The Effects of a High Fat Diet on Leptin mRNA, Serum Leptin and the Response to Leptin Are Not Altered in a Rat Strain Susceptible to High Fat Diet-Induced Obesity¹,²

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ABSTRACT Osborne-Mendel (OM) and S5B/Pl rats differ in their sensitivity to develop obesity when fed a high fat (HF) diet; OM rats become obese, whereas S5B/Pl rats remain thin. We have investigated the possibilities that either an impaired leptin response or resistance to leptin action underlies the sensitivity to this form of obesity in OM rats. In Experiment 1, OM and S5B/Pl rats fed a nonpurified diet were killed at d 0 or were fed either a HF (56% fat energy) or a low fat (LF, 10% fat energy) diet for 2 or 7 d. The HF diet increased serum leptin significantly by d 2 to levels that were similar in both rat strains. At 7 d, leptin levels were lower than at d 2 but remained higher than levels in the d 0 control groups. The leptin mRNA:18S RNA ratio in epididymal adipose tissue increased to higher levels in HF-fed OM rats than in S5B/Pl rats fed that diet. However, although the LF diet had no effect in S5B/Pl rats, it increased leptin mRNA levels in epididymal adipose tissue of OM rats compared with the controls fed the nonpurified diet. In Experiment 2, OM and S5B/Pl rats were fed HF or LF diets for 5 wk. At that time, their feeding response to a range of leptin doses (0, 1, 5 or 10 µg) given intracerebroventricularly was tested after overnight food deprivation. There was a similar dose-dependent reduction in energy intake in response to leptin in both OM and S5B/Pl rats. These responses were independent of the diet. The data suggest that the susceptibility of OM rats to HF diet–induced obesity is not related to either a loss of central sensitivity to leptin or a failure to enhance leptin production acutely, although the failure to maintain chronically increased levels of serum leptin could contribute to the obesity. J. Nutr. 128: 1606–1613, 1998.

KEY WORDS: leptin • dietary fat • obesity • adipose tissue • rats

Leptin, the 16-kDa protein secreted from white adipose tissue, acts as a feedback signal for body fat stores to the hypothalamic centers that control food intake and energy expenditure (Friedman 1997, Gura 1997). This hypothesis was proposed from observations in three rodent strains of obesity that resulted from mutations in either the leptin gene (ob/ob mouse) or in the leptin receptor gene (db/db mouse and fa/fa rat) (Chua et al. 1996, Lee et al. 1996, Zhang et al. 1994). Replacement of normal leptin into the ob/ob mouse is associated with reversal of all of the phenotypic characteristics of the mutant mouse; the obesity is reversed, food intake, serum insulin and serum corticosterone fall; sympathetic drive to brown adipose tissue (BAT) is elevated and the abnormalities in reproductive function may be overcome (Ahima et al. 1996 and 1997, Campfield et al. 1995, Chehab et al. 1997, Collins et al. 1996, Halaas et al. 1995, Pelleymonter et al. 1995). In contrast, the mutation in the intracellular signaling arm of the leptin receptor in db/db mice prevents any response to either endogenous or exogenous leptin (Campfield et al. 1995, Halaas et al. 1995). There may however be some sensitivity to leptin in the obese (fa/fa) rat in which the mutation is in the extracellular binding domain of the receptor (Cusin et al. 1996, Lin et al. 1996a).

With the exception of the ob/ob mouse, all other forms of rodent obesity, whether of genetic, hypothalamic or dietary origin, are characterized by increased leptin mRNA levels in the adipose tissue and increased serum leptin levels (Maffei et al. 1995a, Mizuno et al. 1996, Vydelingum et al. 1995). Similarly, in human obesity, serum leptin levels are correlated with body fat, although there is considerable individual variation at any specific level of body fat (Lonnqvist et al. 1995, Maffei et al. 1995b). Mutations in either the leptin gene or the leptin receptor gene are not a common cause of human obesity (Considine et al. 1996a and 1996b, Stirling et al. 1995). The close association between circulating leptin level and body fat has led to the concept of leptin resistance as a cause of obesity. However, the site of such resistance is unclear. Although the obese Zucker rat appears to retain some sensitivity to central leptin (Cusin et al. 1996a and 1996b, Lin et al. 1996a), dietary

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Abbreviations used: BAT, brown adipose tissue; CNS, central nervous system; CSF, cerebrospinal fluid; HF, high fat; ICV, intracerebroventricular; LF, low fat; OM, Osborne-Mendel; RT-PCR, reverse transcription-polymerase chain reaction.

obesity in mice has been associated with normal responses to intracerebroventricular (ICV) leptin but loss of responses to peripherally administered leptin (Van Heek et al. 1997). This suggests that leptin resistance may be associated with access of leptin to central sites of action.

In an effort to investigate the role of the leptin signaling system in dietary obesity, we have compared the acute effects of feeding a high fat diet in two strains of rat that differ in their susceptibility to become obese on this diet, i.e., the Osborne-Mendel OM rat, which becomes obese when fed a high fat diet, and the SS/Pi rat, which is resistant to this form of obesity and remains thin (Buchanan et al. 1992, Schemmel et al. 1970).

**MATERIALS AND METHODS**

**Animals.** All protocols were reviewed and approved by the Institutional Animal Care and Use Committee.

**Experiment 1.** Twenty-five age matched 13-wk-old OM and SS/Pi rats were selected from the breeding colonies maintained in the animal facility of the Pennington Biomedical Research Center. They were housed in individual hanging wire-mesh cages attached to an automated watering system in rooms with a 12-h light:dark cycle (lights off 1900 h) maintained at 22–23°C. All rats consumed the standardized diet (Rodent Chow 5001, Purina Mills, St. Louis, MO) ad libitum until the beginning of the experiments.

After 2 wk of adaptation to the cages, all rats were weighed and five rats of each strain were killed as zero-time controls. The remaining 20 SS/Pi and 20 OM rats were then provided with either a high fat (HF) or low fat diet (LF) \( (n = 10 \text{ per diet/strain group}) \) in a single food cup that was secured to the wall of their cages. The compositions of these diets are given in Table 1. Food intake and body weight were measured daily after which the rats were given fresh quantities of their individual diets. Half of the rats in each group \( (n = 5) \) were killed after 48 h of consuming the HF or LF diets; the remaining rats were killed after 7 d. Rats were killed by decapitation to allow collection of trunk blood and preparation of serum. Inguinal subcutaneous and epididymal fat was dissected free of surrounding tissue, weighed and then frozen in liquid nitrogen. Serum and fat tissue samples were stored at \(-70°C\) before being used for extraction of RNA or hormone assays, respectively.

**Experiment 2.** Forty-eight Osborne-Mendel and forty-seven SS/Pi males rats weighing between 250 and 325 g were used in this experiment.

**Surgical procedures.** While anesthetized with pentobarbital (40 mg/kg, intraperitoneal), rats were placed in a stereotaxic apparatus with the incisor bar set at \(-3.3 \text{ mm}\) below the interaural line. When necessary, the height of the incisor was adjusted such that bregma and lambda had the same vertical coordinate. The tip of the vertical mounted 21-gauge stainless steel cannula was lowered 7.8 mm ventral to dura at a coordinate 2.2 mm posterior to bregma and directly on midline according to the atlas of Paxinos and Watson (1986). The midsagittal sinus was briefly displaced laterally while the guide cannula was lowered. The guide cannula was fixed in place by use of dental acrylic and stainless steel anchor screws. Each guide was fitted with a 27-gauge obturator that extended 0.5 mm below the tip of the guide cannula. Gentlymcytin \((0.2 \text{ mL}, \text{ intramuscular})\) was administered prophylactically in a single dose immediately before surgery. Verification of cannula placement was performed 5–7 d later by monitoring water consumption in nonwater-deprived rats in response to ICV angiotensin II \((15 \text{ ng})\). Rats that did not consume at least 5 mL of water in 1 h were excluded from the experiment (1 SS/Pi rat was excluded).

**Experimental protocol.** At least 10 d after the ventricular cannulation, the nonpurified diet was replaced with either the high fat (HF) or low fat/high carbohydrate (LF) diet (Table 1) such that half of the OM and SS/Pi rats were fed each diet. Food intake and body weight were monitored weekly. During this period, all animals were handled regularly to adapt them to receiving injections into the third cerebral ventricle. At the end of wk 5, the rats from each group (strain \( \times \text{ diet} \)) were further divided into four weight-matched groups \( (n = 5 \text{ or } 6) \) for a total of 16 groups. One group of OM and SS/Pi rats fed each diet was used each day for four successive days. Rats were food-deprived beginning 6 h after dark onset on the preexperimental day. One hour before dark onset on the experimental day, rats were administered either vehicle \((2 \muL \text{ artificial cerebrospinal fluid (CSF, Harvard Apparatus, Holliston, MA))} \) or 1, 5, or 10 \( \muL \) mouse leptin into the third cerebral ventricle. The injections were done in a counterbalanced fashion. Food was returned to the cages at dark onset and food intake was measured 2 and 4 h later. Two days later, freely fed rats were anesthetized with isoflurane, blood was collected via cardiac puncture, centrifuged and stored at \(-70°C\) until assayed for serum leptin. The mouse leptin used in these studies was a gift from Novartis (Basel, Switzerland).

**Preparation of leptin cDNA probe.** A 320-bp cDNA sequence of rat leptin was obtained by reverse transcription-polymerase chain reaction (RT-PCR) using oligonucleotide primers \((\text{sense, } 5’-\text{AAG ATC CCA GGG AGG AA}; \text{antisense, } 5’-\text{ATC TGC AGC AC})\). The resulting PCR fragment was cloned into a plasmid, linearized with \( \text{EcoRI} \), and ligated into the PstI site of the Bluescript plasmid. The fragment was transferred to nylon membranes (Zeta-Probe, Bio-Rad Laboratories, Hercules, CA). The leptin probe was labeled using a DECAprime II kit (Ambion, Austin, TX) with \( 32P-\text{dATP, } 3 \text{ Ci/mol (111 GBq/ mol)} \) (Amersham, Arlington Heights, IL). Blots were hybridized with \( 50 \text{ gL L NaHPO}_4, \text{pH 7.2}, 0.25 \text{ mol/L NaCl and } 70 \text{ gL SDS for 1 h at 43°C. Hybridization was performed for } 16 \text{ h at } 43°C \text{ in the same prehybridization buffer including } 2×10^4 \text{ dpm (333–666 MBq)} \) of \( ^{32P} \)-labeled probes. Filters were washed at room temperature with \( 2× \text{ SSC/} \text{g L SDS} \) for 10 min, \( 0.5X \text{ SSC/} \text{g L SDS for 5 min.} \)

**Isolation of RNA.** Adipose tissue RNA was extracted by the guanidinium-thiocyanate method (Chomczynski and Sacchi 1987) using Triazol Reagent (Gibco BRL, Gaithersburg, MD). The method was adapted for adipose tissue by including an additional low speed spin to remove fat and by increasing the volume of Triazol Reagent by 100%.

**Northern blots.** Total RNA \((20 \muL)\) was denatured at \(65°C\) for 15 min, electrophoresed on 10 gL agarose/formaldehyde gels and transferred to nylon membranes (Zeta-Probe, Bio-Rad Laboratories, Hercules, CA). The leptin probe was labeled using a DECAprime II kit (Ambion, Austin, TX) with \( ^{32P}-\text{dATP, } 3 \text{ Ci/} \muL \text{(111 GBq/} \muL) \) (Amersham, Arlington Heights, IL). Blots were prehybridized with \( 50 \text{ gL L formamide, 0.12 mol/L Na}_2\text{HPO}_4, \text{pH 7.2, 0.25 mol/L NaCl and 70 gL SDS for 1 h at 43°C. Hybridization was performed for 16 h at 43°C in the same prehybridization buffer including } 2×10^4 \text{ dpm (333–666 MBq)} \) of \( ^{32P} \)-labeled probes. Filters were washed at room temperature with \( 2× \text{ SSC/} \text{g L SDS} \) for 10 min, \( 0.5X \text{ SSC/} \text{g L SDS for 5 min.} \)

### Table 1: Composition of the high fat (HF) and low fat (LF) diets

<table>
<thead>
<tr>
<th></th>
<th>HF</th>
<th>LF</th>
</tr>
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<tbody>
<tr>
<td><strong>g/kg diet</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casin</td>
<td>287.0</td>
<td>220.0</td>
</tr>
<tr>
<td>Starch</td>
<td>238.0</td>
<td>423.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td></td>
<td>181.5</td>
</tr>
<tr>
<td>Corn oil</td>
<td>32.0</td>
<td>24.6</td>
</tr>
<tr>
<td>Crisco shortening</td>
<td>266.0</td>
<td>16.1</td>
</tr>
<tr>
<td>Choline</td>
<td>2.4</td>
<td>1.8</td>
</tr>
<tr>
<td>dl-Methionine</td>
<td>1.4</td>
<td>1.1</td>
</tr>
<tr>
<td>Vitamin mixture (AIN-76)</td>
<td>11.0</td>
<td>8.1</td>
</tr>
<tr>
<td><strong>kJ/g diet</strong></td>
<td>20.06</td>
<td>15.34</td>
</tr>
</tbody>
</table>

1 Proctor and Gamble, Cincinnati, OH.
2 ICN Biomedicals, Aurora, OH.
3 AIN mineral mix 76, ICN Biomedicals, Costa Mesa, CA.
4 AIN vitamin mix 76, ICN Biomedicals, Costa Mesa, CA.
(g·L·SDS) for 10 min, and 0.1X SSC/(g·L·SDS) for 10 min. Filters were stripped and rehybridized to an 18S RNA oligonucleotide probe as a control for gel loading and transfer. The intensities of signals were quantified by phosphorImage analysis (Molecular Dynamics, Sunnyvale, CA) using ImageQuant software.

**Statistics.** The data from Experiment 1 were analyzed by multivariate ANOVA (strain × diet × time). Individual comparisons were made subsequently using Duncan’s test. For Experiment 2, the food intake data in response to leptin administration were analyzed by ANOVA. Because the variances between groups were unequal, the data were log-transformed before ANOVA. Body weight data were analyzed by ANOVA with three factors, strain, diet and time, with time treated as a repeated measure. Plasma leptin data were analyzed using two-way ANOVA with the factors strain and diet. Individual comparisons between means were made using Bonferroni’s post-hoc method.

**RESULTS**

**Experiment 1**

**Food intake.** The quantity of food eaten did not differ in OM rats fed HF and LF diets on d 1 of exposure to the diets but decreased in the HF group subsequently to a level that was significantly lower than that of the LF group. ANOVA showed significant effect of strain (P < 0.0001), time (P < 0.005), time × strain (P < 0.001), time × diet (P < 0.001) (Fig. 1A) but not of diet alone or diet × strain interaction. However, when the data were expressed as energy intake (kJ/d) (Fig. 1B), the OM HF group ate significantly more than the LF group on d 1, but amounts did not differ on subsequent days. S5B/Pl rats showed a similar pattern of response, although intake in the LF-fed group was initially significantly lower than in the HF-fed group. However, by d 4, the energy consumed by the HF and LF groups did not differ, whereas the amount consumed by the HF groups was significantly reduced compared with the LF group. Once again, there was a significant effect of strain (P < 0.001), time (P < 0.003), time × strain (P < 0.001) and time × diet (P < 0.0001) on energy intake, but neither diet alone nor the strain × diet interaction was significant.

**Body weight.** A significant effect of strain (P < 0.001) and diet (P < 0.007) on body weight was apparent. The HF-fed OM rats gained significantly more body weight than the LF-fed OM group (Fig. 2). Diet did not affect weight gain in S5B/Pl rats. The weight of epididymal adipose tissue was greater in OM rats than in S5B/Pl rats (P < 0.0001) (Table 2), but there were no diet or strain × diet effects. In contrast, there were significant effects of both strain (P < 0.005) and diet (P < 0.02) on the weight of the inguinal adipose tissue depot. Again, the strain × diet interaction was not significant. Liver size was also greater in OM rats (P < 0.0001), but there were no effects of diet or strain × diet interaction.

**Serum leptin.** Serum leptin of OM rats fed the nonpurified diet was ~100% greater than that of S5B/Pl rats fed the same diet. Introduction of the HF diet increased serum leptin dramatically within 48 h in OM rats, whereas there was no effect in rats fed the LF diet (Fig. 3). This difference (sixfold) was still evident in OM rats that had been fed the HF and LF diets for 7 d, although at this time, the leptin levels of both OM groups had decreased significantly from those observed at 2 d. In S5B/Pl rats, consuming a HF diet for 48 h increased serum leptin to levels that were different from those of OM rats fed the HP diet, whereas serum leptin in S5B/Pl rats fed the LF diet was unchanged. After 7 d of consumption of the HF diet, plasma leptin of S5B/Pl rats was lower than that observed at 2 d, but remained sixfold greater than that of the LF-fed S5B/Pl rats.

**Leptin mRNA expression.** In the epididymal adipose tissue depot of OM rats, significant effects of strain (P < 0.0001) and strain × diet interaction (P < 0.01) were observed on leptin mRNA:18S RNA ratios. In OM rats, the leptin:18S RNA ratio was greater in the HF group compared with the LF group 48 h after introduction of the diets, but by d 7, there was no difference between the two groups (Fig. 4). However, all values were significantly greater than those of OM rats that were fed the nonpurified diet. Leptin mRNA levels in the epididymal fat pad of S5B/Pl rats fed the nonpurified diet were lower than in the OM rats. After consumption of the HF diet for 48 h, epididymal leptin mRNA:18S RNA ratios in S5B/Pl rats were significantly higher than the levels in S5B/Pl rats fed the LF diet. Although their leptin mRNA levels fell by d 7, the HF-fed group still maintained significantly higher levels than the LF-fed group. Similar changes were observed in the inguinal adipose depots (data not shown).

**Experiment 2**

**Body weight.** OM rats weighed more than S5B/Pl rats during the baseline period, and this difference between the strains...
FIGURE 2 Changes in body weight of OM and S5B/Pl rats fed the high fat (HF) or low fat (LF) diet for 2 or 7 d. Values represent means ± SEM, n = 5. Different letters at a time indicate significant difference of P < 0.05 at the specific time.

was maintained for the 5-wk duration of the experiment, independently of diet (P < 0.0001) (Fig. 5). There were significant effects of strain (P < 0.0001), diet (P < 0.0001) and strain × diet interaction (P < 0.0001) on body weight gain. As expected, HF-fed OM rats gained more weight than their LF-fed counterparts over the 5-wk experimental period. There was no difference in body weight between the S5B/Pl groups fed the two experimental diets for 5 wk.

Energy intake of OM rats. The effect of ICV leptin on short-term (4-h) energy intake of OM and S5B/Pl rats that were food deprived overnight is shown in Figure 6. In OM rats, there was a significant effect of leptin (P < 0.001), with leptin administration reducing energy intake in a dose-dependent manner, independently of diet. Similarly, in S5B/Pl rats, leptin administration produced a dose-dependent reduction in energy intake in S5B/Pl rats (P < 0.001), independently of diet. Unlike OM rats, the three-way interaction was not significant.

Serum leptin. The serum leptin concentrations of OM rats 2 d after the last ICV leptin treatment (Fig. 7) were significantly greater (P < 0.0001) than those of S5B/Pl rats, but neither the effect of diet nor the diet × strain interaction was significant.

DISCUSSION

OM rats become obese consuming a HF diet, whereas S5B/Pl rats remain thin. This study again confirmed this observation. OM rats fed the HF diet gained more weight than those fed the LF diet. In contrast, diet did not affect weight gain in S5B/Pl rats. We did not, unfortunately, measure body composition in the second experiment. However, we can be confident from previous reports that the ~40 g excess weight accumulated by OM rats during this 5-wk period was associated mainly with additional fat deposition (Buchanan et al. 1992, Okada et al. 1992, Schemmel et al. 1970). In the first experiment, when the rats were killed 2 and 7 d after introduction of the

TABLE 2

<table>
<thead>
<tr>
<th>Strain</th>
<th>OM</th>
<th>S5B/Pl</th>
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<tbody>
<tr>
<td>Diet</td>
<td>HF</td>
<td>LF</td>
</tr>
<tr>
<td>Tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epididymal adipose</td>
<td>2.40 ± 0.25a</td>
<td>2.25 ± 0.20a</td>
</tr>
<tr>
<td>Inguinal adipose</td>
<td>2.60 ± 0.38a</td>
<td>1.67 ± 0.22a</td>
</tr>
<tr>
<td>Liver</td>
<td>11.65 ± 0.37a</td>
<td>11.52 ± 0.48a</td>
</tr>
</tbody>
</table>

1 Values represent means ± SEM, n = 5. Different superscript letters in a row indicate significant difference (P < 0.05) among groups.
HF diet, we were unable to identify any significant diet × strain interactions on adipose tissue depot weights. This probably reflects the short duration of that experiment and the interanimal variability in size of adipose depots, which would preclude sufficient statistical power with the number of rats used in this experiment.

Numerous endocrine, metabolic, neurochemical, and behavioral differences have been described between OM and SSB/Pl rats fed HF diets (Lin et al. 1996b, Nagase et al. 1996, Singer et al. 1997, York and Hansen 1997). However, the basis for the differential sensitivity to HF diets remains unclear. The recent identification of the leptin-signaling system and the demonstration that defects in either the production of leptin or the response to leptin will cause obesity prompted the current experiments. Plasma leptin increases with body fat in all species including humans (Lonnqvist et al. 1995, Maffei et al. 1995b, Mizuno et al. 1996, Trayhurn et al. 1995a, Vydelingum et al. 1995), and the coincidence of obesity with high leptin levels has led to the suggestion that leptin resistance is a major cause of obesity. Leptin is thought to inhibit food intake and promote weight loss primarily through its actions in the hypothalamus, which include reduction in neuropeptide Y synthesis (Schwartz et al. 1996) and stimulation of sympathetic drive to BAT (Collins et al. 1996). The current experiments were designed to explore the possibilities that the susceptibility to HF diet induced obesity was related either to the failure to adequately increase leptin synthesis and secretion or to a diminished sensitivity to the effects of central leptin.

Both OM and SSB/Pl rats showed acute changes in adipose tissue leptin mRNA expression and circulating leptin levels in response to the introduction of a HF diet. However, there were interesting divergences in the patterns of response of leptin mRNA levels and serum leptin concentrations. There was a clear diet-dependent response of adipose tissue leptin mRNA levels and serum leptin in the SSB/Pl rats, with HF diets increasing both, compared with the LF diet. The increased production of leptin in HF-fed SSB/Pl rats is clearly consistent with a role of leptin in protecting against obesity in this strain. The increase in leptin gene expression in rats overconsuming a high fat diet has been previously reported (Masuzaki et al. 1995). However, in that study, the rats were killed at a time when there was already a 7% body weight gain, and this excess adiposity would itself be expected to enhance leptin gene expression and circulating leptin levels. The dramatic changes in leptin mRNA and serum leptin levels observed in SSB/Pl rats were evident within 48 h of introduction of the HF diet when neither body weight nor body fat were significantly increased. This acute increase in leptin gene expression and leptin secretion could reflect the initial hyperphagia of SSB/Pl rats eating the HF diet because a similar increase has been observed in rats overfed by tube-feeding (Harris et al. 1996). The SSB/Pl rats given the LF diet showed some initial neophobia, and energy intake was reduced. Despite this, we were unable to detect any decrease in either leptin gene expression or serum leptin levels. Although fasting and weight loss reduce leptin gene expression (Trayhurn et al. 1995b), it is possible that the relatively small changes in energy intake and body weight were insufficient to cause a detectable difference in leptin levels.

The dietary effect on leptin gene expression in OM rats was evident 48 h after introduction of the HF and LF diets and had disappeared by 7 d of exposure to these diets, although the ratio of leptin mRNA:18S RNA was substantially greater in both groups compared with the controls fed the nonpurified diet. Nevertheless, serum leptin levels were substantially greater in the HF group compared with the LF group after 48 h of consuming these diets. These data suggest either that the two adipose depots studied, the epididymal and the inguinal pads, were atypical of the general adipose tissue response to a HF diet or that secretion and/or turnover of leptin may be regulated independently of synthesis in OM rats. The similarity in leptin levels in OM and SSB/Pl rats fed the HF diet, despite clear differences in mRNA expression, would support this argument. Differential responses of leptin gene expression among different adipose depots have been reported (Harris et al. 1996, Ogawa et al. 1995), but, in general, changes in leptin mRNA expression have correlated with changes in circulating leptin levels (Maffei et al. 1995b).

The elevated levels of leptin in OM rats compared with SSB/Pl rats is consistent with their increased body fat. However, in our second experiment, OM rats fed the HF diet for 5 wk had a 7% greater increase in body weight compared with OM rats fed the LF diet, but there was no increase in serum leptin levels, suggesting either that the increase in body fat was insufficient to cause a detectable rise in leptin levels or that OM rats may not regulate leptin levels normally. The failure of OM rats to have a chronically increased leptin level could contribute to their sensitivity to high fat diet-induced obesity.

Although leptin levels have been shown to correlate with body fat in numerous studies of animals and humans (Lonnqvist et al. 1995, Mizuno et al. 1996, Trayhurn et al. 1995),

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**FIGURE 4** Leptin mRNA expression in epididymal adipose tissue depots of OM and SSB/Pl rats fed either a high fat (HF) or low fat (LF) diet. Upper panel: Northern blot of leptin mRNA and 18S RNA from individual animals (L = low fat, H = high fat). Lower panel: the composite data means ± SEM, n = 5. The data at time zero represent values before the HF and LF diets were provided to the other rats. Different letters indicate significant differences (*P* < 0.05).
levels upon introduction of the HF diet in Experiment 2, the data from Experiment 1 showed that there were acute increases in leptin production and secretion in the two strains of rat that were independent of body fat and dietary energy consumed. Further, despite similar acute changes in leptin levels, OM rats fed the HF diet gained more weight in 7 d than S5B/PI rats fed the HF diet, and adipose depot sizes were also increased. These data suggest that dietary fat acutely increases serum leptin and that this response is normal in OM rats. Thus, a failure to secrete leptin acutely does not appear to contribute to the sensitivity of OM rats to HF diet–induced obesity.

Although serum leptin was acutely elevated in OM rats fed the HF diet, this response was no longer evident in Experiment 2 when the rats were fed diets for 5–6 wk. At this time, leptin levels in OM rats were greater than in S5B/PI rats but were independent of dietary fat. Nevertheless, despite increased circulating levels of leptin during initial exposure to a high fat diet and apparently normal sensitivity to central leptin, the OM rats fed the HF diet were not protected against the development of obesity. In the current experiments, we showed that central leptin administration reduced short-term food intake in both OM and S5B/PI rats and did so independently of diet composition and body weight gain. These data suggest that there is no decrease in central leptin sensitivity in obese HF-fed OM rats, relative to OM rats fed a LF diet or to S5B/PI rats. It is possible that the acute feeding response to leptin is not a good index of its proposed chronic tonic role within the central nervous system (CNS) to regulate energy intake and energy expenditure in relation to body energy stores. However, our data are similar to those of Van Heek et al. (1997) who demonstrated that mice that became obese consuming a HF diet retain normal sensitivity to ICV administration of leptin. In their studies, they also showed that the mice that became obese consuming the HF diet lost their responsiveness to peripheral leptin. Such studies suggest that “leptin resistance” may be related to the inability of peripheral leptin to gain access to its central site of action. Leptin transport into the CNS appears to be a saturable process (Banks et al. 1996), suggesting that any insensitivity toward leptin associated with the development of obesity may be related to the inability to increase transport of leptin into the brain. The current conclusion should, however, be qualified. The level of obesity

there is also evidence for acute regulation of leptin levels and leptin gene transcription in response to food deprivation and refeeding, cold, β3-adrenergic agonists, insulin, glucocorticoids and other signals that are independent of changes in body fat (MacDougald et al. 1995, Mantzoros et al. 1996, Murakami et al. 1995, Rentsch and Chiesi 1996, Saladin et al. 1995, Trayhurn et al. 1995a and 1995b). The current studies suggest that HF diets, either directly or indirectly, may also lead to acute changes in leptin gene expression and leptin levels in susceptible strains of rat independently of changes in body fat.

OM rats ate similar amounts of HF and LF diet, which represented more energy than that consumed in the HF group. In contrast, S5B/PI rats exhibited an initial neophobia to the LF diet. However, from d 2 to 7, the amount of LF diet consumed was greater than that of HF diet; thus, there was little difference in energy ingested between the groups. Although recognizing that we did not study temporal changes in leptin...
induced in OM rats was only moderate during the short time frame of this experiment and may not have been sufficient to detect changes in sensitivity.

There are, of course, several possible alternative explanations for the current observations. It is possible that HF feeding itself, either directly or indirectly, may lead to increases in leptin transport into the brain in SSB/P1 rats and that this adaptation is impaired or absent in OM rats. Second, it is possible that HF diets differentially alter the ratio of free to bound leptin in the circulation. Finally, it is also clearly possible that differences in leptin production and/or signaling play a role in the differential sensitivity to obesity between OM and SSB/P1 rats fed a HF diet. Studies of blood-brain barrier leptin transport in these rat strains should clarify those possibilities.

LITERATURE CITED


FIGURE 7 Serum leptin concentrations of OM and SSB/P1 rats fed high fat (HF) or low fat (LF) diets for 5 wk. Values represent means ± SEM; n = 23–24. Different letters indicate significant differences. P < 0.05.


