Long-term effects of diet on leptin, energy intake, and activity in a model of diet-induced obesity

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Roberts, Christian K., Joshua J. Berger, and R. James Barnard. Long-term effects of diet on leptin, energy intake, and activity in a model of diet-induced obesity. J Appl Physiol 93: 887–893, 2002. First published May 31, 2002; 10.1152/japplphysiol.00224.2002.—This study investigated the effect of long-term high-fat sucrose (HFS) or low-fat complex-carbohydrate (LFCC) diet consumption on leptin, insulin, fat cell size, energy intake, and markers of activity to ascertain the role that leptin plays in long-term energy balance in a model of diet-induced obesity. Female Fischer 344 rats were fed either a HFS or LFCC diet ad libitum for a period of 20 mo. Measurements of leptin concentration, insulin concentration, and adipocyte size were performed at 2 wk, 2 mo, 6 mo, and 20 mo. Body weight and energy intake were measured weekly for calculation of feed efficiency. Body temperature and activity levels were assessed over a 5-day period after 12 mo of the dietary intervention. Plasma leptin and insulin concentrations were significantly elevated within 2 wk of HFS diet consumption and remained elevated throughout the course of the study. After 2 mo, the adipocytes of the HFS group were significantly larger and continued to increase in size throughout the course of the study. A significant correlation was noted between leptin and adipocyte cell size ($r = 0.96, P < 0.01$). However, despite elevated leptin, energy intake was similar, and the HFS group weighed significantly more than the LFCC group, as a result of a higher feed efficiency. There were no significant differences in body temperature or activity levels between the groups. These results demonstrate that a HFS diet causes hyperleptinemia and hyperinsulinemia before adipocyte size is increased and suggests that leptin resistance may be present or, alternatively, that leptin does not play a major role in the long-term regulation of energy intake or activity levels in this model.

Conversely, in humans, rarely does a mutation in the leptin gene or the leptin-receptor gene occur (34), and leptin administration results in modest weight loss in obese humans (20). The majority of human obesity results from gene-environment interactions, where dietary factors (i.e., fat and refined carbohydrate) and physical inactivity have long been evidenced to be contributing factors. For example, cross-sectional studies have reported an association between dietary fat and adiposity (3, 23, 32). Many models of diet-induced obesity have demonstrated that animals fed a diet high in fat and/or refined sugar become obese in the absence of excess energy intake (5, 36). It is possible that there may be an alteration in energy expenditure (i.e., decreased thermogenesis or decreased activity levels) that may contribute, in part, to the development of obesity noted in animals fed a high-fat, refined-sugar diet.

Leptin appears to be regulated in humans and animals by short-term alterations in energy intake, because leptin declines with fasting and increases with acute overfeeding (1, 7, 26). The role of leptin in the long-term regulation of body fat via modulation in energy intake and energy expenditure in normal rodents and humans remains unknown. The following experiment was undertaken to examine the long-term effects of diet on leptin, fat cell size, energy intake, and activity in female Fischer 344 rats raised on either a low-fat complex-carbohydrate (LFCC) or a high-fat sucrose (HFS) diet for 20 mo.

METHODS

Animals and diet. All protocols were conducted in accordance with and approved by the University of California, Los Angeles, Animal Research Committee. Inbred female Fischer 344 rats were obtained from Harlan Sprague Dawley (San Diego, CA) at 2 mo of age, and after a 1-wk period during which the animals were fed standard rat chow, the rats were randomly assigned to either the LFCC or HFS diet (at 9 wk of age) and were housed four per cage with a 12-h light cycle starting at 0700 at 75–76°F. Our laboratory used this rat model in previous studies (4, 40). The diets were provided ad libitum with large bowls placed in the cages to ensure that all animals had access to the food. The diets were prepared in powder form by Purina Test Diets (Richmond, IN) and con-
tained a standard vitamin and mineral mix and all essential nutrients. The LFCC diet (Purina 5001) is low in saturated fat and contains mostly complex carbohydrate (starch), whereas the HFS diet is high in saturated and monounsaturated fat (primarily from lard plus a small amount of corn oil) and high in refined sugar (sucrose) as previously published (40). Both groups were studied at various time points for up to 20 mo of dietary intervention. The rats were weighed weekly, and food intake was measured daily (5 days/wk) in both groups for calculation of energy intake and feed efficiency (FE).

Adipocyte morphology. Fat cell size was determined as previously described (5). Briefly, omental fat samples were removed, rinsed in 0.85% NaCl, and placed in 10% phosphate-buffered formalin. The samples were then dehydrated, filtered, and embedded in paraffin according to the method of Sheehan and Hrapchak (44). Sections were then sliced at a thickness of 4 μm at three different depths, at least 200 μm apart, within the same embedded tissue sample. The samples were then affixed to slides, after which video prints were taken with a Codonics VP-3500 video printer attached to a Perspective Systems image-analysis system connected to an Olympus BH2 microscope. By using the video images, the number of adipocytes within a given area was counted, and the number obtained was used to calculate mean cell number and mean cell volume according to calculations described by Lemonnier (27) and Ashwell et al. (2).

Blood chemistries. The rats were fasted overnight to eliminate any confounding effects of the last meal and were anesthetized with 10% chloral hydrate (250 mg/kg ip; University of California, Los Angeles Pharmacy), and blood was drawn via cardiac puncture. Blood samples were immediately centrifuged, and the plasma was separated and frozen at −70°C. Insulin concentration was quantified by using a double-antibody RIA kit obtained from Ventrex Laboratories (Portland, ME). Frozen plasma samples were sent to Linco Research (St. Charles, MO) for quantification of plasma leptin concentration also by using a double-antibody RIA.

Body temperature and activity. Six animals from both groups were analyzed for body temperature and motor activity after 12 mo on their respective diets. With battery-operated, precalibrated biotelmetric transmitters (model VM-FH, Mini Mitter, Sunriver, OR) implanted in the peritoneal cavity, body temperature and motor activity were measured. Transmitter implantation was conducted under halothane anesthesia. After the operation, rats were housed individually and were allowed to recover for 1 wk before measurements were started. The animals were maintained on their respective diets during the recovery period and for 5 additional days of data collection. Output was monitored by a receiver board (model RA-1010) placed under each animals cage and fed into a peripheral processor (BCM100) connected to a personal computer as previously described (52). Body temperature was detected by a sensor imbedded in the transmitter and was recorded at 10-min intervals. Activity was measured by detecting changes in signal that occurred as the animals moved about the cages.

Statistical analysis. Data from the experiments were analyzed by using a Student’s t-test or an ANOVA with GraphPad Prism (GraphPad Software, San Diego, CA). When significant F values were found, post hoc analyses were performed by using a Newman-Keuls multiple-comparison test. Differences were considered statistically significant at P < 0.05. Values are reported as means ± SE with 6–8 rats per group for each study, except the body weight, food intake, and energy intake studies, which contained 16 rats per group.

RESULTS

Plasma leptin, adipocyte size, and plasma insulin. Leptin concentrations were significantly elevated in the HFS compared with the LFCC group after 2 wk on the diets (Fig. 1). Leptin levels continued to rise in the HFS group over the remainder of the study and were significantly elevated at all time points compared with the LFCC group. Leptin, although increased slightly with time, was not significantly different among the LFCC animals, whereas in the HFS group the leptin concentration at 20 mo was significantly higher compared with the other time points (P < 0.05).

Histological analysis of the fat pads revealed that the HFS group exhibited a gradual rise in adipocyte size during the observation period (P < 0.01, ANOVA), and at all time points the values were significantly different from each other. The difference in adipocyte cell size between the groups after 2 wk did not reach significance (LFCC: 2.50 ± 0.18 vs. HFS: 2.85 ± 0.23 μm3 × 105). By 2 mo, however, there was a significant difference in fat cell size (LFCC: 2.50 ± 0.18 vs. HFS: 4.26 ± 0.29 μm3 × 105; P < 0.01), and the HFS cells increased significantly in size at each time point throughout the study (6-mo HFS: 6.27 ± 0.4 μm3 × 105, 20-mo HFS: 10.02 ± 0.58 μm3 × 105; P < 0.01). In contrast, the LFCC adipocytes remained nearly constant in volume throughout the study (6-mo LFCC: 2.51 ± 0.02 μm3 × 105, 20-mo LFCC: 2.46 ± 0.06 μm3 × 105). There was a strong positive correlation between fat cell size and plasma leptin concentration (r = 0.96; Fig. 2).

After 2 wk there was a significant difference in fasting plasma insulin between the LFCC and HFS groups (LFCC vs. HFS, 48.0 ± 11.4 vs. 285.6 ± 63.6 pmol/ml; P < 0.05). The insulin concentration in the HFS group remained significantly elevated at subsequent time points (2 mo: 373.2 ± 105.6 pmol/ml, 6 mo: 336 ± 72.6 pmol/ml, 20 mo: 367.2 ± 99 pmol/ml) compared with the LFCC group (2 mo: 48 ± 11.4, 6 mo: 87 ± 17.4, 20 mo: 81.6 ± 8.4 pmol/ml). In addition, the 2-, 6-, and 20-mo insulin levels of the HFS animals were significantly higher than the 2-wk insulin levels of the LFCC animals (P < 0.05).

Body weight, FE, and energy intake. Figure 3 shows the average weekly body weights, food intake (g/day),
and energy intake (kJ/day) in the two groups for 85 wk. Weight gain for the two groups paralleled each other until week 21, after which the HFS group began to gain weight at an increased rate compared with the LFCC group, in which weight gain slowed (Fig. 3). The difference in body weights was not statistically significant until week 25 (LFCC vs. HFS: 188 ± 1.4 vs. 214 ± 3.4 g; P < 0.01), and the difference remained significant throughout the remainder of the study (final weights: 253.8 ± 6.0 vs. 367.2 ± 9.3 g; P < 0.01).

The food intake data revealed a trend of decreased food and energy intake over the first 10 wk on the diets in both groups, which is consistent with the end of the rapid growth phase of the animals. The intake then reached a plateau and remained steady for the remainder of the study. The energy intake per gram of body weight gain, termed the FE, was calculated as a digestive and metabolic indicator of the ease with which energy consumed was added as body weight. The FE was much lower in the LFCC compared with the HFS group (777 vs. 422 kJ/g; P < 0.01). There was no difference in energy intake in the HFS group over the 20-mo period (average daily intake of HFS: 176.5 ± 3.2 kJ/day; LFCC: 176.3 ± 3.1 kJ/day). The difference in energy intake between the LFCC and HFS groups accounted for 2.5% of the 123-g difference in body weight in the HFS animals.

**Body temperature and activity levels.** Figure 4 illustrates that, when measured at 12 mo, there was no significant difference in body temperature between the HFS and LFCC groups at any time during the day or when averaged over the entire day. However, the temperature in both groups was higher during the night compared with the day because rats, being nocturnal animals, consume most of their food when the lights first go out. The increased activity levels seen in both groups at night, as demonstrated in Fig. 5, may help explain the temperature increases. Although the nighttime activity levels were higher than daytime activity levels, there were no significant differences seen between the HFS and LFCC groups. The body temperature and activity counts are presented in Figures 4 and 5 as an average temperature and total activity per day, respectively, over 5 days.

**DISCUSSION**

Obesity is a condition that has reached epidemic levels in recent years. Flegal et al. (16) estimated that between 1960 and 1994 the proportion of adults in the United States with a body mass index between 30 and 34.9 kg/m² rose by 66%. In addition, it is apparent that the rate of rise in obesity is accelerating, as Mokdad et
al. (33) reported that the proportion of adults in the United States with a body mass index ≥30 kg/m² has increased nearly 50% from 1991 to 1998.

Leptin has been documented to regulate food intake, body temperature, energy expenditure (38), insulin action (6), and blood pressure (49), and thus it might play a role in the metabolic syndrome. Normal animals and humans exhibit leptin expression and secretion that correlate with body fat mass (11, 31) and with adipocyte size in both lean and obese mice (21). The present study is the first prolonged, longitudinal study to document changes in insulin, leptin, food intake, fat cell size, and markers of energy expenditure simultaneously over a 20-mo period with use of an ad libitum protocol of diet-induced obesity. The results of the present study confirm that prolonged consumption of a HFS diet leads to excessive weight gain and adipocyte hypertrophy compared with a LFCC diet. Leptin was highly correlated with fat cell size but was unrelated to food intake or to energy intake and activity levels, as estimated by body temperature and cage movement.

Studies with the ob/ob and db/db mice have clearly established that leptin regulates food and energy intake in genetic models of obesity. In the present study with normal rats, the volume of food consumed by the HFS animals was consistently less than the volume consumed by the LFCC animals (Fig. 3). However, energy intake was similar for the two groups because of the different energy densities of the two diets. Other studies have documented a similar pattern of food consumption in high-fat-fed animals compared with low-fat-fed animals (36). Li et al. (28) demonstrated in male Fischer 344 rats that food intake was constant over 30 mo in animals fed a standard LFCC chow diet. In addition, it has been reported that short-term leptin administration to lean animals causes a reduction of food intake (9). In the present study, leptin was significantly elevated after 2 wk on the HFS diet and continued to rise with long-term consumption of the HFS diet. However, the increase in leptin was unrelated to food intake and suggests the possibility of leptin resistance in the HFS rats. In support of the leptin resistance theory, Van Heek et al. (48) found that resistance to leptin treatment developed in diet-induced obese mice after 16 days of leptin treatment in high-fat-fed (45% fat cal) animals, whereas animals maintained on a low-fat (10% fat cal) diet retained their sensitivity to exogenous leptin administration. Other data suggests that insulin and dietary fat may induce leptin resistance (29). In the study by Lin et al. (29), the authors demonstrated that the hypophagic effect of leptin in low-fat-fed animals was preserved when the animals were naively fed a high-fat diet but not when high-fat-fed animals were fed a low-fat diet. The authors suggested that a signal related to the low-fat, high-carbohydrate diet was needed for the leptin response, rather than an inhibitory response to dietary fat. Additional studies have shown that diets high in dietary fat can increase plasma leptin in as little as 2 days (30); however, insulin alters adipocyte production of leptin (12, 25), and thus changes in plasma leptin may not accurately reflect the presence of leptin resistance and may be secondary to changes in insulin. For instance, Steinberg and Dyck (45) demonstrated that diet-induced peripheral leptin resistance, as measured by leptin’s ability to induce skeletal muscle free fatty acid oxidation, occurs in the absence of changes in plasma leptin.

Another possibility is that, in the long-term, leptin does not regulate food intake per se but rather that it regulates a lack of food intake. In this proposed model, weight loss is the signal that causes a cascade of events beginning with decreased leptin, a drive for increased food intake via hypothalamic mechanisms, and decreased energy expenditure to favor a return to normal body weight. When sufficient energy stores or energy intake has occurred, the metabolic and endocrine alterations are returned to normal. However, further elevation of leptin above a threshold for defense against starvation has no effect on systems of energy homeostasis unless supraphysiological levels of leptin are achieved (41). The relative ineffectiveness in humans of long-term leptin administration for weight control supports this contention (20).

There is evidence that leptin is involved in the regulation of energy expenditure in genetic models of obesity. Leptin administration has been shown to increase energy expenditure and induce weight loss in ob/ob mice by increasing oxygen consumption, body temperature, and locomotor activity (38). However, in rodent models without genetically induced obesity, rather than increasing energy expenditure above basal levels, leptin appears to act primarily to prevent the decline in metabolic rate associated with periods of decreased energy intake (19). This is supported by the present data that demonstrated no difference in body temperature or motor activity between the LFCC and HFS groups despite significant hyperleptinemia in the HFS group. In humans, Wisse et al. (51) reported a positive correlation between leptin and resting energy expenditure during a refeeding period after the subjects were severely energy restricted for 4 wk. In contrast, Rosenbaum et al. (42) found no correlation between changes in serum leptin and changes in energy expenditure in humans. The disparity in results may

Fig. 5. Effect of diet on daily activity. After 12 mo of the dietary intervention, activity levels were measured during the day and during night, and total 24-h counts were averaged for a 5-day period. Values are means ± SE for 6 rats/group.
be explained by the fact that Wisse et al. measured the
leptin at a time when dynamic changes in energy
balance were occurring, whereas Rosenbaum et al.
measured leptin during periods when energy intake
was static. These findings suggest that leptin does not
cause elevations in energy expenditure that would pre-
vent the development of diet-induced obesity.

The correlation between leptin and adipocyte size
found in this study has been demonstrated in several
other studies involving both animals and humans (11,
17). After 2 wk on the HFS diet, without a significant
change in adipocyte cell size, the HFS animals already
exhibited hyperleptinemia compared with the LFCC-
fed animals. The plasma leptin rose throughout the 20
mo in the HFS group and paralleled the increase in
adipocyte cell size. Ahren et al. (1) demonstrated a
similar effect of a high-fat diet on the relationship
between leptin and fat mass in mice maintained on the
diet for 12 mo. In other studies, however, treating
animals with leptin resulted in a paradoxical loss of
adipose tissue stores (6). In some humans, this effect
of leptin on the adipose tissue stores has also been
demonstrated (20). Thus amelioration of diet-induced
metabolic abnormalities (e.g., insulin resistance, hypergly-
cemia, hypertriglyceridemia, obesity) by leptin may
require administration of exogenous leptin as shown by
Buettner et al. (8), who used a recombinant adenovirus
containing the leptin cDNA.

Exposure of Fischer 344 rats to a HFS diet has
previously been shown to cause insulin resistance and
hyperinsulinemia after 2 wk (5). The hyperleptinemia
observed at 2 wk before any change in fat cell size in
the HFS rats is possibly the result of a stimulatory
effect of insulin on ob gene expression. It is known that
insulin is an adipogenic hormone (24), and insulin and
glucose together regulate leptin production and secre-
tion (35). In the rodent model, hyperinsulinemia is
associated with overexpression of ob mRNA, and insu-
lin has been reported to directly elevate leptin mRNA
in rat adipocytes (13, 43).

Normally, obesity is thought to be simply the result
of an energy intake-energy expenditure imbalance. We
measured the metabolizable energy intake of the HFS
and LFCC diets rather than gross energy intake, be-
cause diets high in fiber and unrefined carbohydrates
have a lower metabolizable energy content (50), and
consequently the availability of nutrients from the feed
ingredients is lower in the LFCC diet. This is due to the
increase in energy loss mainly through the feces. Nev-
evertheless, the metabolizable energy intakes in both
groups were nearly equal during the 20-mo study.
However, the HFS group had a higher FE, which may
have contributed to the weight gain, because the di-
gestibility of the diet affects FE and weight gain (10).
The fact that the difference in energy intake only could
explain 2.5% of the weight gain supports this conten-
tion. We suggest that the higher FE is a potential
explanation for the greater weight gain noted in rodent
models despite equivalent energy intakes or even lower
gross caloric intake in animals ingesting high-sucrose
diets, high-fat diets (37), or both (40). The higher res-

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It is probable that other factors are playing a role in
the weight gain. First, protein and carbohydrate pos-
sess a higher thermic effect compared with fat (47),
which may contribute to energy expenditure. Second,
the conversion of excess dietary fat into body fat is a
more efficient process than the conversion of protein or
carbohydrate into body fat (15), and normally carbohy-
drate and protein balances are tightly regulated,
whereas fat balance is not (46). Third, differences in
energy expenditure may be present, because there are
inherent limitations in the extrapolations of activity
and body temperature measured only over 5 days.
Namely, although not statistically different, the cumu-
lative difference over 20 mo in activity and body tem-
perature may have contributed to some of the differ-
ence in body weight. At some point during the course of
the study, it is possible that energy lost as heat may
change to affect energy expenditure. Additionally, be-
cause proton leaks constitute a considerable part of an
organisms’ metabolic rate, differences in the activities
of uncoupling proteins may contribute to the differ-
ences noted between the diet groups (14). Although
probably not an initiating response, as fat accumulates
in the animal, it provides an insulating effect, such
that it would lessen heat dissipation, facilitating the
maintenance of body temperature. Additionally, the
rise in insulin may have played a role in the obesity as
documented by weight gain and adipocyte hypertrophy
in the HFS group. Our laboratory has previously re-
ported that hyperinsulinemia in the HFS diet group
was associated with reductions in skeletal muscle and
elevations in adipose tissue lipoprotein lipase activity,
lipoprotein lipase protein, and very-low-density lipo-
protein-receptor protein expression (39). This would
enhance fat storage, contributing to obesity.

We acknowledge that there are inherent limitations
to our study. First, because we measured the weight of
the food present in bowls for each group, we cannot
rule out the possibility that there may be differences in
the rate of spillage between the groups; however, this
was observed to be minimal. Second, the activity was
determined by using monitors that can only detect
large movements that occurred as the animals moved
to different portions of the cages, which would not
account for animal fidgeting. Third, we measured the
leptin during fasting because metabolic parameters
are normally measured during fasting conditions to
eliminate any potential confounding acute influences
of the last meal, which may result in an inaccurate
reflection of the long-term chronic response. In doing
so, the leptin concentration during nonfasted periods of
the day, which may not have differed between the
groups, was not accounted for. However, there is evi-
dence in obese individuals that feeding does not reduce
leptin levels. For example, Guerci et al. (18) demon-
strated that leptin is not acutely affected with an oral
fat load in normal or obese individuals. The obese
patients had significantly higher leptin than the con-

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trols during fasting and the subsequent 8-h period after the meal was consumed. Furthermore, Imbeault et al. (22) fed a high-fat meal to lean and obese men, and leptin decreased slightly but remained fourfold higher than lean controls in the 8-h period after the meal. Thus we suggest that leptin would be elevated in our HFS rats throughout the day as well as in the fasted state, as we observed.

Overall, the present study has documented that long-term consumption of the HFS diet increases fat cell size and plasma leptin concentration. The increase in leptin noted in the HFS diet-fed animals was associated with elevated plasma insulin levels and was highly correlated with fat cell hypertrophy, but it was unrelated to energy intake or markers of energy expenditure in this model.

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