β-Cell “rest” accompanies reduced first-pass hepatic insulin extraction in the insulin-resistant, fat-fed canine model

Stella P. Kim, Martin Ellmerer, Erlinda L. Kirkman, and Richard N. Bergman

Department of Physiology and Biophysics, Keck School of Medicine of the University of Southern California, Los Angeles, California

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Kim SP, Ellmerer M, Kirkman EL, Bergman RN. β-Cell “rest” accompanies reduced first-pass hepatic insulin extraction in the insulin-resistant, fat-fed canine model. Am J Physiol Endocrinol Metab 292: E1581–E1589, 2007. First published February 6, 2007; doi:10.1152/ajpendo.00351.2006.—During insulin resistance, glucose homeostasis is maintained by an increase in plasma insulin via increased secretion and/or decreased first-pass hepatic insulin extraction. However, the relative importance of insulin secretion vs. 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 insulin secretion and first-pass hepatic insulin extraction during development of obesity and insulin resistance. Six dogs were fed an isocaloric diet with an ~8% increase in fat calories for 12 wk and evaluated at weeks 0, 6, and 12 for changes in 1) insulin sensitivity by euglycemic-hyperinsulinemic clamp, 2) first-pass hepatic insulin extraction by direct assessment, and 3) glucose-stimulated insulin secretory response by hyperglycemic clamp. We found that 12 wk of a fat diet increased subcutaneous and visceral fat as assessed by MR imaging. Consistent with increased body fat, the dogs exhibited a ~30% decrease in insulin sensitivity and fasting hyperinsulinemia. Although insulin secretion was substantially increased at week 6, β-cell sensitivity returned to prediet levels by week 12. However, peripheral hyperinsulinemia was maintained because of 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 are 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.

hyperinsulinemia; insulin resistance; obesity; insulin secretion; insulin clearance

OBESITY IS ASSOCIATED WITH HYPERINSULINEMIA and insulin resistance, both primary risk factors for type 2 diabetes. When the body is confronted with 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 the insulin-resistant state. It is often assumed that increased pancreatic islet cell secretion is the mechanism by which plasma insulin is increased (5, 31, 35). However, the liver can also contribute to hyperinsulinemia by decreasing extraction of insulin (6, 10, 16, 27). Increased secretion and decreased hepatic extraction can make significant contributions to hyperinsulinemia in the insulin-resistant state. Bonora et al. (2) reported hypersecretion of insulin and decreased hepatic insulin extraction in subjects with obesity or mild glucose intolerance, whereas in subjects with more severe impaired glucose intolerance, hyperinsulinemia was induced by decreased hepatic extraction alone. Glucose-intolerant subjects can demonstrate >50% islet cell dysfunction (41), possibly explaining the lack of β-cell compensation in the studies of Bonora et al. However, the importance of β-cell dysfunction vs. reduced liver insulin clearance to compensate 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. In the previous study, β-cell sensitivity to glucose and first-pass clearance of insulin by the liver were calculated from a model and were not directly measured. Given the potential importance of reduced clearance, which can relieve the pancreatic islets of 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 vs. clearance, which together allow maintenance of glucose tolerance within normal limits.

METHODS

Animals

Six male mongrel dogs (27.5 ± 1.5 kg body wt) were housed in the University of Southern California (USC) Keck School of Medicine Vivarium under controlled kennel conditions (12:12-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 of dry chow (36.6% carbohydrate, 26.4% protein, 14.7% fat, and 2.9% fiber; Wayne Dog Food, Alfred Mills, Chicago, IL). Thus the total diet consisted of 3,885 kcal/day: 37.9% from carbohydrate, 26.3% from protein, and 35.8% from fat. At 7–10 days before a period of weight stabilization, chronic catheters were surgically implanted: one was inserted into the jugular vein and advanced to the right atrium for sampling of mixed central venous blood, another was inserted into the femoral vein and advanced to the vena cava for insulin and somatostatin infusion, and another was inserted into 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

Address for reprint requests and other correspondence: R. N. Bergman, Dept. of Physiology and Biophysics, Keck School of Medicine of USC, 1333 San Pablo St. MMR 626, Los Angeles, CA 90033 (e-mail: rbergman@usc.edu).

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After 2–3 wk on the standard diet for weight stabilization (week 0), dogs were fed an isocaloric, increased-fat diet in which 32.9% carbohydrate, 22.9% protein, and 49.2% of total calories was replaced by cooked bacon grease (USC Keck School of Medicine cafeteria). This increased-fat diet consisted of one can of Hill’s Prescription Diet, 715 g of dry chow, and 2 g/kg prediet body weight of cooked bacon grease. This diet consisted of 3,950 kcal/day: 32.9% carbohydrate, 22.9% protein, and 44.3% fat. The animals were maintained on this diet for 12 wk.

**MR Imaging**

During weeks 0, 6, and 12 of the moderate-fat diet, MR imaging scans were performed on the animals (25). One-centimeter axial abdominal images (T1 slices; TR 500, TE 14) were obtained using a General Electric 1.5-T Horizon (version 5.7 software) magnet. The MR images were analyzed using Scion Image (Windows 2000 version Beta 4.0.2, Scion, Frederick, MD), which quantifies fat tissue (121–254 pixels) and other tissue (20–120 pixels) in each slice. Total fat was estimated as the integrated fat or tissue across the MR-imaged 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, with the slice at the level where the left renal artery branches from the abdominal aorta used 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 a Pavlov sling for ≥1 wk before the first experiment. Three protocols (I, II, and III) were performed three times on each animal: at weeks 0, 6, and 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). Before the beginning of each of the tests, animals were fasted for 12 h. At ~7 AM animals were brought to the laboratory and placed in the sling. At ~30 min before 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 ~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 0 min, a somatostatin infusion (1.0 μg·min⁻¹·kg⁻¹; Bachem California) was started to suppress endogenous insulin and glucagon secretion and was continued for the duration of the experiment. Porcine insulin (1.5 mU·min⁻¹·kg⁻¹; Novo Nordisk) was infused into the femoral vein to induce hyperinsulinemia. Glucose was clamped at basal by a variable glucose infusion. The experimental protocol was approved by the USC Institutional Animal Care and Use Committee.

**Diet**

During weeks 0, 6, and 12 of the moderate-fat diet, MR imaging scans were performed on the animals (25). One-centimeter axial abdominal images (T1 slices; TR 500, TE 14) were obtained using a General Electric 1.5-T Horizon (version 5.7 software) magnet. The MR images were analyzed using Scion Image (Windows 2000 version Beta 4.0.2, Scion, Frederick, MD), which quantifies fat tissue (121–254 pixels) and other tissue (20–120 pixels) in each slice. Total fat was estimated as the integrated fat or tissue across the MR-imaged 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, with the slice at the level where the left renal artery branches from the abdominal aorta used 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.

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**Protocol I: assessment of insulin sensitivity by euglycemic-hyperinsulinemic clamp.** Insulin sensitivity was calculated using the following equation (12)

\[
S_1 = \frac{\Delta G_{int}}{\Delta I \times G}
\]

where \(\Delta G_{int}\) 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.** Steady-state plasma insulin concentration was designated the average of the last 30 min of each dose period during the three-step insulin euglycemic clamp. Clearance of insulin from plasma (CI) can be calculated by dividing the insulin infusion rate by the plasma insulin concentration. With the assumption of linear insulin kinetics (11), CI 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
\[
Cl = \frac{1}{m} \quad (2)
\]

First-pass extraction (FPE) of insulin by the liver can be defined as the difference between the clearance rate measured after portal insulin administration (\(Cl_{po}\)) and the clearance rate measured after peripheral insulin administration (\(Cl_{pe}\)) (11), such that

\[
FPE = \frac{Cl_{po} - Cl_{pe}}{Cl_{po}} \quad (3)
\]

By combining Eqs. 2 and 3, first-pass hepatic insulin extraction can then be calculated as

\[
FPE = 1 - \frac{m}{m_{po}} \quad (4)
\]

Protocol III: assessment of \(\beta\)-cell function by graded hyperglycemic clamp. Steady state was designated the last 30 min of each glycemic clamp period during the graded hyperglycemic clamp. Plasma insulin concentration during steady state (ISS) 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 that would result from hyperglycemia. We wished to define an index of islet function that 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 Eq. 4) was defined as

\[
INS_{corr} = \frac{INS_p}{1 - FPE} \quad (5)
\]

where \(INS_p\) is the plasma insulin concentration measured in the peripheral circulation and FPE is fractional insulin extraction across the liver. Then islet \(\beta\)-cell function was quantified as the slope relating the average of the extraction-corrected plasma insulin concentration (\(INS_{corr}\)) to the average plasma glucose level during steady state for each glycemic phase of the experiment. Total AUC over basal (\(AUC_T\)) 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

\[
\text{sensitivity} \times \text{secretion} = \text{constant} \quad (6)
\]

The putative constant is termed the disposition index and reflects the ability of insulin secretory mechanisms to compensate for changes in insulin sensitivity. To compare the contribution of insulin secretion only and insulin secretion + hepatic insulin clearance to insulin resistance, this hyperbolic relation was calculated utilizing the sensitivity measurements (\(S_I\)) as described above and either \(AUC_T\) for \(INS_{corr}\) (insulin secretion only) or \(AUC_T\) for \(INS_T\) (insulin secretion + hepatic insulin clearance).

Statistical Analyses

Values are means ± SE. Statistical analyses were performed with paired Student’s \(t\)-tests or a two-way ANOVA as appropriate for comparisons of week 6 or week 12 with week 0. The \(t\)-tests were performed using Microsoft Excel 2002, and all ANOVAs were performed using Minitab for Windows software (Minitab, 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 weeks 6 and 12 without a significant change in body weight (25). Total trunk fat increased from 16 ± 3% at week 0 to 26 ± 4% at week 6 (\(P < 0.05\) vs. week 0, ANOVA), and this increase in total trunk adiposity was maintained to week 12, during which total trunk fat accounted for 29 ± 6% of total trunk tissue (\(P < 0.05\) vs. week 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 the study. The isocaloric diet with increased fat reduced insulin sensitivity by 32 ± 6% at week 6 (3.9 ± 0.6 and 2.5 ± 0.3 \(\times 10^{-4}\) dl·kg\(^{-1}\)·min\(^{-1}\)·pM\(^{-1}\) at weeks 0 and 6, respectively, \(P < 0.05\), ANOVA), and this decrement persisted for the duration of the study, such that, by week 12, insulin sensitivity had decreased by 36 ± 6% compared with week 0 (2.5 ± 0.4 \(\times 10^{-4}\) dl·kg\(^{-1}\)·min\(^{-1}\)·pM\(^{-1}\) at week 12, \(P < 0.05\), ANOVA). As described previously (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 week 0) at week 12 (\(P < 0.05\) vs. week 0 for both, ANOVA; Table 1).

Although there was a tendency for subcutaneous adiposity to be negatively correlated with insulin sensitivity (\(r = -0.46, P = 0.056\)), only omental fat was found to have a significant negative correlation with insulin sensitivity (\(r = -0.60, P = 0.009\)) during the 12-wk period (Fig. 1).

| Table 1. Body composition, basal metabolic parameters, and factors involved in compensatory hypersinsulinemia at weeks 0, 6, and 12 |
|-----------------|------------|------------|
|                 | Week 0     | Week 6     | Week 12    |
| Total trunk fat | 16±3       | 26±4*      | 29±6*      |
| Omental fat     | 11±2       | 15±2       | 16±3*      |
| Subcutaneous fat| 6±1        | 11±2       | 12±3*      |
| Body weight     | 27.5±1.5   | 28.4±1.6   | 29.0±1.7   |
| Fasting glucose | 96.4±1.9   | 92±2.8     | 94.0±1.6   |
| Fasting insulin | 78±12      | 115±20*    | 126±17*    |
| S_I \(\times 10^{-4}\) dl·kg\(^{-1}\)·min\(^{-1}\)·pM\(^{-1}\) | 3.9±0.6 | 2.5±0.3* | 2.5±0.4* |
| FPE, %          | 59.5±3.1   | 55.6±4.5   | 43.9±3.9*  |
| \(\beta\)-Cell function, pM·mg\(^{-1}\)·dl\(^{-1}\) | 12.4±4.1 | 25.6±5.7* | 12.2±1.9 |
| \(AUC_T\), \(10^4\) pM·min | 2.04±0.66 | 2.98±0.78* | 1.74±0.34 |

Values are means ± SE. \(S_i\), insulin sensitivity; FPE, 1st-pass hepatic insulin extraction; \(AUC_T\), total area under the curve. \(*P < 0.05\); †\(P < 0.005\) vs. week 0 (ANOVA).
Protocol II: First-Pass Hepatic Insulin Extraction

Glucose. Basal glucose was unchanged during the course of the diet (92.7 ± 1.8, 96.5 ± 2.3, and 92.5 ± 1.2 mg/dl at weeks 0, 6, and 12, respectively, P = not significant, ANOVA). Plasma glucose was clamped at basal in all experiments (mean coefficient of variation = 8.5%), and steady-state plasma glucose levels did not differ between infusion protocols, insulin doses, or times on diet (data not shown).

Dose response. The time course of plasma insulin during the peripheral and portal insulin infusion experiments for weeks 0, 6, and 12 is shown in Fig. 2, A, C, and E. Plasma insulin reached steady state within 45 min of each individual insulin dose period during both infusion protocols. The relation between insulin infusion rate and steady-state peripheral insulin concentration (Fig. 2, B, D, and F) was virtually linear for the portal and peripheral infusion experiments, allowing for simple and direct calculation of first-pass hepatic insulin extraction. First-pass hepatic insulin extraction was almost unchanged at week 6 compared with basal (59.5 ± 3.1% vs. 55.6 ± 4.5%, P = 0.45). This differed from the previous study (29), in which insulin clearance as calculated from the exponential rate of decline after insulin injection appeared to decrease beginning at week 6. However, by week 12, first-pass hepatic insulin extraction was substantially and significantly decreased to 43.9 ± 3.9% (P = 0.003 vs. week 0, ANOVA; Fig. 2F), reflecting an overall potential increase of 30% in the probability that an insulin molecule secreted at week 12 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 (Fig. 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, INScorr (Eq. 5), during the clamps at weeks 0, 6, and 12 are depicted in Fig. 3B. β-Cell function, defined as the slope of the line relating average INScorr to the average plasma glucose level during steady state, was 12.4 ± 4.1 pM·mg⁻¹·dl⁻¹ at week 0 (Fig. 4). By week 6, β-cell function per se more than doubled to 25.6 ± 5.7 pM·mg⁻¹·dl⁻¹ (P = 0.006 vs. week 0, ANOVA). This increase in β-cell function was not sustained, despite continued insulin resistance. By week 12, insulin response had renormalized to the prediet value (12.2 ± 1.9 pM·mg⁻¹·dl⁻¹, P = 0.97 vs. week 0, ANOVA). AUCₜ for extraction-corrected plasma insulin concentration throughout the entire experimental protocol (240 min) showed a similar pattern (Table 1). Thus, at week 12, β-cell function was renormalized, yet a compensatory hyperinsulinemia could 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 relation, which is reflected by a hyperbolic function (Fig. 5, Table 2) (23). When examining this hyperbolic relation using only insulin secretory function (i.e., AUCₜ for INScorr), we found that the decrease in insulin sensitivity was compensated by a ~60% increase in insulin secretion, such that the disposition index was unchanged at week 6 (63.6 ± 0.8 and 64.3 ± 1.2 dl/kg at weeks 0 and 6, respectively, P = not significant, ANOVA). Although insulin sensitivity was not further decreased from week 6, by week 12 the disposition index 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 AUCₜ for INSp, the disposition index remains unchanged throughout the study. Thus the consistency of the disposition index 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 of liver, skeletal muscle, and adipose tissue to insulin. This compensatory rise in the plasma concentration of insulin is essential for the maintenance of glucose homeostasis, despite chronic insulin resistance. Studies in obese individuals with insulin resistance have shown 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 in 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 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 fasting insulin. There was a substantial increase in insulin secretion at week 6. At week 12, sensitivity of the β-cells had returned to the basal level. However, there was a substantial reduction in first-pass hepatic insulin extraction, thus maintaining peripheral hyperinsulinemia, despite the transient nature of the increase in insulin release. This dynamic compensatory response to insulin resistance is best characterized by the changes in the insulin secretion-sensitivity relation or the disposition index. After 6

Fig. 2. Plasma insulin vs. insulin infusion rate (A, C, and E) and steady-state peripheral insulin concentration (B, D, and F) during the 3-step insulin euglycemic clamp for measurement of first-pass hepatic insulin extraction at week 0 (A and B), week 6 (C and D), and week 12 (E and F). ○, Peripheral infusion protocol; ○, portal infusion protocol.

AJP-Endocrinol Metab • VOL 292 • JUNE 2007 • www.ajpendo.org
Hyperinsulinemia was sustained, even when blood remained unchanged throughout the study. Compensatory insulin resistance was not totally compensated by increased secretion. However, by including the changes in hepatic insulin extraction are not fully understood. The initial step in insulin secretion has not previously been directly examined. Because of the longitudinal nature of the 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 at week 6.

The liver is known to extract a large percentage (~55%) of insulin secreted by the pancreas under normal conditions. Studies 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 the substantial increase in visceral fat depot and relative insulin resistance of this fat tissue to the antilipolytic actions of insulin are taken into account, it is possible that the liver was subjected to an increase of FFA due to increased portal flux. We 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 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 increased by ~50% in the liver samples from fat-fed dogs. These data are further evidence that the decrease 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 in liver-specific insulin receptor-knockout mice (13, 28) have provided evidence that 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 in obesity (26). Many animal models of obesity have shown increased β-cell mass, and it has been demonstrated that, in humans, β-cell mass is increased by ~50% in obese individuals compared with lean controls (4).

The contribution of decreased hepatic insulin extraction 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 Ca²⁺ channels, resulting in insulin secretion. It has also been shown that FFA can stimulate insulin secretion directly, although the complete mechanism by which this occurs is unknown (17). Although it has been suggested that glucose and FFA may play a role in the accumulation in the liver.

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Table 2. Components of disposition index calculated with and without contribution of hepatic insulin extraction to overall insulin response at weeks 0, 6, and 12

<table>
<thead>
<tr>
<th>Week</th>
<th>Stb $\times 10^4$ (dl/kg$^{-1}$min$^{-1}$pM$^{-1}$)</th>
<th>Insulin AUC$_T$, $\times 10^5$ pM$\cdot$min</th>
<th>DI, dl/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.9±0.6</td>
<td>2.0±0.7</td>
<td>63.6±0.8</td>
</tr>
<tr>
<td>6</td>
<td>2.5±0.3*</td>
<td>3.0±0.8*</td>
<td>64.3±1.2</td>
</tr>
<tr>
<td>12</td>
<td>2.5±0.4*</td>
<td>1.7±0.3</td>
<td>38.8±0.6*</td>
</tr>
</tbody>
</table>

**Secretion only**

<table>
<thead>
<tr>
<th>Week</th>
<th>Stb $\times 10^4$ (dl/kg$^{-1}$min$^{-1}$pM$^{-1}$)</th>
<th>Insulin AUC$_T$, $\times 10^5$ pM$\cdot$min</th>
<th>DI, dl/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.9±0.6</td>
<td>0.8±0.2</td>
<td>25.6±0.4</td>
</tr>
<tr>
<td>6</td>
<td>2.5±0.3*</td>
<td>1.3±0.4*</td>
<td>28.9±0.7</td>
</tr>
<tr>
<td>12</td>
<td>2.3±0.4*</td>
<td>1.0±0.2</td>
<td>21.0±0.2</td>
</tr>
</tbody>
</table>

**Secretion + hepatic extraction**

Values are means ± SE. DI, disposition index. *P < 0.05 vs. week 0 (ANOVA).

Fig. 5. A: disposition index calculated using insulin secretory response only. Note leftward shift (arrow) at week 12, indicating ~35% decrease. B: disposition index calculated using insulin secretory response and hepatic insulin extraction. Note no significant change in disposition index at week 6 or week 12. C. Week 6; ○. week 12. Individual results for each animal are represented by smaller symbols, and average of all animals for each respective week is represented by larger corresponding symbol.

The progression of impaired glucose tolerance to frank type 2 diabetes involves multiple stages, and the development of insulin resistance is a key feature. In the context of type 2 diabetes, the liver plays a critical role in the regulation of insulin metabolism. Increased hepatic triglyceride content results in a 50% decrease in liver insulin clearance, indicating the importance of hepatic insulin resistance in the development of hyperinsulinemia.

Recent studies have highlighted the role of the transmembrane glycoprotein CEACAM1 in the regulation of hepatic insulin clearance. CEACAM1 is involved in the internalization and degradation of insulin by insulin-degrading enzyme, which may be modulated by the insertion in the membrane or internalization of insulin receptor. Previous studies have shown that a change in insulin degradation due to a decrease in liver insulin clearance results in hyperinsulinemia, as well as development of visceral adiposity.

The insulin-degrading enzyme has also been shown to play an important role in the cellular metabolism of insulin. Increased portal FFA results in a greater degree of peripheral hypersinulinemia compared to hepatic insulin extraction, despite only a moderate degree of peripheral insulin resistance, when visceral adiposity was increased by fat feeding. These previous data, in combination with the findings presented here, suggest 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.

In dogs, specific insulin receptor-knockout mice have shown that when hepatic insulin resistance is genetically induced, development of severe hyperinsulinemia due to a decrease in liver insulin clearance and a large mass of β-cells may contribute to development of peripheral insulin resistance. In addition, the aforementioned study in phosphorylation-defective CEACAM1-mutant mice showed that a change in insulin clearance results in hyperinsulinemia without a change in insulin secretion and the subsequent development of hepatic insulin resistance. Also, in dogs, 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 development of hepatic insulin resistance primary or secondary to the decrease in liver insulin clearance when obesity is induced by a moderate-fat diet. 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 induce widespread insulin resistance.

Although many studies have examined putative changes in insulin secretion within the context of pathophysiological conditions such as obesity, insulin resistance, or aging, relatively few studies have examined the changes in insulin extraction. The progression of impaired glucose tolerance to frank type 2 diabetes likely involves a combination of decreased insulin secretion and increased insulin resistance in peripheral tissues.
diabetes has been characterized as the loss of β-cell function in the setting of insulin resistance due to the constant secretory demands on 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, the rate of diabetes is reduced >50% (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 the face of chronic insulin resistance was initially due to an increase in insulin secretion that was not maintained and predict 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 that accompanies β-cell rest while hyperinsulinemia is maintained in the face of chronic insulin resistance.

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GRANTS

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