Decreased visceral adiposity accounts for leptin effect on hepatic but not peripheral insulin action

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Barzilai, Nir, Li She, Lisen Liu, Jiali Wang, Meizu Hu, Patricia Vuguin, and Luciano Rossetti. Decreased visceral adiposity accounts for leptin effect on hepatic but not peripheral insulin action. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E291–E298, 1999.—Leptin decreases visceral fat (VF) and increases peripheral and hepatic insulin action. Here, we generated similar decreases in VF using leptin (Lep), β3-adrenergoreceptor agonism (β3), or food restriction (FR) and asked whether insulin action would be equally improved. For 8 days before the in vivo study, Sprague-Dawley rats (n = 24) were either fed ad libitum [control (Con)], treated with Lep or β3 (CL-316,243) by implanted osmotic mini-pumps, or treated with FR. Total VF was similarly decreased in the latter three groups (Lep, 3.11 ± 0.35 g; β3, 2.87 ± 0.48 g; and FR, 3.54 ± 0.77 g compared with 6.91 ± 1.41 g in Con; P < 0.001) independent of total fat mass (by 12.5%) and food intake. Insulin (3 mU·kg−1·min−1) clamp studies were performed to assess hepatic and peripheral insulin sensitivity. Decreased VF resulted in similar and marked improvements in insulin action on glucose production (GP) (Lep, 1.19 ± 0.51; β3, 1.46 ± 0.68; FR, 2.27 ± 0.71 compared with 6.06 ± 0.70 mg·kg−1·min−1 in Con; P < 0.001). By contrast, reduction in VF by β3 and FR failed to reproduce the stimulation of insulin-mediated glucose uptake (~60%), glycogen synthesis (~80%), and glycolysis (~25%) observed with Lep. We conclude that 1) a moderate decrease in VF uniformly leads to a marked increase in hepatic insulin action, but 2) the effects of leptin on peripheral insulin action are not due to the associated changes in VF or β3 activation.

A major role of a centripetal distribution of adiposity in the pathophysiology of insulin resistance has been suggested by numerous epidemiological studies (9, 25). However, the covariance of central adiposity and insulin resistance may also be due to tightly associated hormonal or metabolic parameters. We have recently reported that the administration of the “anorectic” fat-derived hormone, leptin, to moderately obese rats leads to a selective decrease in intra-abdominal adiposity and to marked improvements in both hepatic and peripheral insulin action (7). This suggests that some of the chronic metabolic effects of leptin might be secondary to the decrease in visceral fat (VF).

Leptin suppresses appetite and augments energy expenditure mainly via its interaction with hypothalamic receptors (41, 42), and it has been postulated that some of the downstream effects of leptin are mediated by the β3-adrenergoreceptor system (17). A mutation in this receptor is associated with insulin resistance (44), morbid obesity (10), and increased visceral adiposity (40). Administration of β3-adrenergoreceptor agonists increases thermogenesis through their action on uncoupling protein 1. Although this action occurs mainly in brown fat, β3-adrenergoreceptors are present in a variety of white fat tissue in humans (26). Because the administration of selective β3-adrenergoreceptor agonists to rodents affects visceral more than subcutaneous fat (20), it is possible that the selective effect of leptin on VF is due, in part, to the activation of this neuronal pathway (12). Furthermore, important metabolic actions of leptin on energy expenditure, substrate partitioning, insulin action, and storage of body fat have also emerged (19, 32, 34).

In fact, whereas chronic administration of leptin improves insulin action in animal models (7, 19, 34), recent studies have also shown acute modulation of insulin action by leptin in vivo (22, 39, 43) and in vitro (11). Thus insulin action may be improved before and independently of the leptin-induced decrease in VF.

To delineate the contribution of the leptin-induced changes in VF to the potent effects of leptin on in vivo insulin action, in the current study we generated similar decreases in VF by alternative means and compared their impact on hepatic and peripheral insulin action. We utilized the β3-adrenergoreceptor agonist CL-316,243, which caused decreased VF (by ~60%) with no changes in food intake and modest decline in total fat mass (~10%) (20), and caloric restriction designed to achieve a similar decrease in VF.

We hypothesize that if decreased VF is solely responsible for the leptin-induced improvement in insulin action, the latter will be independent of the modality by which VF is decreased. Alternatively, leptin may play a direct role in the modulation of peripheral or hepatic insulin action.

MATERIALS AND METHODS

Experimental animals. Four groups of male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) received the following treatment by osmotic minipumps for 8 days: 1) Con (n = 6), saline; 2) Lep (n = 6), recombinant mouse leptin at the rate of ~0.5 mg·kg−1·day−1 (Amgen, Thousand Oaks, CA; >95% pure by SDS-PAGE); 3) β3 (n = 6), a β3-adrenergoreceptor agonist, at the rate of ~0.1 mg·kg−1·day−1 (CL-316,243 provided by Wyeth-Ayerst Research); and 4) FR (n = 6), saline and food restriction at the physiological level of 17 kcal/day. Data obtained from four of the six Lep rats were included in a previous publication (7) and are reported here solely to facilitate comparison with β3 and FR. These rats were selected on the basis of their VF to...
match that obtained with the alternative interventions. Food intake and body weights were measured every 24 h during the 8-day infusion period. Rats were housed in individual cages and subjected to a standard light (6 AM to 6 PM)-dark (6 PM to 6 AM) cycle. Eight days before the in vivo study, rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg body wt), the osmotic minipumps were placed in the subcutaneous interscapular area, and indwelling catheters were inserted in the right internal jugular vein and in the left carotid artery (4, 5, 7, 38, 39). The venous catheter was extended to the level of the right atrium, and the arterial catheter was advanced to the level of the aortic arch.

Body composition. Body composition was assessed as in Refs. 3, 5, and 7. Briefly, rats received an intra-arterial bolus injection of 20 µCi of tritiated-labeled water (3H2O; New England Nuclear, Boston, MA), and plasma samples were obtained at 30-min intervals for 3 h. Steady-state conditions for plasma 3H2O specific activity were achieved within 45 min in all studies. Five plasma samples obtained between 1 and 3 h were used in the calculation of the whole body distribution space of water. VF (i.e., epididymal, perinephric, and mesenteric fat depots) was dissected and weighed at the end of each experiment.

Measurements of in vivo glucose kinetics. Measurements were performed as in Refs. 7 and 39. Briefly, a primed-continuous infusion of HPLC-purified [3-3H]glucose (New England Nuclear; 40 µCi bolus, 0.4 µCi/min) was administered for the duration of the study. Two hours after the basal period, a primed-continuous infusion of somatostatin (1.5 µg·kg\(^{-1}\)·min\(^{-1}\)) and regular insulin (3 µU·kg\(^{-1}\)·min\(^{-1}\)) were administered, and a variable infusion of a 25% glucose solution was started at time 0 and periodically adjusted to clamp the plasma glucose concentration at ~7.5 mM for the rest of the studies. Samples for determination of [3H]glucose specific activity were obtained every 10 min, and plasma samples for determination of plasma insulin, glycerol, and free fatty acid (FFA) concentrations were obtained every 30 min during the study. At the end of the infusions, rats were anesthetized (pentobarbital, 60 mg/kg iv), the abdomen was quickly opened, portal vein blood was obtained, and muscle and liver were freeze-clamped in situ with aluminum tongs precooled in liquid nitrogen.

Rates of glycolysis and glycogen synthesis were estimated as in Refs. 7 and 37. Rates of hepatic glucose fluxes were determined as in Refs. 36, 38, and 29. Gene expression of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G-6-Pase) by RT-PCR were determined as in Ref. 29.

Analytic procedures. Plasma glucose was measured by the glucose oxidase method (GlucoseAnalyzer II, Beckman Instruments, Palo Alto, CA). Plasma corticosterone and insulin (with rat and porcine insulin standards) were measured by radioimmunoassay. Plasma glucagon and leptin (RIA kit, Linco Research, St. Charles, MO) concentrations were measured by radioimmunoassay. The plasma concentration of FFA was determined by an automated kit according to the manufacturer’s specifications (Waco Pure Chemical Industries, Osaka, Japan). Plasma [3H]glucose radioactivity was measured in duplicates in the supernatants of Ba(OH)\(_2\) and ZnSO\(_4\) precipitates of plasma samples (20 µl) after evaporation to dryness to eliminate tritiated water. UDP-glucose and PEP concentrations and specific activities in the liver were obtained through two sequential chromatographic separations, as previously reported (7, 36, 38).

RESULTS

Caloric intake, body weight, and fat distribution. Because our design required matching VF by various experimental means, rats had to be preselected for assignment to each study group according to their body weights. Marked decreases in body weight were anticipated after 8 days of Lep and FR; thus rats were weighed before initiation of treatment. Con and β3 rats weighed 303 ± 19 and 288 ± 18 g, whereas Lep and FR rats weighed 351 ± 3 and 338 ± 6 g. As expected, administration of exogenous leptin decreased food intake by ~50%, and administration of CL-316,243 (β3) resulted in similar food intake as Con (Table 1). Because we had previously shown that pair-feeding to Lep was not sufficient to reproduce the effect of Lep on total abdominal fat (7), in this study FR rats received approximately one-half the caloric consumption of Lep. After these protocols, similar body weight and lean body mass (LBW) were achieved in all groups (Table 1, Fig. 1A), and epididymal, perinephric, and mesenteric fat depots were similarly decreased by all interventions (Table 1, Fig. 1C). Thus the remaining differences in body composition among the groups were due to variations in the amount of total fat mass (Fig. 1B). However, the latter was significantly lower in Lep (34 ± 8 g), β3 (28 ± 9 g), and FR (17 ± 8 g) compared with Con (54 ± 4 g).

Decreasing VF per se markedly enhances hepatic insulin sensitivity. Plasma leptin levels were markedly increased in Lep (39 ± 8 ng/ml) compared with β3, FR, and Con (2 ± 1, 3 ± 1, and 4 ± 1 ng/ml, respectively). During the insulin clamp studies, the plasma glucagon (116 ± 11, 96 ± 18, 125 ± 12, and 102 ± 9 pg/ml) and corticosterone (154 ± 22, 126 ± 28, 168 ± 21, and 186 ± 25 ng/ml in Con, Lep, β3, and FR, respectively) concentrations were similar in all groups. Table 2 displays the basal biochemical parameters in all experimental groups. Postabsorptive (6 h of fasting) plasma glucose concentrations were similar in all groups. However, plasma insulin levels were markedly decreased by interventions and were significantly lower in Lep compared with all other groups. Basal plasma FFA and glycerol levels were similar at basal in all groups. At basal, glucose production (GP, Fig. 2A) was similar in all groups (11.2 ± 0.9, 12.2 ± 1.0, 11.5 ± 0.9, and 12.4 ± 1.3 mg·kg\(^{-1}\)·min\(^{-1}\) in Con, Lep, β3, and FR, respectively). During the insulin clamp, plasma insulin levels

Table 1. Caloric intake and body composition

<table>
<thead>
<tr>
<th></th>
<th>Con (n = 6)</th>
<th>Lep (n = 6)</th>
<th>β3 (n = 6)</th>
<th>FR (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories, kcal/day</td>
<td>69 ± 4</td>
<td>29 ± 3</td>
<td>61 ± 4</td>
<td>17 ± 0*</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>322 ± 15</td>
<td>315 ± 9</td>
<td>290 ± 13</td>
<td>291 ± 16</td>
</tr>
<tr>
<td>Epi, g</td>
<td>3.50 ± 0.54</td>
<td>1.98 ± 0.18</td>
<td>1.90 ± 0.21</td>
<td>1.94 ± 0.23</td>
</tr>
<tr>
<td>Peri, g</td>
<td>1.22 ± 0.27</td>
<td>0.49 ± 0.07</td>
<td>0.59 ± 0.06</td>
<td>0.69 ± 0.16</td>
</tr>
<tr>
<td>Mese, g</td>
<td>2.12 ± 0.39</td>
<td>0.74 ± 0.23</td>
<td>0.66 ± 0.09</td>
<td>0.71 ± 0.09</td>
</tr>
</tbody>
</table>

Values are means ± SE. Caloric intake, body weight (BW), epididymal (Epi), perinephric (Peri), and mesenteric (Mese) fat pads obtained after the study from control rats (Con) and from rats treated for 8 days with leptin (Lep), β3-adrenoceptor agonist (β3), or caloric restriction (FR). *P < 0.01 vs. all other groups.
Effect of decreasing VF with Lep, β3, or FR on gluconeogenesis and glycogenolysis. The direct contribution of plasma glucose to the hepatic glucose 6-phosphate (G-6-P) pool was calculated from the specific activities of UDP-glucose and plasma glucose (Table 3), and it was similar in all groups. Thus decreased VF resulted in similar decreases (to <30% of Con) in the rates of GP, flux through G-6-Pase or total glucose output (TGO), and glucose cycling (GC) in response to physiological hyperinsulinemia. Lep and FR increased the percentage of hepatic G-6-P pool that is derived from PEP-gluconeogenesis (GN), but the rate of GN was similar in all intervention groups (Table 4). In a net sense, the major contribution to the decreased GP in all intervention groups was due to a marked decrease in glycogenolysis (to <20% of Con).

Effect of decreasing VF with Lep, β3, or FR on PEPCK and G-6-Pase gene expression. Multiple densitometric scanning of PCR products (examples shown in Fig. 3A) shows that when the hepatic G-6-Pase and PEPCK mRNA levels were compared with those of Con, they were increased by approximately twofold in Lep and more modestly in FR, but not in β3.

Leptin, but not decreased VF per se, augments insulin action on glucose uptake, glycogen synthesis, and glycolysis. During the insulin clamp studies, glucose uptake (Rg, Fig. 4A) was increased by 63% (P < 0.001) in Lep (17.5 ± 1.1, 28.6 ± 1.3, 19.2 ± 1.3, and 20.7 ± 1.3 mg·kg⁻¹·min⁻¹ in Con, Lep, β3, and FR; respectively). This improvement in peripheral insulin action was accounted for by a twofold increase in the rate of glycogen synthesis (Fig. 4B; 5.6 ± 0.9, 11.6 ± 1.2, 8.7 ± 1.3, and 9.7 ± 1.1 mg·kg⁻¹·min⁻¹ in Con, Lep, β3, and FR; P < 0.001) and by a 26% increase in glycolysis (11.9 ± 1.6, 15.0 ± 1.2, 10.0 ± 1.2, and 11.2 ± 0.3 mg·kg⁻¹·min⁻¹ in Con, Lep, β3, and FR; P < 0.01).

DISCUSSION

In this study we attempted to delineate whether the potent effects of leptin on in vivo insulin action are secondary to the associated changes in body composition. Decreasing VF led to a striking improvement in hepatic insulin sensitivity that was independent of increased to similar levels, and plasma FFA and glycerol levels decreased similarly in all groups (Table 2). Although GP (Fig. 2B) was decreased from basal in all groups, it was about threefold lower (and similar) in all intervention groups (6.1 ± 0.7, 1.2 ± 0.5, 1.5 ± 0.7, and 2.3 ± 0.7 mg·kg⁻¹·min⁻¹ in Con, Lep, β3, and FR, respectively).
time frame of the present study, the effects of leptin on the latter finding indicates that, at least within the reproducing by decreasing VF by alternative means. In line with these observations, the marked stimulation of insulin-mediated glucose uptake, glycolysis, and glycogen synthesis induced by leptin treatment could not be mediated via decreased VF and/or activation of the \( \beta_3 \)-adrenoreceptor system. Furthermore, rapid changes in VF modulate hepatic much more than peripheral insulin action. "Manipulating" body composition. It is well established that weight loss is commonly associated with decreased plasma insulin concentrations and increased insulin sensitivity (8, 13, 15, 16, 27). Early studies in obese mice reported marked improvements in glucose tolerance after leptin treatment (19, 34). However, whereas some reports suggested that the improvement in glucose tolerance may precede the decline in body weight and total fat mass (34, 41), it has been difficult to discern the relative contribution of the associated changes in body composition to the improved glucose tolerance observed with leptin treatment (19, 34). Furthermore, although pair-feeding vehicle-treated rats to the level of leptin-treated rats resulted in similar decreases in body weight and FM, leptin caused a selective and marked decrease in visceral adiposity (7). The latter observation further complicates the interpretation of potential effects of leptin treatment on in vivo insulin action. Administration of \( \beta_3 \)-adrenoreceptor agonists causes marked decreases in circulating leptin concentrations (18, 28, 30); however, consistent with previous reports (20), in the present study food intake was not decreased compared with Con (Table 1). Despite similar caloric total fat mass (FM) and of the modalities used to achieve the "target" VF. This observation provides support for a cause-effect relationship between intra-abdominal deposition of fat and hepatic insulin resistance. Conversely, the marked stimulation of insulin-mediated glucose uptake, glycolysis, and glycogen synthesis induced by leptin treatment could not be reproduced by decreasing VF by alternative means. The latter finding indicates that, at least within the time frame of the present study, the effects of leptin on peripheral insulin action are not likely to be solely mediated via decreased VF and/or activation of the \( \beta_3 \)-adrenoreceptor system. Furthermore, rapid changes in VF modulate hepatic much more than peripheral insulin action.

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intake, \( \beta_3 \) rats gained less weight and their FM was significantly lower than Con rats. This may be due to increased energy expenditure and thermogenesis in this group (20).

To generate similar VF with FR it was necessary to further decrease the caloric intake by \( \sim 50\% \); this intervention resulted in much lower FM than in the other groups. This finding is a dramatic confirmation of the selective effects of Lep and \( \beta_3 \) on intra-abdominal adiposity. This model is also different from the administration of leptin and \( \beta_3 \)-adrenoreceptor agonists, because energy expenditure is expected to be markedly decreased. Thus similar declines and final mass of VF were obtained in the three intervention groups despite differences in food consumption, weight gain, energy expenditure, food intake, and whole body adiposity.

VF and hepatic insulin sensitivity. All interventions that decreased VF resulted in similar fasting plasma glucose levels despite lower plasma insulin levels compared with Con rats, suggesting an improvement in postabsorptive hepatic insulin sensitivity. To directly test whether hepatic insulin sensitivity was improved by decreasing VF, we performed low-dose insulin clamp studies in combination with somatostatin infusions. The plasma glucose, FFA, glycerol, and insulin concentrations during the clamp studies were similar in all groups (Table 2). This procedure also erased the portal-venous insulin gradient, matching peripheral and hepatic insulin levels in all groups. Decreasing VF resulted in a marked decrease in GP during the insulin clamp, indicating heightened hepatic insulin sensitivity (Fig. 2B). This improvement in insulin action was independent of the modalities by which decreased VF was achieved, and it is consistent with other animal models, such as the calorie-restricted “old” rats (2) and rats with surgical removal of VF (6). Although the mechanism(s) wherebyVF regulates insulin sensitivity remain to be delineated, it is evident that the impacts of changes in VF on hepatic glucose fluxes are remarkable. It has been suggested that the unique metabolic characteristics of the intra-abdominal fat depots concern the turnover of glycerol, FFA, and lactate play a role through a “portal effect” (9), i.e., the hepatic load of FFA, lactate, and glycerol can modulate liver glucose metabolism (31, 35). However, it should be noted that, in this experimental model, the peripheral concentrations of these substrates were unchanged during the basal and insulin clamp periods. Although potential effects of long-term differences in plasma FFA, lactate, or glycerol levels on hepatic enzymes cannot be excluded, alternative hypotheses should also be considered for the “cross-talk” between intra-abdominal fat depots and the liver. For example, a fat-derived and secreted peptide, tumor necrosis factor-\( \alpha \) (TNF-\( \alpha \)), causes peripheral and hepatic insulin resistance via its antagonism of early insulin signaling (14, 21).

Consistent with the lower GP, the rates of TGO and GC were also markedly decreased in parallel with the changes in VF. This suggests a marked decrease in the in vivo flux through G-6-Pase. In a net sense, the decreased GP in the intervention groups was mainly the result of a marked suppression of hepatic glycogenolysis, which was most pronounced in the group treated with leptin (Table 2). Overall, whereas hepatic insulin sensitivity improved similarly with all interventions designed to decrease VF, there were some changes in the intrahepatic distribution of hepatic glucose flux and in the gene expression of key hepatic enzymes that appear to be treatment specific. For example, the percent contribution of GN to TGO was increased by Lep — FR — \( \beta_3 \) (Fig. 2C). This is supported by the increased expression of hepatic PEPCK in Lep and FR. Although leptin has similar effects on PEPCK mRNA when administered acutely via a peripheral vein (39) or in a cerebral ventricle (29), it should be pointed out that the decline in plasma insulin concentrations might also contribute to the upregulation of this gene. By contrast, it is noteworthy that acute and chronic stimulation of the \( \beta_3 \)-adrenergic systems has frequently divergent effects. We have previously shown that the acute (6-h) administration of the same \( \beta_3 \)-adrenoreceptor agonist increased the gene expression of G-6-Pase and PEPCK, perhaps via activation of hypothalamic efferent pathway(s) (29). The waning of this effect after more prolonged exposure to the agonist may be due to either the associated marked decline in leptin levels and/or central or peripheral downregulation of the \( \beta_3 \)-adrenergoreceptor system. This also suggests that the stimulation of the \( \beta_3 \)-adrenoreceptor system is not likely to mediate...
the effects of chronic leptin administration on hepatic gene expression.

Unique effects of leptin on peripheral insulin sensitivity. A major effect of insulin in vivo is to stimulate the disposal of glucose into peripheral tissues (mostly in skeletal muscle). During physiological hyperinsulinemia, the rate of tissue glucose uptake in Lep was improved by >60%, whereas only a mild increase in peripheral glucose uptake was noted in the other intervention groups. This improvement in peripheral insulin action was accounted for by about a twofold increase in the rate of glycogen synthesis and by an ~25% increase in the rate of whole body glycolysis. On the basis of epidemiological evidence correlating insulin resistance and hyperinsulinemia with intra-abdominal adiposity (9, 25), it has been suggested that decreasing VF should lead to a marked improvement in the action of insulin on peripheral glucose disposal. Indeed, modest increases in the rates of insulin-mediated glucose uptake and glycogen synthesis were detected when VF was markedly decreased using caloric restriction or β3-adrenergic agonism. However, this improvement could only account for a small fraction (up to 30%) of the effects of leptin on glucose uptake. Thus 8-day leptin administration exerts potent effects on peripheral insulin action, which are largely independent of the associated decrease in VF. Several mechanism(s) may be invoked to account for the enhanced muscle insulin sensitivity in rats treated with leptin. Leptin has been shown to increase skeletal muscle glucose uptake quite rapidly in some rodent studies (22, 43), and activation of early insulin signaling by leptin has been demonstrated in a muscle cell line (23) and in a preliminary report in rats (24). However, acute exposure of skeletal muscle and adipose cells to leptin, with and without insulin, failed to alter the glucose transport system in some studies (46). Thus leptin may augment muscle insulin signaling via a direct action on local receptors or via hypothalamic efferent pathways. An additional explanation may be found in the "lipopenic" effects of leptin (32) and in the close correlation between intramyocellular lipid levels and insulin sensitivity (33). In fact, leptin enhances lipid oxidation and depletes triglyceride stores in preadipocytes, pancreatic β-cells, and muscle (1, 32, 45). The latter effects may be mediated in part via decreased gene expression of acetyl-CoA carboxylase.

Taken together with the hepatic actions of leptin, the above data suggest that a prolonged elevation in circulating leptin favors the storage of energy into glycogen rather than into lipid stores. The latter metabolic adaptation may represent a response to signals generated by leptin in the hypothalamic "lipostat" and/or the results of peripheral actions of the hormone.

In conclusion, decreasing intra-abdominal adiposity by ~60% via three different means results in a dramatic increase in hepatic insulin sensitivity. Conversely, the potent effect of leptin administration on peripheral insulin action cannot be solely explained on the basis of the associated decrease in VF mass. Understanding the biochemical mechanism(s) that are responsible for the specific action of leptin on skeletal muscle glucose disposal should help to clarify the link between nutrient excess, weight gain, and insulin resistance.

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