β-CELL “REST” ACCOMPANIES REDUCED FIRST-PASS HEPATIC INSULIN EXTRACTION IN THE INSULIN RESISTANT, FAT-FED CANINE MODEL

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Running Title: Beta-cell Rest Accompanies Reduced Hepatic Insulin Extraction

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ABSTRACT
During insulin resistance, glucose homeostasis is maintained by increasing plasma insulin via increased secretion and/or decreased first-pass hepatic insulin extraction. However, the relative importance of insulin secretion versus clearance to compensate for insulin resistance in obesity has yet to be determined. This study utilizes the fat-fed dog model to examine longitudinal changes in both insulin secretion and first-pass hepatic insulin extraction during development of obesity and insulin resistance. Six dogs were fed an isocaloric diet with ~8% increase in fat calories for 12 weeks and evaluated for changes in: 1) insulin sensitivity (SI) by euglycemic-hyperinsulinemic clamp, 2) first-pass hepatic insulin extraction by direct assessment and 3) glucose-stimulated insulin secretory response by hyperglycemic clamp at weeks 0, 6 and 12 of fat-feeding. We found that 12 weeks of a fat diet increased both subcutaneous and visceral fat as assessed by magnetic resonance imaging. Consistent with increased body fat, the dogs exhibited a ~30% decrease in SI and fasting hyperinsulinemia. Although insulin secretion was substantially increased at week 6, β-cell sensitivity returned to pre-diet levels by week 12. However, peripheral hyperinsulinemia was maintained due to a significant decrease in first-pass hepatic insulin extraction, thus maintaining hyperinsulinemia despite changes in insulin release. Our results indicate that when obesity and insulin resistance is induced by an isocaloric, increased fat diet, an initial increase in insulin secretion by the β-cells is followed by a decrease in first-pass hepatic insulin extraction. This may provide a secondary physiological mechanism to preserve pancreatic β-cell function during insulin resistance.
INTRODUCTION

Obesity is associated with hyperinsulinemia and insulin resistance, both primary risk factors for Type 2 diabetes. In the face of insulin resistance due to obesity, hyperinsulinemia acts to compensate for decreased sensitivity of insulin responsive tissues such as liver, skeletal muscle, and adipose tissue. This compensatory hyperinsulinemia is essential for the maintenance of normal glucose homeostasis in face of insulin resistance. It is often assumed that increased pancreatic islet-cell secretion is the mechanism by which plasma insulin is increased (5; 31; 35). But the liver can also contribute to hyperinsulinemia by decreasing extraction of insulin (6; 10; 16; 27). In fact both increased secretion and decreased hepatic extraction can make significant contributions to hyperinsulinemia in the insulin resistant state. Bonora et al. (2) reported that subjects with obesity or mild glucose intolerance exhibited both hypersecretion of insulin and decreased hepatic insulin extraction while subjects with more severe impaired glucose intolerance had only decreased hepatic extraction as the cause of their hyperinsulinemia. Glucose intolerant subjects can demonstrate greater than 50% islet-cell dysfunction (41), possibly explaining lack of β-cell compensation in Bonora et al.’s studies. However, the relative importance of β-cell dysfunction versus reduced liver insulin clearance to compensation for insulin resistance in normal individuals remains to be determined. A previous study completed in our laboratory (29) suggested that the relative importance of reduced liver extraction of insulin to hyperinsulinemic compensation is underestimated. But, in the previous study, beta-cell
sensitivity to glucose and first-pass clearance of insulin by the liver was calculated from a model and was not directly measured. Given the potential importance of reduced clearance, which can relieve the pancreatic islets the burden of responding to insulin resistance, we chose to examine the temporal patterns of response to diet-induced insulin resistance with direct measurement techniques. This approach revealed dissociated time-varying changes in secretion versus clearance which together allow maintenance of glucose tolerance within normal limits.

**METHODS**

**Animals.** Six male mongrel dogs (27.5±1.5 kg) were used in the present study. The animals 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 examination by the veterinary staff. Dogs were fed a 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 chow (36.6% carbohydrate, 26.4% protein, 14.7% fat, and 2.9% fiber [Wayne Dog Food, Alfred Mills, Chicago]). Thus, the total diet consisted of 3885 kcal/day; 37.9% from carbohydrates, 26.3% from protein and 35.8% from fat. Seven to 10 days prior to a period of weight stabilization, chronic catheters were surgically implanted. One catheter was inserted in the jugular vein and advanced to the right atrium for sampling of mixed central venous blood; a second catheter was inserted in the femoral vein and advanced to the vena cava for insulin and somatostatin infusion; a third catheter was placed in the
portal vein for insulin infusion. All catheters were led subcutaneously to the back of the neck and exteriorized. Catheters were flushed with heparinized saline (10 U/mL) at least twice a week and the exteriorization site was cleaned with hydrogen peroxide (4%). On the morning of each experiment, acute catheters were inserted into peripheral veins for glucose infusion. The experimental protocol was approved by the USC Institutional Animal Care and Use Committee.

**Diet.** Following 2-3 weeks on the standard diet for weight stabilization (week 0) dogs were fed an isocaloric, increased fat diet in which approximately 9% of total calories was replaced by cooked bacon grease (USC Keck School of Medicine cafeteria). This increased fat diet was comprised of one can of Hill's Prescription Diet, 715 g dry chow, and 2 g/kg pre-diet body weight of cooked bacon grease. This diet consisted of 3950 kcal/day; 32.9% carbohydrate, 22.9% protein, and ~44.3% fat. The animals were maintained on this diet for 12 weeks.

**Magnetic resonance imaging.** During weeks 0, 6 and 12 of the moderate-fat diet, magnetic resonance imaging (MRI) scans were performed on the animals (25). One-cm axial abdominal images (T1 slices; TR 500 TE:14) were obtained using a General Electric 1.5 Tesla Horizon (v5.7 software) magnet. The MRI images were analyzed using Scion Image (Windows 2000 Version Beta 4.0.2; Scion Corporation, Frederick, MD), which quantifies fat tissue (pixel value 121-230) and other tissue (0-120) in each slice. Total fat was estimated as the integrated fat or tissue across the MRI 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, using the slice at the level where the left renal artery branches from the abdominal aorta as a midpoint landmark ± 5 cm. Percent omental fat was calculated as the omental fat divided by the total tissue area in these same slices.

**Experimental protocols.** Animals were familiarized with being in a Pavlov sling at least 1 week before the first experiment. Three protocols (I, II and III) were performed thrice on each animal: at week 0, week 6 and week 12 of the isocaloric elevated fat diet. Thus each animal served as its own control. The three protocols were the euglycemic clamp for insulin sensitivity [Protocol I, previously presented (25)], direct assessment of first-pass hepatic insulin extraction [Protocol II] and glucose-stimulated insulin secretory response by hyperglycemic clamp [Protocol III]. Prior to the beginning of each of the tests animals were fasted for 12 hours. At approximately 7 AM animals were brought to the laboratory and placed in the sling. Approximately 30 min prior to the beginning of any experiment a 19-gauge angiocatheter for infusion of glucose was placed into a saphenous vein and secured.

**Protocol I: assessment of insulin sensitivity by euglycemic-hyperinsulinemic clamp.**

Insulin sensitivity was assessed in the animals as previously reported (25). At $t = -120$ min, a primed continuous infusion of high-performance liquid chromatography-purified
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[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/kg; Bachem California, Torrance, CA) was started to suppress endogenous insulin and glucagon secretion and was continued for the duration of the experiment. Porcine insulin was infused (1.5 mU/kg/min; Novo Nordisk) into the femoral vein to attain hyperinsulinemia. Glucose was clamped at basal 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 ten minutes from –30 to 60 min, every 15 minutes from 60 to 120 min, and every ten minutes from 120 to 180 min.

**Protocol II: assessment of first-pass hepatic insulin extraction.**

Hepatic insulin extraction was calculated by comparing peripheral insulin levels which resulted from insulin infusion either into the systemic circulation, or directly into the portal venous blood. The relative decrement in plasma insulin with portal infusion reflected liver insulin extraction. This double-infusion protocol allows for direct calculation of first-pass hepatic insulin extraction. No assumptions are needed regarding, for example, C-peptide disappearance kinetics, which may vary under different metabolic conditions (15).

In each animal two matched experiments were performed on subsequent days. In every experiment insulin was infused sequentially at 3 doses under euglycemic clamped
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conditions. In matched experiments, insulin was infused either peripherally at: 1.5, 3.0 and 4.5 pmol/min per kg; or in the matched study infused intraportally at twice the rate: 3.0, 6.0 and 9.0 pmol/min per kg. Two basal samples were obtained at \( t = -100 \) and -91 minutes. Starting at \( t = -90 \) min and continuing for the duration of the experiment intravenous somatostatin (1.0 \( \mu \)g/min/kg; Bachem California, Torrance, CA) was given to suppress endogenous insulin and glucagon secretion, and glucagon was replaced intraportally at 1.3 ng/kg/min. Beginning at \( t = 0 \) min, insulin was infused into either the femoral or portal vein. Each rate of infusion was maintained for a period of 90 minutes to attain steady-state plasma insulin levels. Glucose was clamped at basal by a variable glucose infusion. Blood samples were drawn from the jugular catheter every ten minutes.

**Protocol III: assessment of \( \beta \)-cell function by graded hyperglycemic clamp.**

Basal samples were taken at -20, -10, and -1 min. At time \( t = 0 \) min, glucose was infused intravenously (if needed) to clamp plasma glucose at a target concentration of 100 mg/dl until \( t = 60 \) min. From \( t = 61-150 \) min the glucose infusion rate was increased to clamp plasma glucose at 150 mg/dl, and from \( t = 151-240 \) min, glucose was clamped at 200 mg/dL. Blood was drawn from a jugular vein catheter every ten minutes for the duration of the experiment.

In all experimental protocols, samples for assay of insulin were kept on ice in tubes pre-coated with lithium fluoride and heparin (Brinkmann Instruments; Westbury, New York)
containing 50 μL EDTA while samples for FFA and glycerol assays were taken in tubes with EDTA and paraoxon to inhibit lipase activity.

**Assays.** Glucose was measured in duplicate immediately after sampling with an YSI 2700 autoanalyzer (Yellow Springs Instruments; Yellow Springs, Ohio). Insulin was measured by an ELISA adapted for dog plasma (36) and samples for $^3$H-glucose tracer assay were deproteinized using barium hydroxide and zinc sulfate as described by Somogyi (38). Free fatty acids were measured utilizing a colorimetric assay from Wako (NEFA C, Wako Pure Chemical Industries; Richmond, VA) based on the acylation of coenzyme-A.

**CALCULATIONS**

**Protocol I: assessment of insulin sensitivity by euglycemic-hyperinsulinemic clamp.**

Insulin sensitivity was calculated using the following equation (12):

$$S_I = \frac{\Delta G_{\text{inf}}}{\Delta I \times G} \quad (1)$$

in which $\Delta G_{\text{inf}}$ is the increase in glucose infusion at steady state, $\Delta I$ is the insulin increment and $G$ is the target glucose level.

**Protocol II: assessment of first-pass hepatic insulin extraction.**
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Steady-state plasma insulin concentration was designated as the average of the last 30 min of each dose period during the 3-step insulin euglycemic clamp. Clearance of insulin from plasma (Cl) can be calculated by dividing the insulin infusion rate by plasma insulin concentration. Assuming linear insulin kinetics (11), Cl can also be defined as the inverse of the slope (m) of the best-fit line relating insulin infusion rate (in pmol-min⁻¹·kg⁻¹) to steady-state plasma insulin concentration (in pM) or:

\[ Cl = \frac{1}{m} \]  

(2)

First-pass extraction (FPE) of insulin by the liver can be defined as the difference between the clearance rate measured following portal insulin administration versus the clearance rate measured after peripheral insulin administration (11) such that:

\[ \text{FPE} = \frac{\text{Cl}_{po} - \text{Cl}_{pe}}{\text{Cl}_{po}} \]  

(3)

Where Cl_{po} and Cl_{pe} represent the plasma clearance rate after the intraportal infusion and peripheral infusion, respectively. By combining equations 2 and 3, first-pass hepatic insulin extraction can then be calculated as:

\[ \text{FPE} = 1 - \frac{m_{po}}{m_{pe}} \]  

(4)
Protocol III: assessment of β-cell function by graded hyperglycemic clamp.

Steady state was designated as the last 30 min of each glycemic clamp period during the graded hyperglycemic clamp. Plasma insulin concentration during steady state (I_{SS}) was calculated as the average of all samples during the steady-state period. In addition, the integrated area under the curve over basal (I_{AUC}) was calculated during steady-state using the trapezoidal rule.

Measured plasma insulin values reflect not only insulin secretion rate, but also first-pass liver insulin extraction. Thus, a doubling of liver extraction would reduce by one-half the measured plasma insulin levels which would result from hyperglycemia. We wished to define an index of islet function which was independent of possible differences in first-pass liver insulin uptake. Therefore, we corrected the measured plasma insulin levels observed during hyperglycemia to the independently measured first-pass liver insulin extraction rate. Such a calculation makes it possible to define an index of pancreatic insulin release (INS_{corr}) from the dose-response studies independent of possible changes in first-pass liver insulin extraction. Plasma insulin level corrected for measured first-pass liver insulin extraction (from equation 4) was defined as

\[
(5) \quad \text{INS}_{corr} = \frac{\text{INS}_p}{1 - \text{FPE}}
\]
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Where \( \text{INS}_P \) is the plasma insulin concentration measured in the peripheral circulation, and \( \text{FPE} \) is fractional insulin extraction across the liver. Then, islet \( \beta \)-cell function was quantified as the slope relating the area under the curve of the extraction-corrected plasma insulin concentration (\( \text{INS}_{\text{corr}} \)) to the average plasma glucose level during steady-state for each glycemic phase of the experiment. Total AUC over basal (\( \text{AUC}_T \)) for extraction-corrected plasma insulin concentration throughout the entire experimental protocol (240 min) was also quantified for estimation of insulin secretion.

**Disposition Index.** Insulin sensitivity and insulin secretion are related by a hyperbolic function (23) where:

\[
(6) \quad \text{Sensitivity} \times \text{Secretion} = \text{constant}
\]

The putative constant is termed the disposition index and reflects the ability of insulin secretory mechanisms to compensate for changes in insulin sensitivity. In order to compare the contribution of insulin secretion only and insulin secretion + hepatic insulin clearance in compensating for insulin resistance, this hyperbolic relationship was calculated utilizing the sensitivity measurements (\( \text{S}_I \)) as described above and either the total AUC for \( \text{INS}_{\text{corr}} \) (insulin secretion only) or the total AUC for \( \text{INS}_P \) (insulin secretion + hepatic insulin clearance).

**Statistical analyses.** All experimental data are expressed as means ± SE. Statistical analyses were performed with paired Student’s \( t \) tests or a two-way analysis of variance
(ANOVA) as appropriate for comparisons of weeks 6 or 12 with week 0. The $t$ tests were performed using Microsoft Excel 2002 and all ANOVAs were performed using Minitab for Windows software (Minitab Inc., State College, PA).

**RESULTS**

**Body composition.**

As we have previously shown, the isocaloric moderate-fat diet resulted in a substantial increase in percent body fat at both weeks 6 and 12 of the study without a significant change in body weight (25). Total trunk fat increased from 26 ± 5% at week 0 to 38 ± 5% at week 6 ($p<0.05$ vs. wk 0, ANOVA) and this increase in total trunk adiposity was maintained up to week 12, during which total trunk fat accounted for 41 ± 7% of total trunk tissue ($p<0.05$ vs. wk 0, ANOVA). The extensive increase in total trunk fat was due to an accumulation of fat tissue in the omental and subcutaneous depots (Table 1).

**Protocol I: insulin sensitivity.**

Consistent with increased adiposity, the animals developed insulin resistance and fasting hyperinsulinemia over the course of this study. The isocaloric diet with increased fat reduced insulin sensitivity by 32 ± 6% at week 6 of the diet (wk 0: 3.9 ± 0.6 vs wk 6: 2.5 ± 0.3 $\times 10^{-4}$ dL·kg$^{-1}$·min$^{-1}$·pM$^{-1}$; $p<0.05$, ANOVA) and this decrement persisted for the duration of the study such that by week 12, insulin sensitivity had decreased by 36 ± 6% in comparison to week 0 (wk12: 2.5 ± 0.4 $\times 10^{-4}$ dL·kg$^{-1}$·min$^{-1}$·pM$^{-1}$; $p<0.05$, ANOVA). As described in a previous publication (25), these changes in
insulin sensitivity were due to the development of severe hepatic insulin resistance as well as a moderate degree of resistance at the periphery. Fasting insulin levels increased by 49 ± 18% at week 6 and remained increased (+68 ± 7% above wk 0) at week 12 of the study (p<0.05 vs wk 0 for both, ANOVA) (Table 1).

Although there was a tendency for subcutaneous adiposity to be negatively correlated with $S_i$ ($r = -0.46$, $p=0.056$), only omental fat was found to have a significant negative correlation with insulin sensitivity ($r = -0.60$, $p=0.009$) during the twelve week period (Figure 1).

**Protocol II: first-pass hepatic insulin extraction.**

**Glucose.** Basal glucose was unchanged during the course of the diet (wk 0: 92.7 ± 1.8, wk 6: 96.5 ± 2.3, wk 12: 92.5 ± 1.2 mg/dL; $p=NS$, ANOVA). Plasma glucose was clamped at basal in all experiments (mean coefficient of variation of 8.5%) and steady state plasma glucose levels did not differ between infusion protocols, insulin dose or time on diet (data not shown).

**Dose-response.** The time course of plasma insulin during both the peripheral and portal insulin infusion experiments for week 0, 6 and 12 are shown in Figure 2 (left panel). Plasma insulin reached steady-state within 45 minutes of each individual insulin dose period during both infusion protocols. The relationship between insulin infusion rate and steady-state peripheral insulin concentration (Figure 2; right panel) was
virtually linear for both the portal and peripheral infusion experiments, allowing for simple and direct calculation of first-pass hepatic insulin extraction (FPE). First-pass hepatic insulin extraction was almost unchanged week 6 compared to basal (59.5 ± 3.1% versus 55.6 ± 4.5%, p=0.45). This differed from the previous study (29) in which insulin clearance as calculated from exponential rate of decline after insulin injection appeared to decrease beginning at week 6. However, by week 12 of the diet, first-pass hepatic insulin extraction was substantially and significantly decreased to an extraction rate of 43.9 ± 3.9% (p=0.003 vs. wk 0, ANOVA) (Figure 2F), reflecting an overall potential increase of 30% in the probability that an insulin molecule secreted at 12 weeks would survive the hepatic transit on one pass and reach the systemic circulation.

**Protocol III: β-cell function by graded hyperglycemic clamp.**

**Insulin secretory function.** Plasma glucose was well clamped at the target glucose levels of 100, 150 and 200 mg/dL (Figure 3A) and glucose infusion rates did not differ between weeks 0, 6 and 12 (data not shown). The time courses of plasma insulin corrected for first-pass hepatic extraction, \( \text{INS}_{\text{corr}} \) (equation 3), during the clamps at weeks 0, 6, and 12 are depicted in Figures 3B. β-cell function, defined as the slope of the line relating \( I_{\text{AUC}} \) to the average plasma glucose level during steady-state, was found to be 12.4 ± 4.1 pM per mg/dL at week 0 (Figure 4). By week 6, β-cell function per se more than doubled to 25.6 ± 5.7 pM per mg/dL (p=0.006 vs. wk 0, ANOVA). This increase in β-cell function was not sustained despite continued insulin resistance. In fact, by week 12 of the diet, insulin response had renormalized to the pre-diet value
(12.2 ± 1.9 pM per mg/dL; p=0.97 vs. wk 0, ANOVA). Total AUC over basal (AUC$_T$) for extraction-corrected plasma insulin concentration throughout the entire experimental protocol (240 min) also showed a similar pattern (Table 1). Thus, at 12 weeks beta cell function was renormalized and yet a compensatory hyperinsulinemia could still be maintained solely due to reduced liver insulin extraction and delivery of a larger fraction of secreted insulin to the systemic circulation.

**Disposition Index.**

The ability of the liver to compensate for insulin resistance during a moderate fat diet can be seen in the insulin secretion-sensitivity relationship which is reflected by a hyperbolic function (Figure 5, Table 2) (23). When examining this hyperbolic relationship using only insulin secretory function (i.e. the total AUC for INS$_{corr}$), we found that the decrease in insulin sensitivity was compensated by ~60% increase in insulin secretion such that DI was unchanged at week 6 (wk 0: 63.6 ± 0.8 vs. wk 6: 64.3 ± 1.2 dL/kg; p=NS, ANOVA). Although insulin sensitivity was not further decreased from week 6, by week 12 of the study DI appeared to decrease by 36%, from 64.3 ± 0.8 to 38.7 ± 0.6 (p<0.05, ANOVA) because the increase in insulin secretion was not sustained. However, when changes in hepatic insulin clearance are included in the calculation for overall insulin response by using the total AUC for INS$_P$, the DI remains unchanged throughout the study. Thus, the consistency of DI is due to a combination of enhanced insulin secretion and decreased hepatic insulin clearance.
DISCUSSION

Hyperinsulinemia is a hallmark of insulin resistance, a primary risk factor in the development of Type 2 diabetes. In an insulin resistant state such as obesity, hyperinsulinemia can compensate for the decreased responsiveness to insulin by liver, skeletal muscle, and adipose. This compensatory rise in the plasma concentration of insulin is essential for the maintenance of glucose homeostasis in face of chronic insulin resistance. Studies in obese individuals with insulin resistance have shown that there is increased secretion by the β-cell and decreased extraction of insulin by the liver, both of which can contribute to the maintenance of hyperinsulinemia (1; 20; 27; 32). However, it has been demonstrated that the contribution from the β-cell and the liver to hyperinsulinemia can differ depending on the level of obesity and/or glucose tolerance (37). It appears that these compensations are not static but rather, temporal in nature.

In the present study, we sought to directly examine in a longitudinal manner the temporal pattern of changes that occurred in both insulin secretion and first-pass hepatic insulin extraction during the development of insulin resistance and obesity in dogs fed a diet with a moderate increase in total fat content and virtually no change in total caloric consumption.

When dogs were fed an isocaloric diet with a moderate increase in total fat content, there was a significant accumulation of total trunk body fat due to both subcutaneous and visceral fat despite little change in body weight. Consistent with the increase in body fat, the dogs exhibited a decrease in insulin sensitivity as well as increases in
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fasting insulin. There was a substantial increase in insulin secretion at week 6 of the diet. However, at week 12, sensitivity of the β-cells had returned to the basal level. But, there was a substantial reduction in first-pass hepatic insulin extraction thus maintaining peripheral hyperinsulinemia despite the transient nature of the increase seen in insulin release. This dynamic compensatory response to insulin resistance is best characterized by the changes seen in the insulin secretion-sensitivity relationship, or the disposition index. After 6 weeks of moderate fat feeding, the disposition index remained unchanged despite the decrease in insulin sensitivity due to the considerable increase overall in insulin secretion. But by week 12 of the study the disposition index appeared to decline because the insulin resistance was not totally compensated by increased secretion. However, by including the changes in hepatic insulin clearance into the overall insulin response we found that the DI, based upon total insulin appearance in systemic blood, remained unchanged throughout the study. Compensatory hyperinsulinemia was sustained even when beta cell function was renormalized by a reduction in liver insulin extraction and delivery of a considerably larger fraction of secreted insulin to the systemic circulation, leading to maintenance of the disposition index.

The contribution of decreased hepatic insulin extraction in relation to increased secretion has not previously been directly examined in a longitudinal manner. Studies have demonstrated that increased insulin secretion may be the predominant mechanism by which insulin is increased during obesity and insulin resistance (24; 32).
Under normal conditions, it has classically been thought that insulin release is stimulated by entry of glucose into the β-cell, which induces closure of K⁺ channels, depolarization and opening of voltage-gated calcium channels resulting in the secretion of insulin. It has also been shown that free fatty acids can stimulate insulin secretion directly, although the complete mechanism by which this occurs is yet unknown (17). And although it has been suggested that both glucose and free fatty acids may play a role in the upregulation of insulin secretion often seen in obesity and insulin resistance, fasting glucose and FFA levels (data not shown) were unchanged in this study. In addition to an increase in the function of β-cells, it has also been suggested that increased mass of β-cells may be responsible for increased insulin secretion during obesity (26). Many animal models of obesity have shown increased beta cell mass and it has been demonstrated that in humans, beta cell mass is increased by ~50% in obese individuals compared to lean controls (4). Due to the longitudinal nature of this study, we were unable to measure any potential changes in β-cell mass. However, it would be of great interest to examine whether this may have accounted for the upregulation in insulin secretion seen at week 6 of this study.

The liver is known to extract a large percentage (~55%) of insulin secreted by the pancreas under normal conditions. Studies done in isolated hepatocytes (39) and in situ on perfused liver (40) have shown that FFA can impair insulin binding and degradation, and in vivo (42) intraportal administration of FFA results in a decrease in hepatic insulin extraction. Although there was no measurable change in fasting levels
of FFA in the present study, when taking into account the substantial increase in visceral fat depot and relative insulin resistance of this fat tissue to the anti-lipolytic actions of insulin, it is possible that the liver was subjected to an increase of FFA due to increased portal flux. We have previously reported (21) that gene expression of the transcription factor sterol regulatory element-binding protein 1 (SREBP-1) is increased by ~40% in the livers of fat-fed animals and there is also a tendency for fatty acid binding protein (FABP) to be higher, suggesting that uptake and possibly synthesis of fatty acids may be increased in the liver. Furthermore, it was found that liver triglyceride of increased by ~50% in the liver samples taken from fat fed dogs. These data give further evidence that the decrease we see in hepatic insulin extraction in this study may be due to an elevation in portal vein FFA and accumulation in the liver.

The mechanisms responsible for decreased hepatic insulin extraction are not fully understood. The initial step in insulin degradation by the liver is binding to a cell membrane receptor and is partially mediated by the availability of membrane-bound insulin receptors (18). Studies done in liver specific insulin receptor knockout (LIRKO) mice (13; 28) have provided evidence that receptor-mediated degradation in the liver accounts for a majority of total insulin clearance. Previous studies in our laboratory have found that in addition to the increase in liver triglyceride content, there is a 50% decrease in liver insulin receptors in fat-fed dogs compared to control (21). Furthermore, it has been shown that selective elevation of portal FFA results in a greater degree of peripheral hypersinulinemia than peripheral administration of FFA (8).
This suggests that perhaps increased portal FFA may have a role in decreasing insulin receptor binding in the liver in the fat fed dog model. The insulin-degrading enzyme (IDE) has also been shown to play an important role in the cellular metabolism of insulin (9). Both the insertion in the membrane or internalization and degradation of insulin by IDE may be modulated by the presence of FFA in the portal vein. Another potential mechanism involved in the regulation of hepatic insulin clearance is the transmembrane glycoprotein, CEACAM1 (previously referred to as pp120), which has been implicated in mediating insulin endocytosis and degradation (7; 14; 30). Poy et al. (33) reported that functional inactivation of CEACAM1 in the liver of mice with a dominant-negative, phosphorylation-defective CEACAM1 mutant resulted in hyperinsulinemia due to an impairment in insulin clearance with secondary hepatic insulin resistance as well as the development of visceral adiposity and an increase in liver triglyceride.

We have previously reported (25) the development of hepatic insulin resistance and fasting hyperinsulinemia despite only a moderate degree of peripheral insulin resistance when visceral adiposity is increased by fat-feeding. This previous data in combination with the findings presented here suggests that an increase in visceral adiposity can result in the development of hepatic insulin resistance and hyperinsulinemia due to a decrease in insulin extraction by the liver with relatively minor changes in peripheral insulin sensitivity. Studies done in LIRKO mice (13; 28) have shown that when hepatic insulin resistance is genetically induced, there is development of severe hyperinsulinemia due to a decrease in liver insulin clearance and a larger mass of β-
cells which may contribute to the development of peripheral insulin resistance. In addition, the aforementioned study in phosphorylation defective CEACAM1 mutant mice (33) has shown that when insulin clearance is altered, there is resultant hyperinsulinemia without a change in insulin secretion and the subsequent development of hepatic insulin resistance. It has also been shown in dogs (19; 34) that when pancreatic insulin delivery bypasses the liver completely and is diverted directly to the systemic circulation, hyperinsulinemia results with the subsequent development of peripheral insulin resistance. Our results indicate that when obesity is induced by a moderate fat diet, there is development of hepatic insulin resistance either primary or secondary to the decrease in liver insulin clearance. Although decreased hepatic extraction of insulin may serve as a compensatory mechanism for insulin resistance, it is possible that a continued decrease in hepatic insulin extraction – which results in a chronic increase in insulin delivery to the periphery – may eventually in time induce widespread insulin resistance.

Although there has been many studies published examining putative changes in insulin secretion within the context of pathophysiological conditions such as obesity, insulin resistance or aging, there have been relatively few studies examining the changes in insulin extraction. The progression of impaired glucose tolerance to frank Type 2 diabetes has been characterized as the loss of β-cell function in the setting of insulin resistance due to the constant secretory demands placed upon the pancreas to overproduce insulin during chronic insulin resistance (22). Indeed it has been shown
that when elevated secretory demands on the pancreas are alleviated by improvement of insulin sensitivity with thiazolidinedione treatment in high-risk Hispanic women, there is an over 50% reduction in the rate of diabetes (3). Therefore it is of interest to examine whether decreased hepatic extraction might offer a potential therapeutic target to upregulate insulin availability, which may in turn preserve β-cell function, and perhaps delay or prevent the development of Type 2 diabetes.

In conclusion, we have shown that when dogs are fed an isocaloric diet with a moderate increase in fat, there is a significant accumulation of visceral adipose tissue without a change in body weight. Associated with the increase in adiposity, dogs developed fasting hyperinsulinemia and insulin resistance. The mechanism by which fasting hyperinsulinemia was maintained in face of chronic insulin resistance was initially due to an increase in insulin secretion which was not maintained and returned to pre-diet levels despite ongoing insulin resistance. However, despite the transient nature of increased insulin secretion, compensatory hyperinsulinemia is sustained due to a decrease in insulin extraction by the liver. Our results indicate that the liver plays a crucial role by decreasing its extraction of insulin, and we speculate that this reduction in insulin extraction may act as a second line of defense which accompanies β-cell rest while hyperinsulinemia is maintained in face of chronic insulin resistance.
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FIGURE LEGENDS

**Figure 1.** Relationships between insulin sensitivity versus (A) percent omental fat tissue and (B) percent subcutaneous fat tissue at weeks 0 (white circles), week 6 (black circles) and week 12 (white squares) of the diet.

**Figure 2.** Plasma insulin levels (shown on left) and dose-response relationship between insulin infusion rate and steady-state peripheral insulin concentration (shown on right) during the 3-step insulin euglycemic clamp for measurement of first-pass hepatic insulin extraction at (A-B) week 0, (C-D) week 6 and (E-F) week 12. The peripheral infusion protocol is depicted in black circles and the portal infusion protocol is depicted by white circles for both the time course and dose-response graphs.

**Figure 3.** (A) Plasma glucose levels during the stepwise hyperglycemic clamp at weeks 0 (white circles), 6 (black circles), and 12 (white squares). (B) Time course of plasma insulin levels (corrected for hepatic extraction) during the hyperglycemic clamp at weeks 0 (white circles), 6 (black circles) and 12 (white squares).

**Figure 4.** β-cell function represented as the integrated area under the curve of plasma insulin concentrations (corrected for hepatic extraction) during steady-state as a function of steady-state plasma glucose levels during the graded hyperglycemic clamp at week 0 (white circles), week 6 (black circles), and week 12 (white squares).
**Figure 5.** Week 0 (white circles), week 6 (black circles) and week 12 (white squares) - individual results for each animal are represented by the smaller symbols and the average of all animals for each respective week is represented by the larger corresponding symbol. (A) When the disposition index is calculated using insulin secretory response only, there appears to be a leftward shift (as shown by the arrow) at week 12, indicating an approximate 35% decrease. (B) When the disposition index is calculated using both insulin secretory response and hepatic insulin extraction, there is no significant change in the disposition index at either week 6 or week 12.
TABLE LEGENDS

**Table 1.** Body composition, basal metabolic parameters and factors involved in compensatory hypersinulinemia at weeks 0, 6, and 12 weeks of fat-feeding. *P<0.05, **P<0.005 vs. week 0, ANOVA.

**Table 2.** Components of the disposition index calculated with and without the contribution of hepatic insulin extraction to the overall insulin response at weeks 0, 6, and 12 of the increased fat diet. *P<0.05 vs week 0, ANOVA.
REFERENCES


41. **Weyer C, Bogardus C and Pratley RE.** Metabolic characteristics of individuals with impaired fasting glucose and/or impaired glucose tolerance. *Diabetes* 48: 2197-2203, 1999.

FIGURE 2

Beta-Cell Rest by Reduced Hepatic Insulin Extraction

Plasma Insulin (pM)

Time (min)

Infusion Rate (pmol/kg/min)
FIGURE 3

A) Plasma Glucose (mg/dL)

B) Insulin (pM)
FIGURE 4

Plasma Glucose (mg/dL) vs. Insulin (pM)
FIGURE 5

A) Disposition Index (Secretion Only)

B) Disposition Index (Secretion + Hepatic Extraction)
<table>
<thead>
<tr>
<th></th>
<th>WEEK 0</th>
<th>WEEK 6</th>
<th>WEEK 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total trunk fat (%)</td>
<td>26 ± 5</td>
<td>38 ± 5*</td>
<td>41 ± 7*</td>
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<tr>
<td>Omental fat (%)</td>
<td>11 ± 2</td>
<td>15 ± 2</td>
<td>16 ± 3*</td>
</tr>
<tr>
<td>Subcutaneous fat (%)</td>
<td>6 ± 1</td>
<td>11 ± 2</td>
<td>12 ± 3*</td>
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<tr>
<td>Body weight (kg)</td>
<td>27.5 ± 1.5</td>
<td>28.4 ± 1.6</td>
<td>29.0 ± 1.7</td>
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<tr>
<td>Fasting glucose (mg/dL)</td>
<td>96.4 ± 1.9</td>
<td>92.3 ± 2.8</td>
<td>94.0 ± 1.6</td>
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<tr>
<td>Fasting insulin (pM)</td>
<td>78 ± 12</td>
<td>115 ± 20*</td>
<td>126 ± 17**</td>
</tr>
<tr>
<td>$S_I (\times 10^{-4} \text{ dL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \text{pM}^{-1})$</td>
<td>3.9 ± 0.6</td>
<td>2.5 ± 0.3*</td>
<td>2.5 ± 0.4*</td>
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<tr>
<td>First-pass hepatic insulin extraction (%)</td>
<td>59.5 ± 3.1</td>
<td>55.6 ± 4.5</td>
<td>43.9 ± 3.9**</td>
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<td>Insulin secretion</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>$\beta$-cell function (pM per mg/dL)</td>
<td>12.4±4.1</td>
<td>25.6±5.7*</td>
<td>12.2±1.9</td>
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<tr>
<td>$AUC_T (\times 10^5 \text{ pM} \cdot \text{min})$</td>
<td>2.04 ± 0.66</td>
<td>2.98 ± 0.78*</td>
<td>1.74 ± 0.34</td>
</tr>
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</table>
## Table 2

<table>
<thead>
<tr>
<th></th>
<th>$S_I$ ($\times 10^{-4}$ dL/kg/min/pM)</th>
<th>Insulin AUC$_T$ ($\times 10^5$ pM·min)</th>
<th>DI (dL/kg)</th>
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<tr>
<td><strong>Secretion Only</strong></td>
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<tr>
<td>Week 0</td>
<td>3.9 ± 0.6</td>
<td>2.0 ± 0.7</td>
<td>63.6 ± 0.8</td>
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<tr>
<td>Week 6</td>
<td>2.5 ± 0.3*</td>
<td>3.0 ± 0.8*</td>
<td>64.3 ± 1.2</td>
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<tr>
<td>Week 12</td>
<td>2.5 ± 0.4*</td>
<td>1.7 ± 0.3</td>
<td>38.8 ± 0.6*</td>
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<tr>
<td><strong>Secretion + Hepatic Extraction</strong></td>
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<tr>
<td>Week 0</td>
<td>3.9 ± 0.6</td>
<td>0.8 ± 0.2</td>
<td>25.6 ± 0.4</td>
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<tr>
<td>Week 6</td>
<td>2.5 ± 0.3*</td>
<td>1.3 ± 0.4*</td>
<td>28.9 ± 0.7</td>
</tr>
<tr>
<td>Week 12</td>
<td>2.5 ± 0.4*</td>
<td>1.0 ± 0.2*</td>
<td>21.0 ± 0.2</td>
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