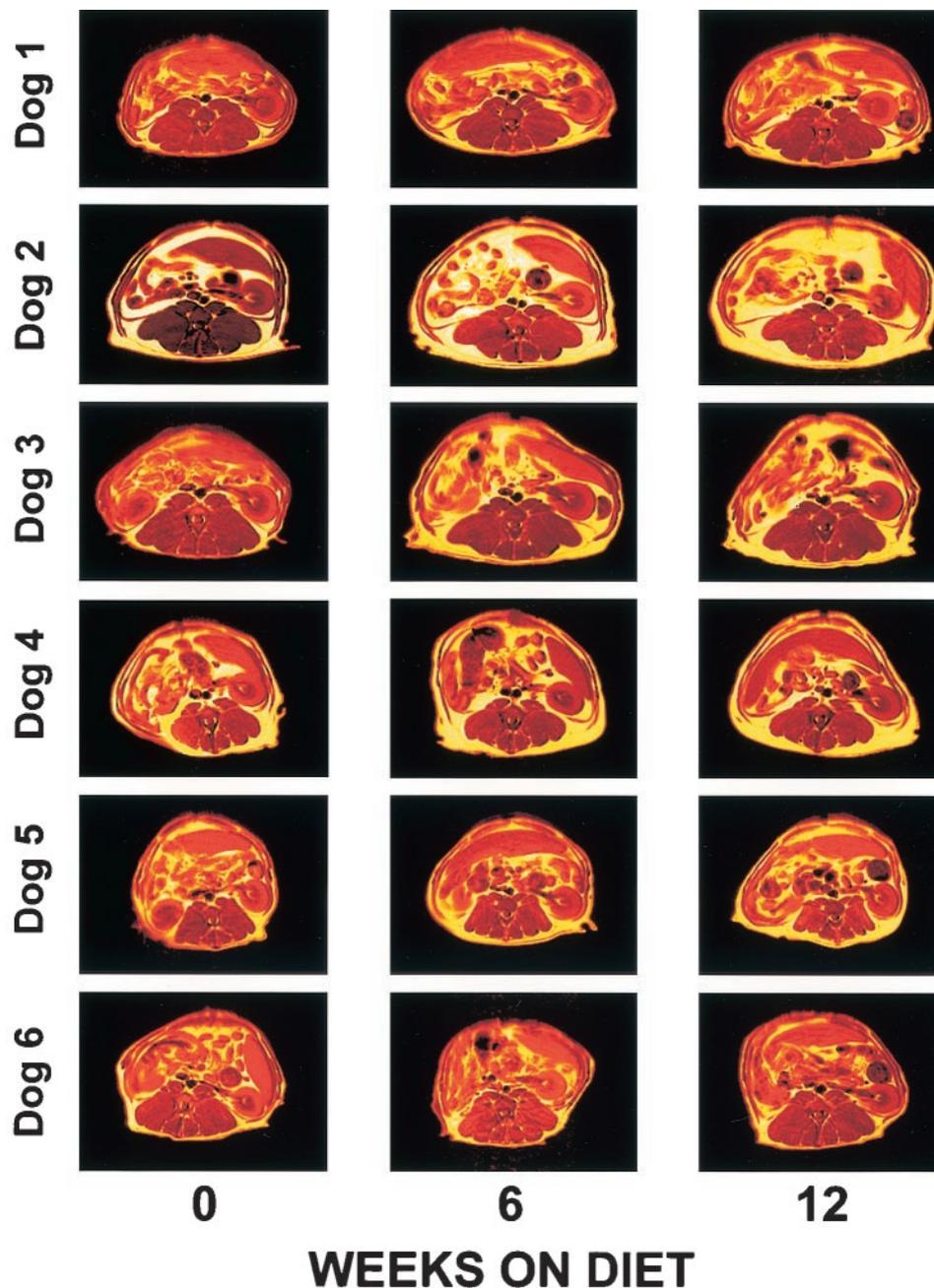
FIG. 2. Axial MRI images taken at the level of the left renal artery branching from the abdominal aorta, used as a midpoint landmark for the area used in quantifying omental and subcutaneous fat. Images are inverse T1-weighted; adipose tissue appears as yellow, and all other tissue appears red. Adipose tissue showed accumulation in both the omental and subcutaneous compartments.

min from 120 to 180 min. Samples for assay of insulin and [3-^{3}H]D-glucose were kept on ice in tubes precoated with lithium fluoride and heparin (Brinkmann Instruments, Westbury, NY) containing 50 μL EDTA, whereas samples for FFA and glycerol assay were taken in tubes with EDTA and paraoxon to inhibit lipase activity.

Assays. Glucose was measured immediately after sampling with a YSI 2700 autoanalyzer (Yellow Springs Instruments, Yellow Springs, OH). FFAs were measured using a colorimetric assay based on the acylation of CoA using a kit from Wako (NEFA C; Wako Pure Chemical Industries, Richmond, VA). Insulin was measured by an ELISA adapted for dog plasma, and samples for ^{3}H-glucose tracer assay were deproteinized using barium hydroxide and zinc sulfate as previously described (15).

Calculations. The time course of endogenous glucose production (EGP) and the rate of glucose disappearance (R_d) was calculated using Steele's model with a labeled glucose infusion, as previously described (14). Derivatives of all time course data were calculated with OOPSEG (16). Basal was defined as the average of four samples taken every 10 min from $t = -30$ to 0 min, and steady

state was defined as the average of four samples taken from $t = 150$ to 180 min.

Statistical analyses. All experimental data are expressed as the means ± SE. Statistical analyses were performed with paired Student's t tests or a two-way ANOVA as appropriate for comparisons of weeks 6 or 12 with week 0. The t tests were performed using Microsoft Excel 98, and all ANOVAs were performed using Minitab for Windows software (Minitab, State College, PA).

RESULTS

Body composition. The isocaloric moderate-fat diet resulted in a substantial increase in body fat at both week 6 and week 12 of the study, as assessed by MRI. Total trunk fat (Figs. 1A and 2) increased from 831 ± 240 cm^3 at week 0 to 1,415 ± 307 cm^3 at week 6 ($P < 0.05$ vs. week 0, ANOVA), and this increase in total trunk adiposity was

TABLE 1
Basal metabolic parameters

	Week 0	Week 6	Week 12
Glucose (mg/dl)	96.4 ± 1.9	92.3 ± 2.8	94.0 ± 1.6
Insulin (pmol/l)	78 ± 12	115 ± 20*	126 ± 17†
Glucose uptake $(\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1})$	2.6 ± 0.5	3.3 ± 0.5	3.3 ± 0.5
Glucose production $(\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1})$	2.1 ± 0.3	3.1 ± 0.5	3.1 ± 0.5
FFAs (mmol/l)	0.41 ± 0.03	0.50 ± 0.07	0.42 ± 0.05

Data are means $\pm$ SE. *$P < 0.05$ vs. week 0, ANOVA; †$P < 0.01$ vs. week 0, ANOVA.

maintained up to week 12, during which total trunk fat was $1,734 \pm 543 \text{ cm}^3$ ($P < 0.05$ vs. week 0, ANOVA), reflecting a more than twofold increase in total trunk body fat over the 12-week period. Thus, increased fat content of the diet resulted in visceral adiposity, despite no increase in daily caloric intake.

When comparing the contribution of the omental and subcutaneous fat depots to total body fat (Fig. 1*B*), we found that within the defined axial region of the trunk, omental fat volume at week 0 was $282 \pm 62 \text{ cm}^3$, whereas subcutaneous fat volume was $159 \pm 46 \text{ cm}^3$. At week 6 of the diet, omental fat volume had increased by $57 \pm 27\%$, whereas subcutaneous fat had increased by $127 \pm 60\%$ from week 0. By week 12, there was a significant increase in both omental and subcutaneous fat compared with week 0, such that omental fat volume had increased by $76 \pm 27\%$, and subcutaneous fat was increased by $182 \pm 47\%$ ($P < 0.05$, ANOVA).

Although the mass of other tissue beds (i.e., muscle and organs) were not assessed, if there were no changes in body composition, the increase in trunk fat would account for an increase of <1 kg in body weight. Consistent with this, despite the large increase seen in total trunk fat volume, there was very little change in body weight, which was 27.5 ± 1.5 kg at the beginning and 29.0 ± 1.7 kg after 12 weeks of the diet ($P = $ NS, paired t test) (Fig. 1*C*).

Glucose. Despite a twofold increase in total trunk fat (see above), fasting glucose was not at all changed over the course of the diet (Table 1). This is consistent with previous results (11). In addition, glucose was well clamped during each experiment throughout the study ($P = $ NS; basal versus steady state, paired t test), and the average time course for plasma glucose during the clamp did not differ between weeks 6 or 12 from week 0 (data not shown; $P = $ NS, paired t test).

Insulin. In sharp contrast to glucose, fat feeding caused an increase in fasting insulin over the course of the diet (Table 1). At week 0, fasting insulin was 78 ± 12 pmol/l. By week 6, fasting insulin had increased by $49 \pm 18\%$ (115 ± 20 pmol/l; $P < 0.05$ vs. week 0, ANOVA). Fasting insulin had further increased to 126 ± 17 pmol/l ($P < 0.01$ vs. week 0, ANOVA) by week 12, signifying a $68 \pm 17\%$ increase when compared with week 0. Thus, an isocaloric elevated fat diet causes hyperinsulinemia, despite absolutely no detectable change in fasting glucose.

When comparing the time course data (Fig. 3*A*) and calculating the steady-state values of plasma insulin during the clamp (Table 2), we found that compared with week 0 (540 ± 62 pmol/l), there was an increase in the steady-state
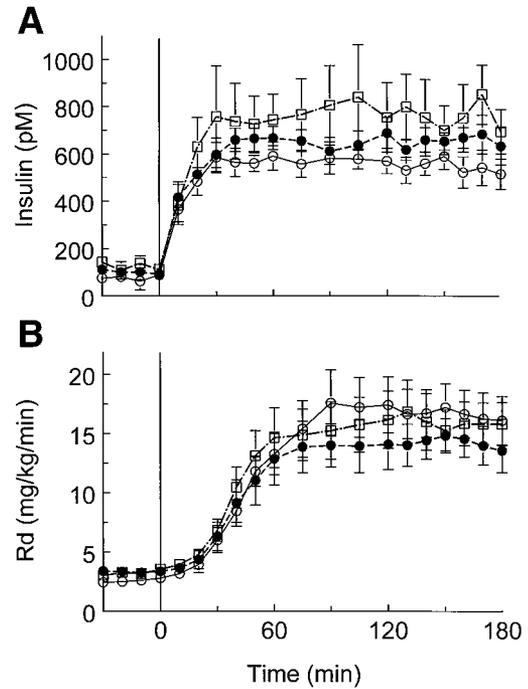

FIG. 3. Time course data of (*A*) plasma insulin concentrations and (*B*) glucose uptake during the clamp at week 0 (○), week 6 (●), and week 12 (□).

concentrations of plasma insulin during the clamps that became evident at week 6 (671 ± 46 pmol/l; $P < 0.05$ vs. week 0, ANOVA) and was further increased at week 12 (735 ± 104 pmol/l; $P < 0.05$ vs. week 0, ANOVA), indicating a reduction in the metabolic clearance rate (MCR) of insulin during fat. MCR was reduced from 0.35 ± 0.03 min^{-1} at week 0 to 0.27 ± 0.02 min^{-1} at week 6 ($P < 0.05$, ANOVA). This 22% reduction remained until week 12 of the diet, during which the MCR was 0.27 ± 0.04 min^{-1} ($P < 0.05$ vs. week 0, ANOVA).

Peripheral insulin action. Despite visceral adiposity and fasting hyperinsulinemia, there was no apparent change in the insulin-stimulated glucose uptake (R_d) measured during clamps over the course of this study. R_d at week 0 during steady state (Table 2 and Fig. 3*b*) was 16.7 ± 2.0 mg $\cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ and did not decrease over the course of the study (14.2 ± 1.6 and 15.7 ± 1.8 mg $\cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for week 6 and 12, respectively; $P = 0.16$ and $P = 0.53$ vs. week 0, respectively; ANOVA). The combination of similar steady-state glucose uptake in the face of a modest increase in steady-state insulinemia during the clamp at weeks 6 and 12 in comparison to week 0 does suggest mild insulin

TABLE 2
Steady-state meatabolic parameters

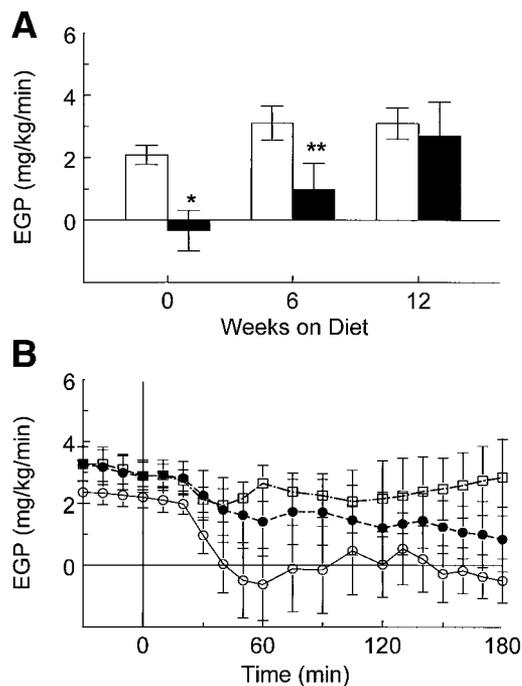|	Week 0	Week 6	Week 12
Glucose (mg/dl)	96.4 ± 2.9	97.5 ± 3.9	94.4 ± 1.5
Insulin (pmol/l)	540 ± 62	671 ± 46*	735 ± 104*
Glucose uptake $(\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1})$	16.7 ± 2.0	14.2 ± 1.6	15.7 ± 1.8
Glucose production $(\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1})$	-0.3 ± 0.6	1.0 ± 0.8	2.7 ± 0.9*
FFAs (mmol/l)	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.01

Data are means $\pm$ SE. *$P < 0.05$ vs. week 0, ANOVA.

FIG. 4. *A*: Basal (□) and steady-state (■) glucose production rates as assessed during the clamp at weeks 0, 6, and 12. *$P < 0.05$, **$P < 0.005$ basal versus steady-state, paired t test. *B*: Time-course data of glucose production during week 0 (○), week 6 (●), and week 12 (□).
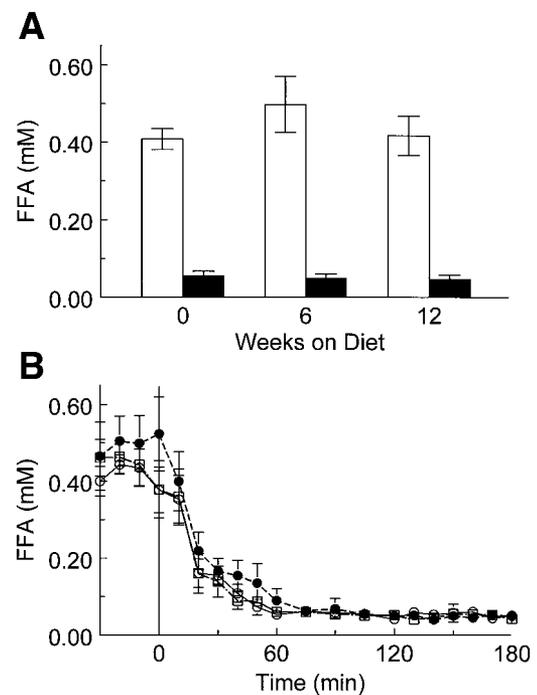

FIG. 5. *A*: Basal (□) and steady-state (■) plasma FFA levels as assessed during the clamp at weeks 0, 6, and 12. *B*: Time-course data of plasma FFAs during week 0 (○), week 6 (●), and week 12 (□).

resistance in peripheral tissues. When normalizing the rates of glucose uptake for plasma insulin levels during the steady-state period of the clamp, we found that although there was a tendency for insulin-stimulated glucose uptake to decrease with fat-feeding (3.4 ± 0.6, 2.2 ± 0.3, and $2.4 \pm 0.4 \times 10^{-2}$ mg $\cdot$ kg^{-1} $\cdot$ min^{-1} $\cdot$ pmol^{-1} $\cdot$ l for week 0, 6, and 12, respectively; $P = 0.07$ for week 6 vs. week 0 and $P = 0.08$ for week 12 vs. week 0, ANOVA), this decrease was not significant.

Hepatic insulin action. Basal glucose production rates as calculated from the clamp did not change throughout the course of the study, despite fasting hyperinsulinemia (2.1 ± 0.3, 3.1 ± 0.5, and 3.1 ± 0.5 mg $\cdot$ kg^{-1} $\cdot$ min^{-1} for week 0, 6, and 12, respectively; $P = $ NS vs. week 0, ANOVA) (Table 1). Supporting hepatic insulin resistance, there was a severe impairment in insulin's ability to suppress EGP during the steady-state period of the clamp (Table 2, Fig. 4) that became clearly evident at week 12 of fat feeding. At week 0 of the study, insulin was able to completely suppress steady-state EGP during the clamp from 2.1 ± 0.3 to -0.3 ± 0.6 mg $\cdot$ kg^{-1} $\cdot$ min^{-1} (100% suppression, $P = 0.02$, paired t test). At week 6 of the study, glucose production was still partially suppressed by insulin from a basal rate of 3.1 ± 0.5 to a rate of 1.0 ± 0.8 mg $\cdot$ kg^{-1} $\cdot$ min^{-1} during steady state (68% suppression, $P = 0.002$, paired t test). However, by week 12 of the study, there was no change from basal glucose production rates during steady state (3.1 ± 0.5 vs. 2.7 ± 0.9 mg $\cdot$ kg^{-1} $\cdot$ min^{-1} for basal vs. steady state, respectively; $P = 0.70$, paired t test), indicating the complete insulin resistance of the liver vis a vis the ability of insulin to suppress glucose production during the clamp.

FFAs. Fasting values of FFAs were unchanged over the course of the diet (0.41 ± 0.03, 0.50 ± 0.07, and 0.42 ± 0.05 mmol/l for week 0, 6, and 12, respectively; $P = $ NS vs. week 0, ANOVA) (Table 1). In addition, insulin's ability to reduce FFAs (Table 2 and Fig. 5) was not changed throughout the study as FFAs during steady state were equally suppressed at the three observation periods (0.06 ± 0.01, 0.05 ± 0.01, and 0.05 ± 0.01 mmol/l for week 0, 6, and 12, respectively; $P = $ NS vs. week 0, ANOVA). Thus, failure to suppress FFA could not explain the apparent total insulin resistance of the liver observed at week 12.

DISCUSSION

Hepatic insulin resistance has been found to exist in both obese nondiabetic and obese type 2 diabetic patients and is considered to be a dominant component in the pathogenesis of fasting hyperglycemia in type 2 diabetes (17). It has been suggested that hepatic insulin resistance may be directly related to visceral adiposity, independent of total fat mass (18). However, despite the number of studies examining the relationship between obesity and hepatic insulin resistance, there is little known regarding the relative time course in the development among obesity, hepatic insulin resistance, and peripheral insulin resistance. Therefore, we felt it was of significant importance to examine the temporal pattern in the relative development of hepatic and peripheral insulin resistance and fat accumulation in a model more reminiscent of human obesity by using a diet with only a modest increase in fat content and relatively no change in total caloric intake. Longitudinal studies may help to reveal the causality in the development of insulin resistance and associated risk factors with obesity.

To address these issues, six normal dogs were fed an ~8% increase in total fat calories for a period of 12 weeks. The diet was isocaloric, so that animals had a significant increase in both omental and subcutaneous adiposity

without a change in body weight. Associated with this increase in adiposity, the dogs exhibited fasting hyperinsulinemia and a decrease in insulin sensitivity. Although there was only a moderate defect in peripheral insulin sensitivity, we found that there was a complete failure of insulin to suppress glucose production during a hyperinsulinemic-euglycemic clamp. It therefore appears that when adiposity is increased by an isocaloric moderate-fat diet, total hepatic insulin resistance will develop relative to insulin suppression of glucose output with only relatively minor changes in peripheral insulin sensitivity. This suggests that hepatic insulin resistance is primary in the establishment of the metabolic syndrome associated with visceral adiposity.

We used an isocaloric diet to isolate effects of increased fat intake from an increase in total caloric intake. As such, we found that even with only a very modest increase in fat intake and virtually no change in total caloric consumption, there was a more than twofold increase in total trunk body fat from week 0 due to an accumulation in both subcutaneous and omental fat, without a significant change in body weight.

As expected, animals exhibited fasting hyperinsulinemia during the course of this study, suggesting insulin resistance. Despite this, we observed no significant impairment in the steady-state rate of glucose uptake during the clamp at weeks 6 and 12 when compared with week 0. However, there was a modest increase in the steady-state plasma levels of insulin at weeks 6 and 12 because of a reduction in the MCR, indicating that insulin's ability to stimulate glucose uptake was moderately impaired over the course of the study. In contrast to the minor defect found in peripheral insulin sensitivity in the present study, studies performed in other animal models of obesity (19,20) have shown that glucose uptake is severely impaired when compared with control groups. However, studies performed using rat models of dietary obesity often use an extremely high-fat diet not typically found in the human diet, which results in large changes in both body weight and body fat accumulation, which may therefore account for the large defect seen in peripheral insulin sensitivity. For example, Kraegen et al. (21) used a diet with very high fat (60%) and a calorie content not generally seen in the human diet. They also reported initial hepatic insulin resistance; however, in their study, unlike the present one, substantial peripheral insulin resistance followed. Kraegen et al. did not report changes that may have occurred in body weight and body composition. In support of their results, additional studies in our laboratory (M.E., unpublished data) have found that feeding a hypercaloric high-fat (6 g/kg body wt) diet for 12 weeks induces both hepatic and peripheral insulin resistance. Therefore, it is most likely that the relatively small degree of peripheral insulin resistance we observed in the present study was due to the more modest physiological nature of the diet. It is also possible that we may have observed accentuated peripheral insulin resistance if we had observed the animals beyond the 12-week window. Our data, along with that of Kraegen et al., argue that it is resistance of the liver that develops first with the advent of adiposity, and peripheral insulin resistance is a secondary phenomenon requiring a higher-fat diet or longer exposure.

The most noteworthy change found in this study is the development of hepatic insulin resistance and substantial fat accumulation with only a modest increase in dietary fat, suggesting that even a minute increase in fat intake can pose an elevated risk for the development of hepatic insulin resistance. However, we cannot discount the possibility that the changes observed in this study in both body fat accumulation and hepatic insulin resistance may be specific to this animal model. It may be that the dog is very sensitive to rather small changes in dietary fat intake, and that the same increase in fat consumption in humans might not result in the same degree of fat accumulation without a change in body weight as seen here. Additionally, we have found that the relative contribution of visceral fat versus subcutaneous fat to the total fat volume in the dog at baseline accounts for ~65% of the total fat volume. Studies completed in humans (22,23) have found that on average, visceral fat accounts for ~20% of total fat volume, although this number is dependent on many factors, such as age, sex, and ethnicity. Therefore, the extreme response of complete hepatic resistance seen in this study may be attributable to the rather large contribution of the visceral adipose depot to total trunk body fat in the dog in comparison to humans, which may render the dog model more prone to changes in hepatic insulin sensitivity due to the large distribution of visceral fat to total body fat.

The mechanisms underlying the development of hepatic insulin resistance are not well understood. FFAs are known to act as an important mediator in the suppression of glucose production (15,24) and have been implicated in playing a key role in the development of insulin resistance and type 2 diabetes (25). Elevated plasma FFA levels have been shown to decrease insulin's ability to both suppress glucose production and stimulate glucose uptake (26,27). It could be suggested that insulin resistance of the adipocyte might be the primary cause of hepatic insulin resistance in the present study—that failure to suppress FFA could result in failure to suppress EGP. However, in the present scenario of modest-fat feeding, we found that insulin did not lose its ability to suppress systemic FFA levels. Therefore, we may reject the hypothesis that insulin resistance of the total fat mass of the body was responsible for the severe hepatic insulin resistance. On the other hand we did not measure portal vein FFA concentrations, and the possibility remains that despite suppression of systemic FFA, there was an increase in the flux of FFA to the liver which could not be suppressed by insulin. It has previously been demonstrated in humans (28) that under hyperinsulinemic conditions, a greater proportion of systemic FFA are derived from splanchnic tissue, indicating that systemic FFA may not be an accurate representation of FFA entering the portal circulation. In addition, several labs including ours (7,29,30) have shown that the omental adipose tissue more resistant than subcutaneous fat to insulin's suppression of lipolysis. This gives further evidence suggesting that an increase in this fat depot associated with FFA flux (and possible alterations in adipokine levels) to the liver would support an increase in glucose production. Although there is much discussion of the role of visceral fat in development of insulin resistance, the possible contribution of subcutaneous fat in the development of hepatic insulin resistance cannot be discounted. Along with an increase in visceral adiposity, the animals in

this study also exhibited a substantial increase in the subcutaneous fat depot, resulting in a more than twofold increase in total trunk fat over the course of the fat diet, which may have also contributed to a rise in portal FFAs. It has previously been demonstrated (31) that upper-body subcutaneous rather than visceral adipose tissue may be the source for increased FFA delivery to the liver. Therefore, although we were unable to directly measure portal levels of FFAs in the present study, it appears that increases in both visceral and subcutaneous fat may have contributed to an increase in FFA delivered to the liver, and it is this portal flux of lipid moieties that accounts for the hepatic resistance.

Although the mechanism(s) by which FFAs inhibit insulin action is unknown, numerous studies performed in vitro and in vivo have shown that FFAs are capable of increasing gluconeogenesis (32), either by increasing substrate availability (the Randle hypothesis) or via induction of the gluconeogenic enzymes glucose-6-phosphatase and phosphoenol pyruvate carboxykinase (33). More recently, evidence (34) has shown that even during hyperinsulinemic conditions, an elevation in plasma FFAs can inhibit insulin suppression of glycogenolysis, possibly because of the suppression of insulin's action in promoting hepatic glycogen cycling (35). It has been postulated by Shulman (36) that FFAs may play a more direct role in modulating glucose production by inhibiting insulin signaling in the liver. Studies performed in mice with liver-specific overexpression of lipoprotein lipase (37) have shown that an increase in fatty acids delivered to the liver results in an increase in the liver triglyceride content associated with an impairment of insulin's ability to suppress EGP due to a defect in insulin-stimulated liver glycogen synthesis. In addition, these studies demonstrated a corresponding decrease in insulin-stimulated insulin receptor substrate-2–associated phosphatidylinositol 3-kinase activity (a key step in the activation of glycogen synthase activity), thereby implicating a direct effect of FFAs on insulin resistance via the insulin signaling pathway. Data obtained in vitro in our laboratory (M. Kabir, unpublished results) has demonstrated that there is a ~40% increase in the liver triglyceride content in dogs fed a moderate-fat diet for 12 weeks when compared with controls (5.83 ± 0.68 vs. 4.01 ± 0.36 µmol/g wet wt for fat-fed vs. control animals, respectively; $P = 0.003$, $n = 6$ for both, unpaired t test), giving further evidence to the possibility that an increase in portal vein FFAs may be a primary factor involved in the development of hepatic insulin resistance. In addition, it has been shown that hepatic fat content is closely correlated with hepatic insulin sensitivity (38) and that elevated liver fat content may be directly associated hepatic insulin resistance, independent of overall obesity (39). These results provide further evidence that the impairment we see in insulin's ability to suppress glucose production in this fat-fed dog model may be caused by the direct effect of increased portal FFAs delivered to the liver from visceral adipose tissue via the portal vein. This increase in FFA delivery can lead to an increase in liver triglyceride content, ultimately resulting in defects along the insulin signaling pathway and an impairment of insulin action on the liver in suppressing glucose production during clamped conditions.

Although there is much evidence implicating elevated portal FFAs as the driving force behind the dysregulation of hepatic glucose production, we cannot discount the possibility of other adipose-secreted factors (e.g., adiponectin, IL-6, TNF-α, resistin, etc.) that may also be involved. Studies have shown that levels of TNF-α and IL-6 are elevated in obese individuals with insulin resistance (40), and that these secretory factors are elevated to a higher degree in individuals with abdominal obesity than those with peripheral obesity (41,42). In vitro studies completed in rat hepatocytes (43) have shown that IL-6 is able to mediate hepatic glucose metabolism by inhibiting insulin-stimulated glycogen synthesis. TNF-α treatment in rats has been shown to impair insulin action on the liver and periphery (44), and it has been suggested (45) that TNF-α may specifically play a role in the development of hepatic insulin resistance by inhibiting signal transduction at the receptor level. Interestingly, additional evidence has shown that in subcutaneous adipose tissue, although both TNF-α and IL-6 are expressed, only IL-6 is released from this fat depot (46), suggesting that subcutaneous fat may also play an important role in mediating liver glucose production via IL-6 secretion. Obesity has also been linked to decreased levels of adiponectin (47), which is known to both enhance insulin's ability to suppress glucose production and decrease the storage of triglycerides in the liver (48), and may therefore be directly involved in the development of hepatic insulin resistance associated with obesity. Alternatively, Fruebis et al. (49) demonstrated that adiponectin increases β-oxidation of FFAs in muscle, suggesting that the adiponectin may have an indirect effect on hepatic insulin sensitivity by modulating the level of FFAs delivered to the liver (50). Thus, it may be possible that a decrease in adiponectin as a result of increased adiposity may be responsible for the extreme hepatic insulin resistance seen in this study, either by direct or indirect mechanisms. Studies to examine the possible involvement of these factors as well as the involvement of FFAs in the development of hepatic insulin resistance are currently under investigation in our laboratory.

In conclusion, our results indicate that hepatic insulin resistance may be the primary event in the development of whole-body insulin resistance associated with increased adiposity. Moreover, it appears that even with only a very moderate increase in fat intake and no change in total caloric consumption, there is significant accumulation of both subcutaneous and omental fat without a change in body weight. This suggests an increased risk for the development of hepatic insulin resistance even for those considered nonobese based solely on body weight or BMI.

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