Short-term, high-fat diets lower circulating leptin concentrations in rats

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ABSTRACT

Background: Leptin is produced in proportion to body fat mass and can act on the brain to induce satiety and regulate adipose tissue mass; factors other than adipose tissue mass may influence circulating leptin concentrations.

Objective: We explored the possibility that short-term, moderately high-fat diets induce weight gain by producing inappropriately low circulating leptin concentrations.

Design: Female Hooded Wistar rats were fed either a moderately high-fat diet or control diet. Body weight, energy intake, body composition, and fasting plasma leptin were compared after 4 and 14 wk of dietary treatment.

Results: After 4 wk, abdominal fat mass was 38% greater in rats fed the high-fat diet than in those fed the control diet (P < 0.01). However, plasma leptin concentrations were 24% lower in animals fed the high-fat diet (P < 0.05), resulting in significantly lower plasma leptin concentrations per unit abdominal fat mass than in control animals (P < 0.005). From 4 to 14 wk, animals fed the high-fat diet gained twice as much weight and consumed 32 kJ/d more than controls (both P < 0.05). At 14 wk, plasma leptin concentrations per unit abdominal fat mass were 27% lower in rats fed the high-fat diet (P = 0.058) and there was a significant negative association between leptin concentrations per unit abdominal fat mass and body weight (r = 0.44, P < 0.05).

Conclusions: In the short term, a moderately high-fat diet is associated with lower than expected circulating leptin concentrations, which correlate with a higher body weight. A high-fat diet may therefore contribute to weight gain by reducing leptin secretion in adipose tissue.

KEY WORDS Energy intake, satiety, leptin, body weight, high-fat diet, adipose tissue, rats

INTRODUCTION

Long-term, high-fat diets can induce overconsumption and weight gain; however, the mechanism by which this occurs is unknown (1). Leptin is a circulating protein produced in proportion to adipose tissue mass (2) that can act on the brain to increase satiety (3). Therefore, a persistent reduction in either the secretion or action of leptin may cause weight gain by sending an inappropriate signal to the brain, resulting in a reduced satiety response. Mice with well-established diet-induced obesity have hyperleptinemia (4), yet are hyperphagic (5) and expend less energy (6), suggesting that dietary fat may cause weight gain by limiting the action of leptin. However, leptin insensitivity only appears in diet-induced obese AKR mice once body fat increases to 25% above normal, suggesting that it is a secondary phenomenon (7). It is also unclear whether central leptin insensitivity is associated with diet-induced obesity; some studies show impaired suppression of food intake after intracerebroventricular administration of leptin (8), whereas others have found a normal response to intracerebroventricular leptin (7, 9). This paper explores the alternative possibility that early gain in fat mass induced by a high-fat diet may be due to low leptin secretion by adipose tissue.

There are factors other than total-body fat mass that can influence circulating leptin concentrations. For example, a paper published recently in this Journal showed that food restriction in humans causes an adiposity-independent decrease in plasma leptin concentration (10). Jenkins et al (11) showed that reduced carbohydrate intake, but not reduced fat intake, was associated with a pronounced fall in serum leptin concentration over a 4-wk period in obese human subjects. Therefore, dietary manipulations appear to influence leptin secretion through mechanisms other than just by altering fat mass. The aims of this study were to investigate whether a short-term, moderately high-fat diet induces overeating and weight gain, and whether these changes are associated with an inappropriately low secretion of leptin by adipose tissue.

MATERIALS AND METHODS

Animals

Female Hooded Wistar rats aged between 20 and 22 wk were purchased from Monash University Animal Facility (Melbourne). Animals were housed individually with a 12-h light-dark cycle maintained by artificial lighting (lights on at 0600) and a constant...
room temperature of 20–22°C. These experiments were approved by the Royal Melbourne Hospital Animal Ethics Committee.

**Experimental protocol**

Animals were divided into 2 weight-matched groups (227 ± 4 and 229 ± 4 g) and were given either a moderately high-fat diet ($n = 19$) or a low-fat control diet ($n = 22$). Animals fed the moderately high-fat diet consumed 10 mL of a fat emulsion (Intralipid; Kabi Pharmacia, AB, Stockholm) containing 20% triacylglycerol, 1.2% phospholipid, and 2.25% glycerol daily and had unlimited access to a standard nonpurified laboratory diet (Barastock Products, Pakenham, Australia). This resulted in a diet consisting of ≈36% of energy as fat, 51% of energy as carbohydrate, and 13% of energy as protein. The fat emulsion was provided at 0900 each day in a dish and was always readily consumed within 2 h. It consists primarily of purified soybean oil, which is composed mostly of linoleic acid (52%) and oleic acid (30%). The diet of the control group consisted of the nonpurified diet provided ad libitum (3% fat, 77% carbohydrate, and 20% protein). Groups of animals ($n = 12$ for the high-fat diet and $n = 14$ for the control diet) were maintained with the diets for 4 wk, whereas the remaining 7 animals in each group continued to consume the diets for an additional 10 wk.

**Body weight and composition measurements**

Four weeks and 14 wk after the start of the study, body weights and food intake were measured at 0900. The estrus cycle of female rats is known to cause fluctuations in body weight and food intake (12) so daily recordings were averaged over a 4-d period. At the end of the dietary period, animals were fasted overnight, then anesthetized by using intraperitoneal pentobarbital (60 mg/kg body wt, Nembutal; Boehringer Ingelheim, Sydney, Australia) and kept warm with a heating lamp. Thirty minutes later, blood was taken by heart puncture and tissues were collected. All visible white adipose tissue (WAT) from the left side of the body was collected and weighed. This WAT weight was doubled to give an estimate of abdominal WAT mass. Single depot infrarenal and subcutaneous WAT and total intrascapular brown adipose tissue were also weighed.

**Blood analyses**

Blood collected by heart puncture was collected in EDTA-coated tubes and centrifuged at 1200 × g and 4°C for 10 min. Plasma leptin was analyzed by using a rat radioimmunoassay (Linco Research, St Charles, MO) as described previously (13). Plasma insulin was measured by a double-antibody radioimmunoassay (Pharmacia, Uppsala, Sweden). The glucose oxidase method was used to determine plasma glucose with a glucose analyzer (Yellow Springs Instrument Company, Yellow Springs, OH).

**Statistical analysis**

Data are expressed as means ± SEMs. Analysis of variance with 2 factors, diet and time, was used for comparisons and post hoc least-significant-difference tests were used to compare individual means (SPSS for WINDOWS 8.0; SPSS Inc, Chicago). Two-tailed Student’s $t$ tests were used to compare variables when a significant interaction was detected between diet and time. Linear regression analysis was performed by using Microsoft EXCEL for WINDOWS 95 (version 7.0; Microsoft Corp, Redmond, WA). The slopes and elevations of regression lines were compared by a method that involves the use of a Student’s $t$ test in a fashion analogous to that of testing for 2 differences between 2 population means (14).

**RESULTS**

The responses of animals to 4 wk of dietary intervention are shown in Table 1 ($n = 7–19$ for the high-fat diet and $n = 7–22$ for the control diet). Although dietary fat intake was 82 kJ/d higher in the rats fed the high-fat diet ($P < 0.001$), body weight and total energy intake did not differ after 4 wk from that of the animals fed the control diet. Total energy intake was maintained in the fat emulsion–fed rats because they consumed less nonpurified diet. This resulted in a decrease in dietary carbohydrate and protein intakes (by 45 and 11 kJ/d, respectively; $P < 0.001$). Both the high-fat and control diets met the dietary requirements of rats (15). Despite there being no significant difference in body weight between the 2 groups, animals fed the high-fat diet had a significant increase in abdominal WAT mass (38%) and infrarenal WAT mass (2-fold increase) than

**FIGURE 1**. Correlation between abdominal white adipose tissue mass and circulating plasma leptin concentrations in rats fed a moderately high-fat diet ($\triangle r = 0.82, P < 0.01$) or a control diet ($\Delta; r = 0.81, P < 0.01$) for 4 wk. Linear correlations were significantly different ($P < 0.001$).
the animals fed the control diet. Not all adipose tissue regions responded in this manner; subcutaneous VAT and brown adipose tissue mass were unaffected by the short-term, high-fat diet.

After 4 wk, plasma leptin concentrations were 24% lower in the animals fed the high-fat diet than in those fed the control diet (Table 1). The fasting leptin concentration is plotted as a function of abdominal WAT mass after 4 wk of the diets in Figure 1. There was a positive relation between abdominal WAT mass and circulating leptin concentration for both the animals fed the control diet and those fed the high-fat diet. However, the correlation for the animals fed the high-fat diet was significantly different from that for the animals fed the control diet. At 4 wk, the animals fed the high-fat diet had significantly lower plasma leptin concentrations per unit of abdominal WAT mass than the control animals (0.15 ± 0.01 compared with 0.24 ± 0.02 μg·L−1·g−1; P = 0.005). It should be emphasized that plasma was collected after a 12-h overnight fast. Similar leptin responses were seen after only a 4-h fast but the differences between the rats fed the high-fat diet and those fed the control diet were less dramatic data not shown). After 4 wk, fasting plasma glucose concentrations were not significantly different between animals fed the high-fat diet and those fed the control diet (6.8 ± 0.3 compared with 6.9 ± 0.4 mmol/L), nor were plasma insulin concentrations (Table 1).

The responses of animals to 14 wk of dietary intervention are shown in Table 2 (n = 6–7 for the high-fat diet and n = 7 for the control diet). From 4 to 14 wk, animals fed the control diet gained only 12 ± 6 g whereas the animals fed the high-fat diet gained twice as much body weight (28 ± 5 g; P < 0.05). Over this time, total daily energy intake dropped by 28 ± 8 kJ in the control-fed rats but was unchanged in the rats fed the high-fat diet (4 ± 8 kJ), resulting in a 28% higher total energy intake in the rats fed the high-fat diet than in those fed the control diet at 14 wk. Dietary protein and carbohydrate intakes were not significantly different between animals fed the high-fat and control diets at 14 wk. Abdominal and infrarenal WAT mass were significantly higher in the animals fed the high-fat diet than in those fed the control diet. Longer-term fat feeding also caused significant increases in intrascapular brown adipose tissue mass and subcutaneous WAT mass. However, it is possible that the increase in intrascapular brown adipose tissue mass in the animals fed the high-fat diet may have been at least partly due to increased WAT infiltration. Brown adipose tissue mass was the only variable for which a diet-by-time interaction was found.

At 14 wk, there was still less leptin secreted per unit abdominal WAT mass in the rats fed the high-fat diet than in those fed the control diet (0.11 ± 0.01 compared with 0.15 ± 0.02 μg·L−1·g−1; P = 0.058) and this was negatively related to body weight (Figure 2). That is, those animals that had less circulating leptin per unit of fat mass had a higher body weight. There was a negative association between leptin secreted per unit of abdominal WAT mass and body weight for the rats fed high-fat and control diets after 4 and 14 wk.

When data from both time points and all animals were combined, significant correlations were found between abdominal WAT mass and total energy intake (r = 0.36, P < 0.05) and between abdominal WAT mass and dietary fat intake (r = 0.44, P < 0.01). However, no correlations were found between abdominal WAT mass and either dietary carbohydrate or dietary protein intake. Dietary fat, carbohydrate, and protein intakes all correlated significantly with the concentration of leptin secreted per unit of abdominal WAT mass after 4 wk (Table 3). At 14 wk, the only correlation that remained significant was a negative correlation between total dietary fat and leptin secreted per unit of abdominal WAT mass.

Fasting plasma insulin concentrations were not affected significantly by diet at 14 wk (Table 2). Plasma insulin increased over time from 4 to 14 wk in both the animals fed the high-fat diet (by 25%; P < 0.005) and those fed the control diet (by 28%; P < 0.01). At 14 wk, the plasma glucose concentration was not significantly different between the animals fed the high-fat diet and those fed the control diet (7.1 ± 0.3 compared with 7.4 ± 0.3 mmol/L). When data from both time points and all animals were combined, there was a weak positive correlation between circulating plasma leptin and plasma glucose concentrations (r = 0.42, P < 0.05); however, no correlations were found between plasma leptin and plasma insulin concentration. Therefore, higher plasma glucose and insulin concentrations were not associated with lower leptin concentrations per unit of abdominal WAT mass.

### Table 2
<table>
<thead>
<tr>
<th></th>
<th>Control diet</th>
<th>High-fat diet</th>
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<tbody>
<tr>
<td>Body energy (kJ/d)</td>
<td>250 ± 6</td>
<td>268 ± 4</td>
</tr>
<tr>
<td>Total energy intake (kJ/d)</td>
<td>197 ± 8</td>
<td>253 ± 8</td>
</tr>
<tr>
<td>Dietary fat intake (kJ/d)</td>
<td>5.9 ± 0.3</td>
<td>88.9 ± 0.3</td>
</tr>
<tr>
<td>Dietary carbohydrate intake (kJ/d)</td>
<td>151.4 ± 6.4</td>
<td>132.9 ± 8.1</td>
</tr>
<tr>
<td>Dietary protein intake (kJ/d)</td>
<td>39.3 ± 1.6</td>
<td>34.5 ± 2.2</td>
</tr>
<tr>
<td>Abdominal VAT (g)</td>
<td>11.00 ± 1.56</td>
<td>16.13 ± 1.5</td>
</tr>
<tr>
<td>Infrarenal VAT (g)</td>
<td>1.33 ± 0.19</td>
<td>2.22 ± 0.18</td>
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<tr>
<td>Subcutaneous VAT (g)</td>
<td>1.39 ± 0.18</td>
<td>1.81 ± 0.10</td>
</tr>
<tr>
<td>Intrascapular VAT (g)</td>
<td>0.19 ± 0.02</td>
<td>0.26 ± 0.01</td>
</tr>
<tr>
<td>Plasma leptin (μg/L)</td>
<td>1.83 ± 0.46</td>
<td>2.11 ± 0.24</td>
</tr>
<tr>
<td>Plasma insulin (pmol/L)</td>
<td>44.2 ± 15.0</td>
<td>34.8 ± 3.7</td>
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- 1 Different from control diet, P = 0.052.
- 2 Different from control diet, P = 0.052.
- 3–4 Significantly different from control diet; 3 P < 0.05, 4 P < 0.001.
- 5 P < 0.01, 6 P < 0.005.

![Figure 1](image1.png)

![Figure 2](image2.png)
DIETARY FAT AND LEPTIN IN RATS

TABLE 3
Correlations (R) between total energy intake, dietary fat, dietary carbohydrate, and dietary protein intake with plasma leptin secreted per unit of abdominal white adipose tissue at 4 and 14 wk

<table>
<thead>
<tr>
<th></th>
<th>4 wk</th>
<th>14 wk</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>(n = 15)</td>
<td>(n = 10)</td>
</tr>
<tr>
<td>Total energy intake</td>
<td>0.25</td>
<td>-0.43</td>
</tr>
<tr>
<td>Dietary fat</td>
<td>-0.64*</td>
<td>-0.62*</td>
</tr>
<tr>
<td>Dietary carbohydrate</td>
<td>0.84*</td>
<td>0.46</td>
</tr>
<tr>
<td>Dietary protein</td>
<td>0.84*</td>
<td>0.46</td>
</tr>
</tbody>
</table>

*P < 0.05.
**P < 0.001.

DISCUSSION

Our study showed that short-term, high-fat diets are associated with reduced leptin secretion. This reduced leptin secretion may contribute to the subsequent weight gain we observed in the animals fed the high-fat diet. On the basis of recent findings in the literature, we propose 2 mechanisms that may explain why a high dietary fat intake is associated with lower than expected circulating leptin concentrations.

Decreases in insulin-stimulated glucose metabolism have been shown to be associated with decreases in leptin expression and secretion in isolated adipocytes (16). This may explain why fasting decreases leptin concentrations (10, 16), and it may explain the lower leptin concentrations we observed after high-fat diets because high-fat feeding can decrease insulin-stimulated glucose uptake into adipocytes (17).

Increases in lipolysis have also been associated with decreased leptin synthesis in several studies (18, 19). This may explain decreased leptin synthesis after fat feeding because increased lipolysis has been documented after high-fat diets (20, 21). The mechanism by which increased lipolysis may decrease leptin synthesis is not known but may involve the activation of peroxisome proliferator-activated receptors by certain fatty acids. These receptors are fat cell-specific transcription factors capable of activating adipocyte differentiation. Exposure of mice to a high-fat diet has been shown to increase peroxisome proliferator-activated receptor γ expression (22). This could explain the reduced leptin secretion in the animals in our study because peroxisome proliferator-activated receptor γ can reduce leptin expression in adipose tissue (23). It is also possible that activation of cyclic AMP or β-agonists by a high-fat diet (24, 25) may act directly to suppress leptin secretion (18, 19).

Insulin has been identified as a possible mediator of leptin secretion. Insulin has been shown to increase leptin messenger RNA gene expression (26) and to increase circulating leptin concentrations (27, 28). We found no relation between circulating insulin and leptin concentrations, suggesting that a high-fat diet does not affect leptin secretion by altering insulin secretion. However, our animals were studied in a fasted, anesthetized condition and different results may have been seen had blood samples been taken from conscious, fed rats.

Diets high in fat have been shown to lower satiety, leading to overconsumption and weight gain (29, 30). Our data suggest that this may be due in part to reduced leptin concentrations. This is supported by a study by Surwit et al (5) in which obesity-prone animals were shown to have lower circulating leptin concentrations than did obesity-resistant mice when fed a high-fat diet. It is also supported by a recent study by Havel et al (31) in which lower 24-h circulating leptin concentrations were observed in human subjects after consumption of high-fat, low-carbohydrate meals. The effect of dietary fat on leptin may be dependent on the type of fat consumed because different sources of fat can have varying effects on lipolysis (32) and adipose tissue glucose uptake (33). We postulate that these 2 factors may be responsible for the reduction in leptin after dietary fat. (In our study, the main source of fat was polyunsaturated linoleic acid and monounsaturated oleic acid.) The effect of dietary fat on leptin may also be dependent on the length of the dietary treatment. Studies have shown that consumption of a high-fat diet for 12 d does not affect circulating leptin concentrations (34), whereas consumption of a high-fat diet by animals for 5 mo did elevate circulating leptin concentrations (35), which may have been due to the development of leptin insensitivity.

In conclusion, a fat-rich diet may contribute to early weight gain not only because it provides the substrate for triacylglycerol accumulation, but also because food intake increases as a result of the reduction in leptin secretion by adipose tissue.

REFERENCES