

Longitudinal Compensation for Fat-Induced Insulin Resistance Includes Reduced Insulin Clearance and Enhanced β -Cell Response

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Central adiposity is highly correlated with insulin resistance, which is an important risk factor for type 2 diabetes and other chronic diseases. However, in normal individuals, central adiposity can be tolerated for many years without development of impaired glucose tolerance or diabetes. Here we examine longitudinally the mechanisms by which glucose tolerance can be maintained in the face of substantial insulin resistance. Normal dogs were fed a diet enriched with moderate amounts of fat ($2 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$), similar to that seen in modern "cafeteria" diets, and the time course of metabolic changes in these animals was examined over 12 weeks. Trunk adiposity as assessed by magnetic resonance imaging increased from 12 to 19%, but body weight remained unchanged. Insulin sensitivity (S_I) as determined by frequently sampled intravenous glucose tolerance tests was measured over a 12-week period. S_I decreased 35% by week 1 and remained impaired for the entire 12 weeks. Intravenous glucose tolerance was reduced transiently for 1 week, recovered to baseline, and then again began to decline after 8 weeks. First-phase insulin response began to increase after week 2, peaked by week 6 (190% of basal), and then declined. The increase in insulin response was due partially to enhanced β -cell function (22%) but due also to an ~50% reduction in insulin clearance. This compensation by insulin clearance was also confirmed with insulin clamps performed in fat-fed versus control dogs. The present study confirms the ability of the normal individual to compensate for fat-induced insulin resistance by enhanced insulin response, such that the product of insulin sensitivity \times secretion is little changed. However, the compensation is due as much to reduced insulin clearance as increased β -cell sensitivity to glucose. Reduced hepatic extraction of insulin may be the first line of defense providing a higher proportion of secreted insulin to the periphery and sparing the β -cells during compensation for the insulin-resistant state. *Diabetes* 49:2116–2125, 2000

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AIR_{G(0-19)}, acute insulin response to glucose from 0 to 19 min; ANOVA, analysis of variance; AUC, area under the curve; DI, disposition index; FFA, free fatty acid; FSIGTT, frequently sampled intravenous glucose tolerance test; IGT, impaired glucose tolerance; MRI, magnetic resonance imaging; S_G , glucose effectiveness; S_I , insulin sensitivity; USC, University of Southern California.

There is abundant evidence that diabetes and obesity are increasing in the U.S. population (1–3). Because adiposity is related to insulin resistance (4–6), and insulin resistance is a risk factor for type 2 diabetes and cardiovascular disease, a consensus has emerged that increased adiposity is responsible for the increased incidence of type 2 diabetes and its associated morbidity (metabolic syndrome or syndrome X [7]). In addition, evidence has accumulated indicating that visceral adiposity in particular is associated with insulin resistance and the metabolic syndrome (8,9).

Despite overwhelming evidence demonstrating association between insulin resistance, visceral adiposity, and metabolic risk, there is little evidence directly demonstrating that central adiposity in fact causes insulin resistance. In addition, there is little understanding of the mechanisms underlying the relationship among visceral adiposity, insulin resistance, and risk.

To examine the acute effects of adiposity, it is useful to choose a model that allows for longitudinal assessment of the temporal changes in the factors that determine glucose tolerance. Such an approach is problematic in rodents, in which extensive metabolic phenotyping is difficult, and in human subjects, in whom repeated assessments of metabolic function cannot easily be made. Therefore, in the present study, we have for the first time examined the time course of metabolic changes in the conscious dog model repeatedly over an extended period of time (12 weeks). The model that we have chosen is representative of modest obesity. The animals' diet was enriched with fat, but because the enrichment was modest, there was no significant weight gain over the 12 weeks, despite a 50% increase in the central fat depot. In these animals, we have been able to measure insulin sensitivity (S_I) repeatedly, as well as other factors that determine glucose tolerance: β -cell response and insulin clearance. These experiments have resulted in a revealing portrait of the time-dependent changes that follow moderate diet-induced obesity: there is a phasic response to adiposity wherein an initial compensation for insulin resistance by the β -cells is relieved by a decreased clearance of insulin, a mechanism that may spare the β -cells from stress related to compensation for induced insulin resistance.

RESEARCH DESIGN AND METHODS

Animals. Six male mongrel dogs ($28.2 \pm 1.7 \text{ kg}$) were used in the present study. The animals were housed under controlled kennel conditions (12-h light/dark cycle) in the University of Southern California (USC) Medical School Vivarium. Dogs were fed a standard diet of a half-can (~200 g) of Hill's Prescription Diet (8.5%

protein, 5% fat, 1% fiber, and 74% moisture [Hill's Pet Nutrition, Topeka, KS] and ad libitum dry food (up to 900 g per day; 25% protein, 9% fat, 49% carbohydrate, and 17% fiber [Wayne Dog Food; Alfred Mills, Chicago]). Thus, the total diet consisted of ~3,700 calories: 25% from carbohydrates, 52% from protein, and 23% from fat.

Diet. After the investigators gave them a clean bill of health, animals were accepted into the study. During a 2-week period (Fig. 1), animals were given two frequently sampled intravenous glucose tolerance tests (FSIGTTs). During this time, animals were fed the normal diet consisting of 23% of calories from fat. After 1–2 weeks on the normal diet, the dogs were begun on a higher-fat diet, which consisted of the above diet supplemented with 2 g/kg body wt of cooked bacon grease supplied by the USC–Keck School of Medicine cafeteria. This change increased the potential calories of the diet to ~4,300: 22% from carbohydrates, 45% from protein, and 33% from fat. The animals were continued on this diet for 12 weeks.

Magnetic resonance imaging. Before and on the fourth and eighth weeks of the high-fat diet, magnetic resonance imaging (MRI) scans were performed on the dogs. Preanesthesia was induced with subcutaneous acepromazine (0.1 mg/kg body wt [Bio-ceutic, St. Joseph, MO]) and atropine sulfate (0.04 mg/kg, 1/120 grain [Western Medical Supply, Arcadia, CA]), followed by intravenous anesthesia with a cocktail of ketamine HCl (10 mg/kg [Phoenix Pharmaceutical, St. Joseph, MO]) and diazepam (0.2–0.5 mg/kg [Abbott Laboratories, North Chicago]). Thirty 1-cm axial abdominal images (T1 slices; TR:500, TE:14) were obtained using a General Electric 1.5 Tesla Horizon (v5.7 software) magnet. These images were analyzed using ScionImage (Windows 95 Version Beta 3b; Scion Corporation, Frederick, MD), which quantified fat tissue (pixel value 0–100) and other tissue (101–230) in each slice. Total trunk fat and tissue were estimated as the integrated fat or tissue across all 30 slices. Percent fat was then calculated as the total trunk fat divided by the total trunk tissue. Omental fat was defined as fat within the peritoneal cavity in the slice at the level where the left renal artery branches from the abdominal aorta. Percent omental fat was defined as the omental fat divided by the total tissue area in the same slice.

FSIGTTs. The FSIGTTs were performed as previously described before and throughout the high-fat diet (10). Although it was not possible to perform FSIGTTs on every dog every week, 7 to 10 FSIGTTs were performed on each dog; a total of 48 FSIGTTs were done (Table 1). Glucose and insulin doses were determined based on the animals' prediet body weight. Animals were familiarized with the Pavlov sling at least 1 week before the first FSIGTT. At approximately 7 A.M. on the day of the FSIGTT, animals were brought to the laboratory and placed in the Pavlov sling. A 19-gauge angiocatheter was placed in a saphenous vein and secured. Approximately 20 min later, basal sampling was begun. After three baseline samples (–20, –10, and –1 min), 0.3 g/kg body wt of glucose (50% dextrose, 454 mg/ml) was injected into the saphenous vein ($t = 0$). Subsequently, insulin was injected ($t = 20$ min, 0.03 U/kg porcine insulin; Novo Nordisk, Copenhagen). We took 28 additional blood samples at $t = 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 140, 160,$ and 180 min for assay of glucose and insulin. Samples were taken into chilled tubes coated with lithium fluoride and heparin containing 50 μ l EDTA, immediately centrifuged, and the plasma separated. Glu-

cose was measured with a YSI 2700 autoanalyzer (Yellow Springs Instruments, Yellow Springs, OH) and the rest of the plasma stored at -20°C for further analysis. Insulin was measured by an enzyme-linked immunosorbent assay originally developed for human serum or plasma by Novo-Nordisk and adapted for dog plasma. The method is based on two murine monoclonal antibodies that bind to different epitopes on insulin, but not to proinsulin. Materials for the insulin assay, including the dog standard, were provided by Novo-Nordisk. C-peptide was measured by Linco Research (St. Charles, MO) using their radioimmunoassay. C-peptide was measured only on experiments 0, 1, 3, 6, 8, 10, and 12 from 5 of the 6 animals.

Basal values. Fasting values were defined as the measure taken of the second basal sample ($t = -10$ min). To minimize day-to-day variability of assays, all fasting samples from a single animal were measured in the same assay.

Calculations

Minimal model parameters. S_i and glucose effectiveness (S_G) were calculated by fitting the glucose profiles from the FSIGTTs using Minmod (Version 3.0, 1994). The acute insulin response to glucose was calculated as the area under the curve of the insulin concentrations above the average of the basal values, from 0 to 19 min after the glucose injection ($\text{AIR}_{\text{G}(0-19)}$). The disposition index (DI), which represents a measure of insulin responsiveness corrected for changes in S_i (11), was calculated as the product of the average S_i and $\text{AIR}_{\text{G}(0-19)}$ from each experiment week.

C-peptide levels were also measured as an estimate of β -cell function. The area under the curve for C-peptide was calculated using the trapezoidal rule (above basal) from 0 to 10 min after the glucose injection at $t = 0$, and it should be proportional to the insulin secreted in response to glucose, independent of insulin clearance: This assumes that C-peptide clearance is not altered during fat feeding. This value is also reported as a percentage of the week 0 C-peptide response.

Insulin clearance. Effect of fat feeding on metabolic clearance of insulin was measured several ways. First, parameters reflecting insulin clearance were estimated from the FSIGTT results. At 20 min, exogenous insulin was injected. A parameter reflecting the metabolic clearance rate of insulin (in minutes^{-1}) was estimated by fitting the insulin profile from $t = 20$ to 180 to an exponential decay curve on SlideWrite Plus Version 4.0 (Advanced Graphics Software, Carlsbad, CA). Data were fit to the equation:

$$\text{Insulin} = \text{Insulin}_{\text{Basal}} + \text{Insulin}_{\text{max}} \times e^{-kt}, \quad (1)$$

where insulin is the insulin concentration in picomoles per liter, and $\text{Insulin}_{\text{Basal}}$, $\text{Insulin}_{\text{max}}$, and k are the fitted basal insulin (picomoles per liter), peak insulin (picomoles/liter), and insulin clearance (minutes^{-1}), respectively. Equation 1 assumes that endogenous insulin secretion is rapidly suppressed by the insulin injection (12), and that small changes in insulin secretion after this time point are not likely to significantly affect the decay curve.

A second independent index of insulin clearance was obtained by comparing fasting insulin and C-peptide levels. Because fasting C-peptide and insulin both increase proportionally with fasting insulin secretion, whereas insulin levels are also inversely proportional to insulin clearance, the ratio of fasting insulin to fasting C-peptide should be inversely proportional to insulin clearance. Likewise, whereas changes in the acute C-peptide response to glucose, as reflected in the areas under the curve, are determined primarily by insulin secretion, changes in the insulin profiles reflect both insulin secretion and clearance. The insulin area under the curve from $t = 0$ to 10 min was calculated using the trapezoidal rule (above basal) and its relative change from week 0 was reported as a percentage.

Finally, insulin clearance was measured directly on a separate group of 7 dogs fed the high-fat diet for ~12 weeks and 8 dogs fed a normal diet (13). This assessment was performed under euglycemic clamped conditions in anesthetized animals with somatostatin and constant systemic insulin infusions (13). Insulin clearance (milliliters per kilogram per minute) was calculated as the insulin infusion rate (picomoles per kilogram per minute) divided by the steady-state plasma insulin concentration (picomoles per milliliter).

Hyperbolic function and disposition index. Secretion versus sensitivity are related by a hyperbolic function:

$$\text{Secretion} \times \text{sensitivity} = \text{constant} \quad (2)$$

or

$$S_i \times \text{AIR}_{\text{G}(0-19)} = \text{DI} \quad (3),$$

for which S_i and $\text{AIR}_{\text{G}(0-19)}$ are defined above, and the putative constant on the right-hand side is termed the disposition index (DI). The latter constant represents the ability of the insulin secretory mechanism to compensate for changes in S_i .

The progress of the hyperbolic relationship (equation 3) in the present longitudinal study was monitored by plotting sequential biweekly average mea-

TABLE 1
Schedule of FSIGTTs

Week	Animal #						Tests (n)
	8	9	10	11	12	13	
–1	—	—	X	X	X	X	4
0	X	X	X	X	X	X	6
1	X	X	X	X	X	X	6
2	—	—	X	X	X	X	4
3	X	X	—	—	—	—	2
4	—	—	X	—	X	X	3
5	X	X	—	—	—	—	2
6	X	X	X	X	X	X	6
7	X	X	—	X	X	—	4
8	X	X	X	X	X	—	5
9	X	X	—	—	—	—	2
10	X	X	X	X	X	X	6
12	X	—	X	X	X	—	4
Total	10	9	9	9	10	7	54

Weeks when FSIGTTs were performed are marked with an X.

TABLE 2
Fasting values

	Week of diet		
	0	6	12
Weight (kg)	27.8 ± 1.4	29.0 ± 2.0	28.9 ± 3.2
Glucose (mg/dl)	91.8 ± 1.8	89.5 ± 2.8	89.9 ± 2.1
Insulin (pmol/l)	70.7 ± 12.7	121.3 ± 28.1	127.3 ± 26.6
FFA (mmol/l)	0.67 ± 0.12	0.51 ± 0.06	0.49 ± 0.07
Glycerol (mg/dl)	1.0 ± 0.2	1.1 ± 0.2	0.9 ± 0.2

Data are means ± SD.

measurements of S_I and $AIR_{G(0-19)}$ against one another, which allowed for examination of whether equation 2 holds longitudinally for the fat-fed dog model. **Statistics.** Linear interpolation was used to estimate values between weeks for which FSIGTTs were not performed. For the two animals that did not undergo experiments on week 12, the values from week 10 were used in place of those from week 12. This allowed repeated-measures analysis of variance (ANOVA) of all outcome variables, separating the effects of animal and week. Linear trend tests were performed using the generalized estimating equation method to test for effects of the enriched fat diet on the outcome variables. Individual weekly comparisons to prediet values (week 0) were made using the Bonferroni method to correct for multiple comparisons. With this correction, P values <0.0045 (0.05 divided by 11, to correct for 11 comparisons) are considered significant. P values of borderline significance (e.g., <0.05 but >0.0045) are occasionally reported as a tendency so that the reader can judge their importance. Because MRI scans were performed only on weeks 0, 4, and 8, a different ANOVA was used to compare body fat data for weeks 4 and 8 with those for week 0, and paired t tests were used to compare weeks 4 and 8 with week 0. Unpaired t test was used to compare insulin clearance calculated from the clamp data from a previous study (13). All ANOVA and linear trend tests were performed using SAS Version 8 (SAS Institute, Cary, NC) on an IBM-compatible computer. The t tests were performed using Microsoft Excel 97.

RESULTS

Body composition. The higher-fat diet did not significantly increase animals' weights over the 12-week period. Though mean body weight increased by 1 kg from 27.8 ± 1.4 to 28.9 ± 3.2 kg over the 12 weeks of fat feeding, this increase was not significant ($P = NS$, linear trend and comparisons to week 0, Table 2, Fig. 1). Despite no net change in weight, there was a profound increase in total trunk body fat as revealed from the MRI images ($P < 0.01$, ANOVA, Fig. 2). Trunk fat increased from 12.3 ± 3.1 to $18.9 \pm 3.6\%$ by 4 weeks on the high-fat diet ($P < 0.01$). The increase in percent body fat was maintained through week 8, which was the final MRI measurement ($17.8 \pm 4.0\%$, $P < 0.01$). This increase in body fat was due to an increase in both omental and subcutaneous fat (Fig. 1). Increased body fat without an increase in weight implies a decrease in volume of muscle or organ tissue; however, these other tissue volumes were not independently assessed. However, an increase in total body fat from 12 to 18% with no other changes in body composition would have caused an increase of only 2 kg in body weight. Thus, the modest increase in fat content of food from 23 to 33% of calories caused a highly significant increase in trunk fat content despite no net change in body weight of the animals.

Glucose tolerance. Intravenous glucose tolerance, assessed by the K_G value (Fig. 3), was not significantly altered by fat feeding ($P = NS$, linear trend). K_G tended to decrease after 1 week of fat feeding ($P = 0.01$) but reapproached control levels the second week. Despite fat feeding, K_G did not differ significantly from basal through week 12, although glucose tolerance appeared to be declining toward week 12, at which

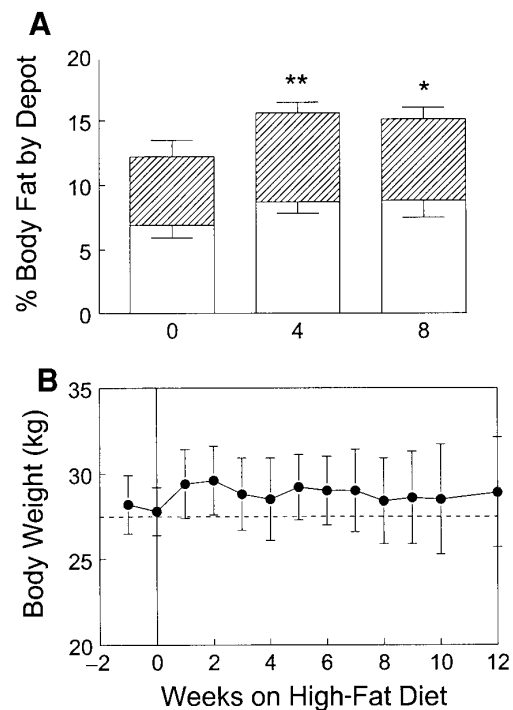


FIG. 1. **A:** Omental (\square) and subcutaneous (\boxplus) body fat, calculated as the area percent of the axial slices shown in Fig. 2. **B:** Body weight. There was a tendency for increased body weight with fat feeding. However, only one dog gained >1 kg over the 12-week high-fat feeding period. * $P < 0.05$ vs. week 0; ** $P < 0.005$ vs. week 0.

point it was $2.5 \pm 0.2 \text{ min}^{-1}$ ($P = NS$ vs. 3.2 ± 0.5 at week 0). Thus, fat-fed animals maintained glucose tolerance for at least the first 12 weeks of this high-fat diet.

Fasting values. Associated with increased central fat, basal plasma insulin showed a tendency to increase with fat feeding ($P = 0.065$, linear trend test). By 3 weeks, mean insulin had risen from 71 ± 13 to $110 \pm 25 \text{ pmol/l}$ ($P = 0.13$, Table 2, Fig. 4). Fasting insulin reached $121 \pm 28 \text{ pmol}$ at 6 weeks ($P = 0.048$), suggesting substantial insulin resistance at that time. Insulin was still elevated ~80% on week 12 of the diet ($P = 0.028$). Fasting glucose concentration was not significantly different from week 0 at any week during the diet ($P = NS$, linear trends and multiple comparisons) except for one point at week 7, when fasting levels dipped to 86.0 ± 3.2 ($P = 0.039$). Free fatty acid (FFA) also did not increase over the course of the higher dietary fat period but, in fact, decreased with fat feeding ($P < 0.0001$ linear trend test, $P < 0.0045$ week 10 vs. week 0). Thus, from basal measurements, we found no evidence that increases in fasting glucose or FFA were the stimulus for the increase in plasma insulin. Fasting glycerol concentration was not significantly affected by fat feeding ($P = NS$).

S_I . The average FSIGTTs for weeks 0, 1, 3, 6, and 12 are reproduced in Fig. 5. Note the trend for increasing insulin response and slightly slower decline of postinjection glucose after insulin as the period of fat feeding increased. These changes suggest insulin resistance; this suggestion was supported by the calculation of S_I . Commencing the higher-fat diet caused S_I to decline ($P < 0.0001$ linear trend, Table 5, Fig. 6); S_I tended to decline in week 1 ($P = 0.03$, not significant by Bonferroni correction) and decreased ~50% by week 2 ($P < 0.0045$); the reduction in S_I was maintained

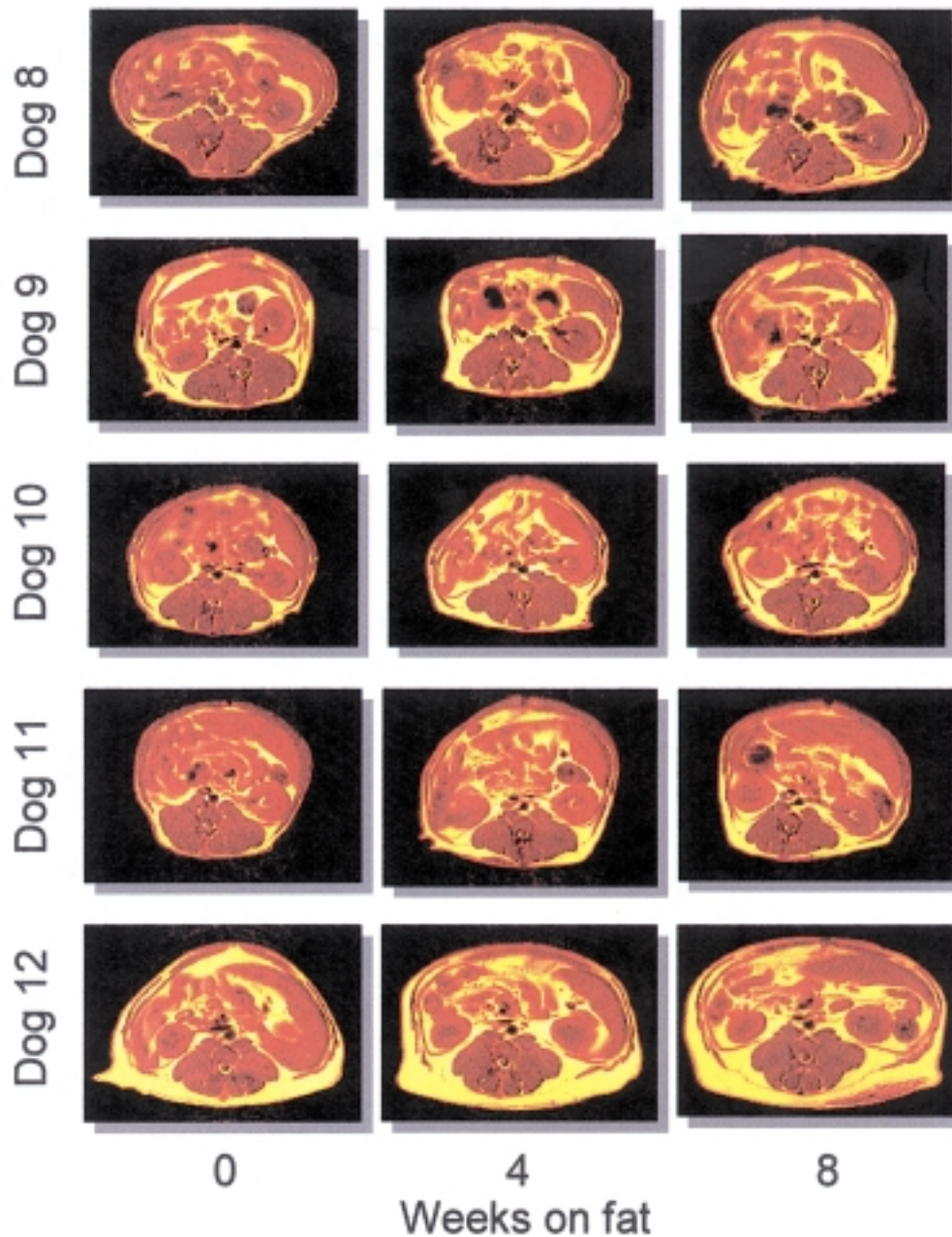


FIG. 2. Axial MRI images taken at the level of the left renal artery branching from the abdominal aorta. In these inverse T1-weighted images, adipose tissue shows up as yellow, whereas other tissue appears red. Adipose tissue accumulated in both the subcutaneous (outside the peritoneum) and omental (inside the peritoneum) compartments.

for the entire 12-week dietary period ($P < 0.0045$, weeks 2–12 vs. basal). S_G (the effect of glucose to suppress its own production and increase its own disposal) showed a trend toward increasing with fat feeding from 0.041 ± 0.004 to 0.051 ± 0.006 at week 12 ($P < 0.0001$ linear trend) but was not significantly different from week 0 throughout the diet ($P = NS$).

Insulin response. It was expected that insulin response would immediately compensate for the decline in sensitivity. In fact, $AIR_{G(0-19)}$ showed a trend of increasing with fat feeding ($P < 0.0005$, linear trend). However, surprisingly, $AIR_{G(0-19)}$ did not change at all in week 1 ($P = NS$, Table 5) and only began to increase by week 3 ($P = NS$), 2 weeks after the

reduction in S_I . The $AIR_{G(0-19)}$ peaked at week 6 ($P < 0.0045$) and then declined again toward the prediet value ($P = 0.07$, week 12 vs. week 0, not significant by Bonferroni correction). Table 3 compares the 0–10 min AUC (integrated area under the curve, above basal) for insulin to that of C-peptide. The AUC for insulin changed little until week 3, at which time it had increased 12% ($P = NS$). This measure of insulin response peaked at 6 weeks at 164% of basal ($P < 0.0045$) after which it moderated, settling ~40% above basal at 12 weeks ($P = 0.02$ vs. week 0, not significant by Bonferroni correction; $P < 0.0001$ linear trend test). This increase of insulin response, however, was not mirrored by the C-peptide AUC. The maximum change in C-peptide AUC was 122% of basal

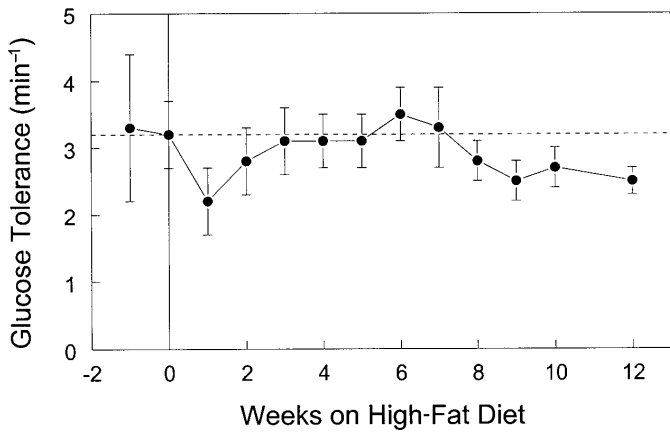


FIG. 3. Glucose tolerance (K_G , in min^{-1}) was significantly reduced after 1 week of fat feeding, but it thereafter remained at baseline levels until declining again at 12 weeks.

at 6 weeks ($P = 0.13$ vs. week 0; $P = 0.17$ linear trend test). Thus, AUC for insulin increased considerably more than the increase from 0 to 10 min in stimulated C-peptide (64 vs. 22%, respectively).

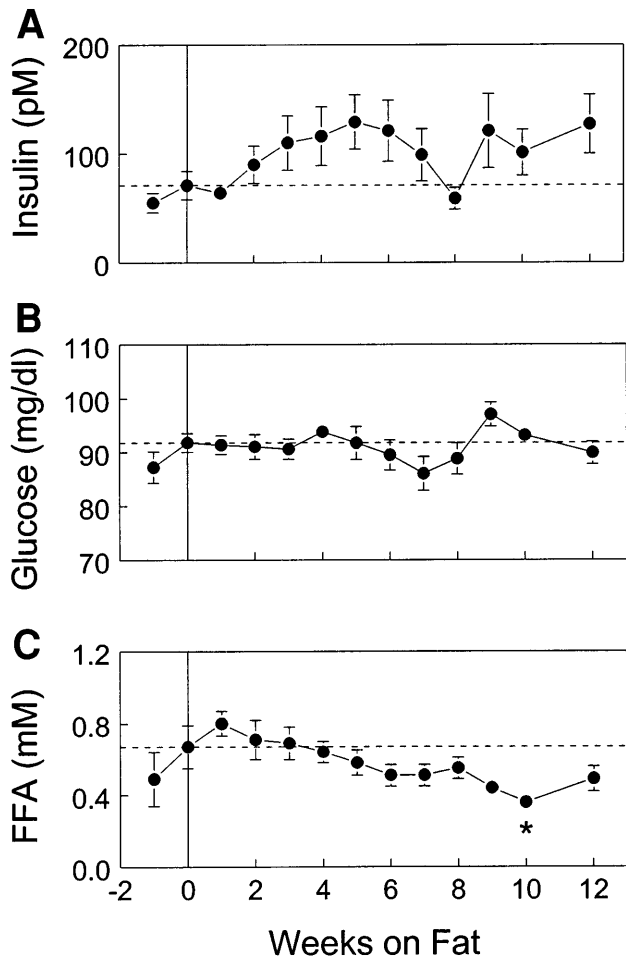


FIG. 4. Basal fasting levels of insulin (A), glucose (B), and FFA (C). For reference, the week 0 value is represented by a dashed line. * $P < 0.0045$ vs. week 0.

Insulin clearance. Although increased β -cell responsiveness is partly responsible for the compensation of insulin response, an additional mechanism is the reduction of insulin clearance. There was a suggestion of reduced insulin clearance from the fasting insulin-to-C-peptide ratio, which was double its baseline value at weeks 3 and 12 ($P = 0.08$ week 3 vs. 0, not significant by Bonferroni correction; $P = \text{NS}$, week 12 vs. 0; Table 3); however, no significant effect of fat feeding was detected by the linear trend test ($P = \text{NS}$). Additionally, the observation that stimulated insulin response increased much more than C-peptide is consistent with a reduction in insulin clearance due to fat feeding, which would have allowed a greater proportion of insulin to escape first-pass degradation by liver. To examine the possibility that clearance was reduced, we exploited the exponential rate of decline of insulin after injection at $t = 20$, as described in RESEARCH DESIGN AND METHODS. This decline was exponential (Fig. 7) with r^2 values of insulin versus time (after 20 min) exceeding 0.923 in all cases. Fat feeding caused a slow decline in insulin clearance ($P = 0.07$ linear trend, $P < 0.0045$ weeks 5–9 and 12 vs. week 0; Fig. 6, Table 4); the reduction in clearance was 50% by week 6. These data support the concept that a reduction in insulin clearance rate contributed to the increased plasma insulin response during fat feeding.

Insulin clearance was also measured directly in seven dogs fed the same high-fat diet (three from the present study) and

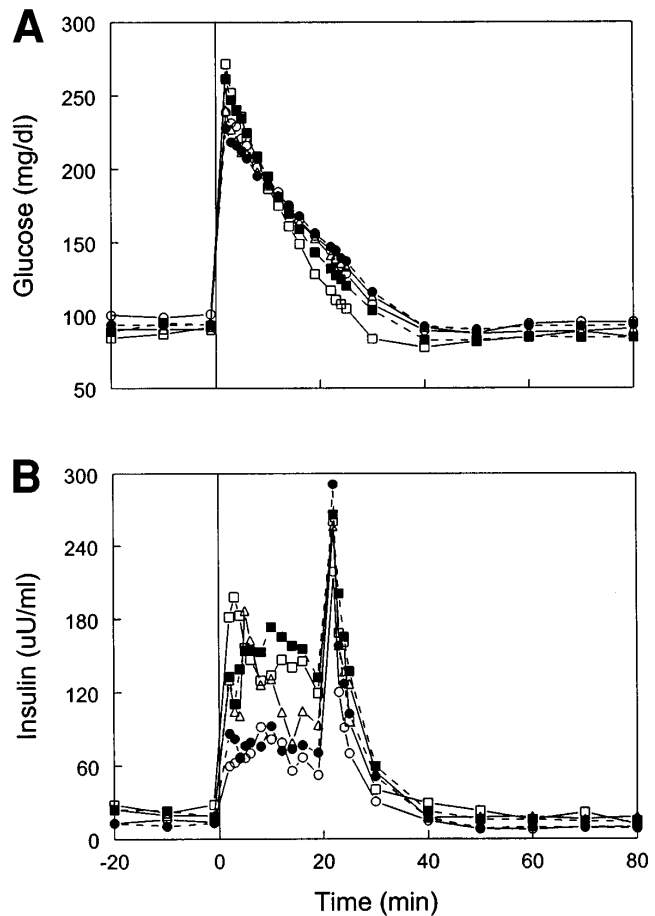


FIG. 5. Raw glucose (A) and insulin (B) data from FSIGTTs performed at weeks 0 (\circ), 1 (\bullet), 3 (\square), 6 (\blacksquare), and 12 (\triangle) of the high-fat diet. Data are presented only up to 80 min after the glucose injection for clarity.

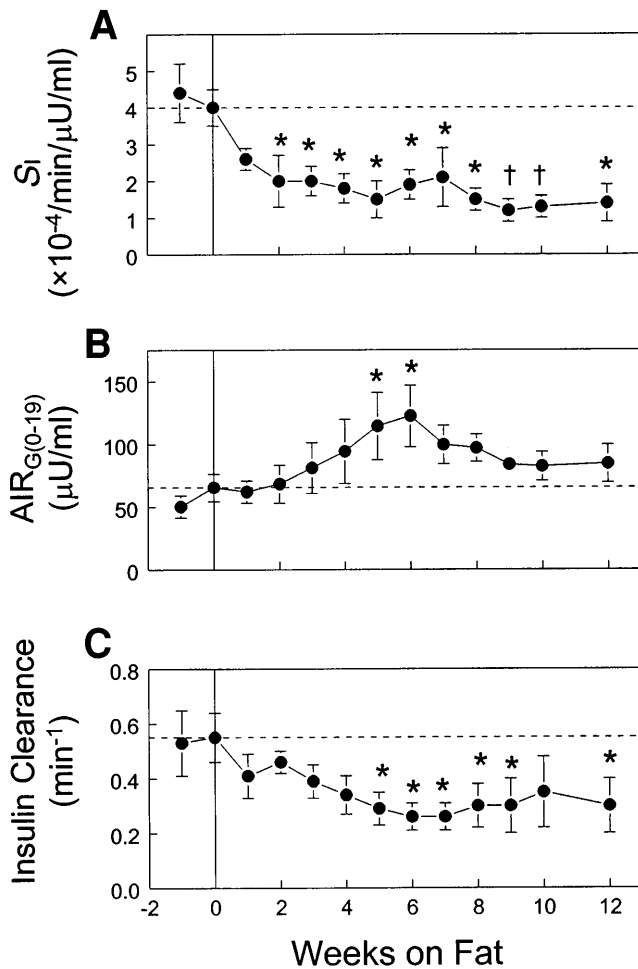


FIG. 6. FSIGTT results: S_1 (A), the $AIR_{G(0-19)}$ (B), and insulin clearance (C). S_1 is calculated from the minimal model (see text); $AIR_{G(0-19)}$ is the area of insulin concentration over basal for the 19 min after glucose is injected; insulin clearance is calculated from the exponential decay curve fitted to the decline in insulin levels after the insulin bolus at $t = 20$ min. * $P < 0.0045$, † $P < 0.0001$ vs. week 0.

eight control animals (13), as described in RESEARCH DESIGN AND METHODS. Despite similar insulin infusion rates, systemic insulin levels were higher in the fat-fed animals than in the controls (Table 4). Insulin clearance was 12.4 ± 1.0 in the con-

trol animals and $10.1 \pm 0.5 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in the animals fed the high-fat diet ($P < 0.05$, unpaired t test). This represents an $\sim 20\%$ lower insulin clearance in the fat-fed animals.

Secretion-sensitivity relationship. The multiphasic response to fat feeding can be seen in the insulin response-sensitivity relationship, which has been shown to reflect a hyperbolic function in cross-sectional studies (14–16). The time course of the components of the DI and the time course of DI itself are in Table 5, and the secretion-sensitivity relationship (as percent basal) is in Fig. 8. Because the DI calculated from the averaged S_1 and $AIR_{G(0-19)}$ is not the same as the average DI, the actual average DIs (as a percent of week 0) are included in the figure. The decrease in S_1 during the first 1- to 3-week period (A to B) occurred with little compensation in insulin response; thus, DI declined by $\sim 33\%$, from 252 ± 75 to 161 ± 50 . Compensation of insulin response then maximized during weeks 4–6 (C), but because of worsening insulin resistance, this compensation failed to increase DI further. Thus, we observed less-than-complete compensation of insulin response when insulin resistance was induced with fat feeding. Interestingly, after compensation peaked at 6 weeks, there was a further decline in $AIR_{G(0-19)}$ (D and E); thus, the compensation of the β -cells was time dependent and was not maintained despite continuation of the diet and the relative constancy of insulin resistance.

DISCUSSION

Dogs were fed a diet enriched with a moderate amount of fat for 12 weeks. The animals' trunk body fat increased by 50% without a significant change in body weight. Despite the increase in central adiposity and the resultant insulin resistance, intravenous glucose tolerance was maintained (except for a brief decline) for 7 weeks. Also, there was no increase in fasting plasma glucose or FFA for the entire feeding period. Therefore, we report a model of central adiposity, limited weight gain, insulin resistance, but maintenance of normal glucose tolerance. Unlike models of fat overfeeding and massive weight gain (17–20), the present model may be representative of modest weight gain in normal adults and therefore reveal the normal mechanisms by which homeostasis in response to central adiposity is maintained.

Compensation for insulin resistance turned out to be a surprisingly dynamic process consisting of several phases. Although not identical, these phases bear resemblance to

TABLE 3
Basal and stimulated insulin and C-peptide (pmol/l)

	Weeks on high-fat diet						
	0	1	3	6	8	10	12
Basal							
Insulin	71 ± 13	65 ± 8	110 ± 25	121 ± 28	59 ± 10	101 ± 22	127 ± 27
C-peptide	142 ± 13	117 ± 6	133 ± 14	169 ± 6	151 ± 18	170 ± 15	158 ± 18
Ratio	0.43 ± 0.07	0.57 ± 0.09	0.86 ± 0.20	0.76 ± 0.18	0.39 ± 0.08	0.62 ± 0.13	0.86 ± 0.21
AUC							
Insulin	409 ± 79	366 ± 60	476 ± 112	$669 \pm 135^*$	561 ± 82	483 ± 86	582 ± 123
Percent of week 0	100 ± 0	94 ± 8	112 ± 8	164 ± 14	151 ± 21	126 ± 12	142 ± 16
C-peptide	222 ± 26	191 ± 21	196 ± 19	269 ± 39	206 ± 34	223 ± 33	243 ± 43
Percent of week 0	100 ± 0	89 ± 12	89 ± 3	122 ± 11	96 ± 17	103 ± 16	107 ± 10

Data are means \pm SD. AUC is the integrated area under the curve over $t = 0$, from 0 to 10 min in pmol/l. Ratios represent the mean of the fasting insulin-to-C-peptide ratios for the week. * $P < 0.0045$ vs. week 0.

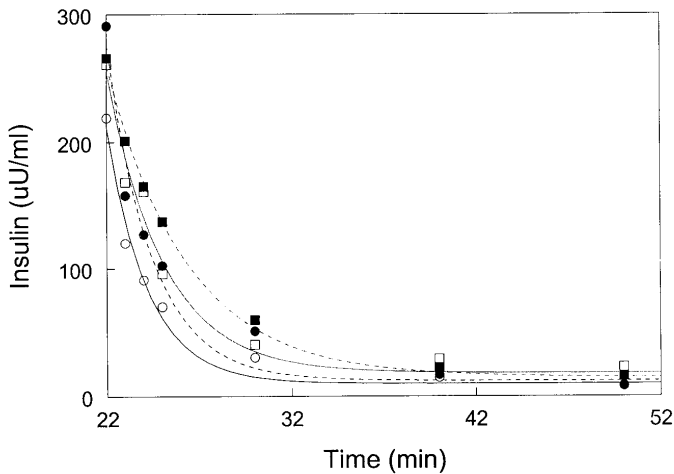


FIG. 7. Insulin profiles after the insulin bolus for different weeks on fat feeding (see text for calculations; week 0 [○], 1 [●], 3 [□], and 6 [■]). Lines show the best fit of the average data. Week 12 fit was similar to that of week 6 and is omitted for clarity.

those defined by Howard and Yasuda (21) in spontaneously obese rhesus monkeys:

1) The first week after dietary fat was increased, there was very little change in the acute insulin response to glucose, despite a modest decline in S_T . A slight decline in insulin clearance was countered by a decline in insulin secretion, such that there were no changes in fasting insulin or glucose-stimulated insulin secretion.

2) By 3 weeks on the diet, there was a severe insulin resistance that was in fact compensated by a substantial increase in the plasma insulin response and fasting hyperinsulinemia. However, these changes were due primarily to decreasing clearance, because insulin secretion still showed no compensation. Thus, for at least the initial 3 weeks on the high-fat diet, glucose tolerance was maintained in the presence of severe insulin resistance without any apparent β -cell compensation.

3) Insulin secretion showed a modest increase by 6 weeks, which, combined with the low clearance, yielded an insulin response greater than twice the prediet level. Fasting insulin was also more than double its prediet level.

4) Surprisingly, the increase in insulin secretion did not last, tending to regress back toward the prediet values after 6 weeks. Although this secondary reduction could be considered a "failure" of the β -cells (22–25), it is also possible that this is a normal physiological mechanism to preserve β -cell function by reducing insulin clearance, and calling less upon

the pancreas to secrete insulin in the presence of chronic insulin resistance.

Compensation for insulin resistance induced by the extra fat involved increasing insulin secretion and decreased insulin clearance. Cross-sectional support for these changes has been shown in other models of insulin resistance. Increased insulin secretion has been observed in animal (26) and human (4,27) obesity. Insulin clearance was reduced in obesity (28–31), insulin resistance without obesity (31), impaired glucose tolerance (IGT) (32), and diabetes (33). Decreased insulin clearance has also been observed with high-fat feeding in rats (34) and was suggested in a model of fat-fed dogs (19). Thus, the model of insulin resistance developed in this study is consistent with experimental and natural states of insulin resistance reported in the literature with respect to the compensation for insulin resistance.

The mechanism of decreased insulin extraction after fat feeding is not understood. Insulin extraction is impaired by elevation of FFA (35), suggesting that the hyperlipidemia associated with these states may play a role. Fasting FFA levels, however, were never observed to increase and, in fact, tended to decrease in the present study. It is possible that the delivery rate of FFA to the liver is increased in the fat-fed dogs, and this may alter liver FFAs or triglycerides, which may play a role in reducing insulin clearance. Also, incretins have also been shown to lower hepatic insulin extraction (36,37) and may therefore help delay the onset of IGT with progressing insulin resistance. Incretins glucagon-like peptide 1, gastric inhibitory polypeptide, and cholecystokinin were not measured in the present study, but it is provocative to consider that incretins could play a role coordinating the reduction in insulin clearance and increase in secretion observed.

Reduction in β -cell responsiveness with feeding of fat to rodents (38,39) as well as humans (40) has been interpreted as toxicity to the β -cells due to lipemia. However, an alternative interpretation emerges from the present study. Normally, half of secreted insulin is degraded by liver. Although we did not determine in the present study whether the halving of insulin clearance was due primarily to liver extraction, because such a large fraction of insulin is cleared by liver, it is reasonable to suggest that fractional extraction of insulin by liver was reduced. Therefore, reduction of liver insulin extraction may be interpreted as a mechanism by which less stress is placed on the β -cells by diverting a larger percentage of secreted insulin to the systemic circulation to enhance glucose uptake and suppress lipolysis. Therefore, reductions in β -cell secretion with fat-induced insulin resistance should be interpreted with caution, in that they may represent normal physiology rather than pathophysiology.

TABLE 4
Steady-state insulin concentrations and total insulin clearance in seven dogs fed a high-fat diet and eight control-fed dogs

	Basal insulin ($0.2 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)		High insulin ($1.2 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)		Average
	Insulin (pmol/l)	Clearance ($\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	Insulin (pmol/l)	Clearance ($\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	Clearance ($\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)
Control	101.4 ± 9.6	12.5 ± 1.1	608.3 ± 45.8	12.3 ± 0.9	12.4 ± 1.0
Fat fed	114.5 ± 7.2	10.7 ± 0.6	$783.2 \pm 57.2^*$	$9.5 \pm 0.7^*$	$10.1 \pm 0.5^*$

Data are means \pm SD. * $P < 0.05$ vs. controls, unpaired t test.

TABLE 5
Components of the DI

Week	S_I (* 10^{-4} min $^{-1}$ /μU/ml)	AIR _{G(0-19)} (μU/ml)	DI (min $^{-1}$)
-1	4.42 ± 0.77	50.5 ± 8.9	236.2 ± 80.0
0	3.98 ± 0.54	65.7 ± 10.8	267.3 ± 69.3
1	2.57 ± 0.28	62.3 ± 9.0	161.0 ± 29.4
2	1.99 ± 0.68*	68.5 ± 15.1	154.8 ± 56.4
3	2.01 ± 0.43*	81.2 ± 20.2	167.1 ± 59.6
4	1.76 ± 0.40*	94.3 ± 25.4	141.2 ± 49.0
5	1.54 ± 0.48*	114.4 ± 26.8*	133.8 ± 39.5
6	1.94 ± 0.44*	122.5 ± 24.5*	187.3 ± 28.0
7	2.07 ± 0.80*	99.6 ± 15.5	170.9 ± 47.5
8	1.48 ± 0.33*	96.9 ± 10.9	134.8 ± 30.6
9	1.16 ± 0.32†	83.9 ± 6.8	91.1 ± 26.7*
10	1.29 ± 0.29†	82.4 ± 11.5	110.3 ± 29.2*
12	1.40 ± 0.55*	84.6 ± 15.0	145.8 ± 66.1

Data are means ± SD. * $P < 0.0045$, † $P < 0.0001$ vs. week 0.

Rocchini et al. (17,19) have previously fed dogs a very-high-fat diet that included 900 g cooked beef fat (8,100 extra kcal/day). The dogs in our laboratory would not voluntarily eat this amount of fat, and the physiological significance of such a diet may be questioned. Therefore, we used a moderate and physiologically realistic diet that was well tolerated (~55 g/day or ~500 extra kcal/day). Using the very-high-fat diet of Rocchini et al., Kaiyala et al. (20) found no effect of high-fat feeding on insulin secretion, despite a decline in S_I similar to that witnessed in the present study. Additionally, Kaiyala et al. did not follow the changes in metabolic variables longitudinally during fat feeding. Their approach failed to uncover the polyphasic nature of the physiologic response, including the polyphasic plasma insulin response as well as the fundamental importance of changed insulin clearance, which they did not measure. Thus, the longitudinal nature of the present study, the more physiological diet, and the measurement of insulin clearance allowed us to fully characterize the progression of and compensation for a physiological induction of insulin resistance in a model that can be argued to represent more faithfully the modest central adiposity that afflicts a significant fraction of the U.S. population (1–3).

The signals that modulate the observed increase in insulin secretion are not known (41). The present data indicate that elevated fasting glucose levels are not the signal for β -cell compensation, because fasting euglycemia was maintained throughout the diet, and glucose tolerance declined only after 10 weeks. In preliminary results, postprandial glucose was also not increased in this fat-fed canine model (42). FFAs have been suggested by McGarry (43) and others as both signaling an increase in insulin secretion but also as impairing the β -cell after prolonged exposure. Because fasting FFA levels did not increase in this study but, in fact, decreased in the presence of elevated insulin levels, they cannot be responsible for the observed changes in insulin levels. Other possibilities to be considered are elevated portal vein FFAs or triglycerides, or incretin moieties. Finally, it may be insulin resistance of the β -cells themselves, as recently suggested by Withers et al. (41). Future studies must address the signal for the upregulation of insulin secretion, because failure of this signal may play an important role in the pathogenesis of IGT and/or type 2 diabetes.

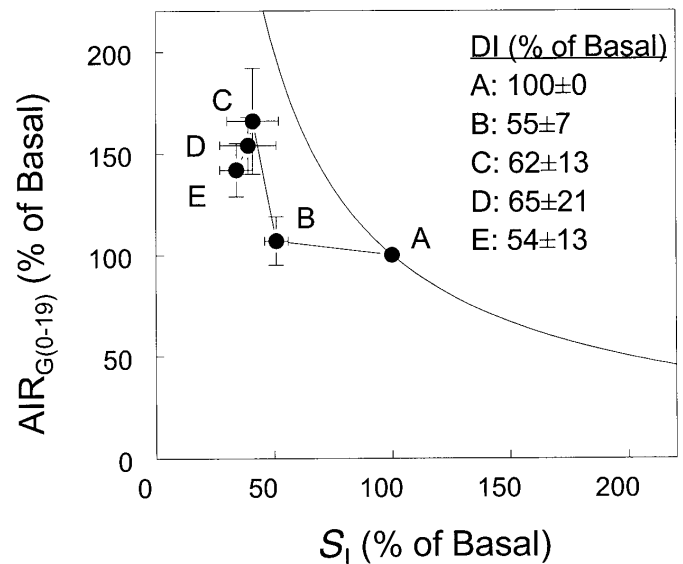


FIG. 8. The AIR_{G(0-19)} versus S_I , both as a percentage of their predict baseline values. The hyperbola represents the curve on which the animals would have to remain to maintain a constant DI (the function AIR_{G(0-19)} × S_I = 100%). A, average of weeks -1 and 0; B, weeks 1–3; C, weeks 4–6; D, weeks 7–9; and E, weeks 10–12.

Much of the confusion regarding the roles of the β -cell and insulin clearing tissues in insulin resistance is likely due to a failure to examine insulin response in relation to the relative S_I . The DI, which is defined as the product of insulin response (AIR_{G(0-19)}) and S_I , has been proposed as a more appropriate measure of relative insulin responsiveness. In a healthy person, a decline in S_I should be compensated for by an increase in insulin secretion and a decrease in extraction, such that the acute insulin response to glucose would increase and the DI would not change. Evidence for an inverse relationship between insulin response and sensitivity has come primarily from cross-sectional studies (14).

We expected that the decrease in S_I would be compensated by an increase in insulin response, maintaining a constant DI (14). The AIR_{G(0-19)} showed no change within the first 2 weeks of the diet, despite the 45% decline in S_I by this time. Thus, when insulin response and S_I were plotted against each other, there was an initial shift laterally off of the baseline, “normal” hyperbola, and the DI decreased by ~45% (Fig. 8). Insulin response began to increase after the third week, peaking at approximately double its basal value. However, because of a continuing decline in S_I , the increased insulin response did not correct the DI, but managed only to prevent a further decline in this value. This was seen visually as a move to the upper left with no change in the distance from the hyperbola. In the final 6 weeks of the diet, insulin response declined with no further decrease in S_I , seen as a vertical drop on the S_I versus the acute insulin response to glucose curve. Thus, the DI declined by a total of ~50% by the end of the 12 weeks. It is clear that the response to increased fat intake is more complex than previously predicted. It will be of interest to examine whether similar complex mechanisms would result from insulin resistance induced by other environmental insults such as fructose feeding or steroids.

The present study may give some insight into the progression of obesity and insulin resistance in humans, though one

must always use caution when comparing animals to humans. A study by Sims et al. (44) in which volunteers were fed a diet supplemented with 1,100 kcal of fat, demonstrated a 20% increase in body weight over a period of 4–6 months. These subjects developed insulin resistance, but the compensation was not as complete as that witnessed in the present study; fasting plasma insulin levels increased only 50%, and there was a slight increase in fasting plasma glucose levels. This less-complete compensation may be secondary to the much-higher exposure to dietary fat given in the study by Sims et al. It is interesting to note that despite the fasting hyperglycemia, intravenous glucose tolerance in the human volunteers declined by only 30%—the same relative change as observed in the present study. This finding implies a role for other factors such as S_G in determining glucose tolerance.

Although some investigators have reported that the progression to diabetes is determined primarily by a β -cell defect (23,24), others have found evidence for a primary defect in S_I (45,46). It is possible that the discrepancies in these findings are due to failure to quantify β -cell function and insulin extraction independently, and failure to examine insulin response with respect to S_I (i.e., DI). In fact, recent studies have found evidence that the DI is more strongly associated with IGT and diabetes than is either insulin response or S_I alone (15,16). The present study supports the concept that insulin resistance may be the first abnormality seen with a high-fat diet. Glucose tolerance was maintained in the present study because of compensation by the β -cells and insulin clearing tissues. IGT was seen only when both S_I and relative β -cell function were impaired. This result suggests that insulin resistance may develop in many individuals but leads to IGT or diabetes only in those who either have or are susceptible to developing a defect in insulin secretion or clearance. It appears that a greater focus on the relationship between insulin resistance and control of extraction of insulin by the liver as a compensating mechanism is warranted.

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