Leptin prevents obesity induced by a high-fat diet after diet-induced weight loss in the marsupial *S. crassicaudata*

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Leptin prevents obesity induced by a high-fat diet after diet-induced weight loss in the marsupial *S. crassicaudata*. Am J Physiol Regul Integr Comp Physiol 286: R734–R739, 2004; 10.1152/ajpregu.00240.2003.—The aims of this study were to determine in the marsupial *Sminthopsis crassicaudata*, the effects of leptin on food intake, body weight, tail width (a reflection of fat stores), and leptin mRNA, after caloric restriction followed by refeeding ad libitum with either a standard or high-fat preferred diet. *S. crassicaudata* (n = 32), were fed standard laboratory diet (LabD; 1.01 kcal/g, 20% fat) ad libitum for 3 days. On days 4–10, animals received LabD at 75% of basal intake and then (days 11–25) were fed either LabD or a choice of LabD and mealworms (MW; 2.99 kcal/g, 30% fat); during this time, half the animals (n = 8) in each group received either leptin (2.5 mg/kg) or PBS intraperitoneally two times daily. On day 26, animals were killed and fat was removed for assay of leptin mRNA. At baseline, body weight, tail width, and food intake were similar in each group. After caloric restriction, body weight (P < 0.001) and tail width (P < 0.001) decreased. On return to ad libitum feeding in the PBS-treated animals, body weight and tail width returned to baseline in the LabD-fed animals (P < 0.001) and increased above baseline in the MW-fed animals (P < 0.001). In the LabD groups, tail width (P < 0.001) and body weight (P < 0.001) decreased after leptin compared with PBS. In the MW groups, the increase in tail width (P < 0.001) and body weight (P = 0.001) were attenuated after leptin compared with PBS. The expression of leptin mRNA in groups fed MW were greater in PBS than in leptin-treated animals (P < 0.05). Therefore, after diet-induced weight loss, leptin prevents a gain in fat mass in *S. crassicaudata*; this has potential implications for the therapeutic use of leptin.

adipose tissue; resistance; *Sminthopsis crassicaudata*

Obesity is difficult to treat because even when weight loss is achieved the vast majority of individuals regain the adipose tissue lost (25). The resistance to a reduction of fat mass is consistent with a strong physiological drive to conserve fat stores (25). Leptin, the protein product of the ob gene, is involved in a signaling pathway from adipose tissue that acts to regulate body fat stores by modulating food intake and energy expenditure (35). Leptin circulates in proportion to total body fat mass (25, 28), and its level rises with increasing adiposity (14, 15, 25, 27). This finding has been interpreted to indicate that leptin “resistance” occurs (25).

The leptin system is more sensitive to both caloric restriction and a decrease in fat mass than to an increase in fat mass (2, 17). Adiposity is associated with increasing leptin levels, and the administration of leptin (at least peripherally) does not prevent diet-induced obesity in animals (17, 21, 31, 34). Accordingly, it appears that leptin is more important as a defense against weight loss, permitting fat mass to increase to a maximum genetic potential when food is abundant (17, 25). The disappointing data from clinical trials of leptin in humans (20) are consistent with this view of leptin physiology, which predicts that the exogenous administration of leptin should prevent an increase in fat mass, after prior caloric restriction or diet-induced weight loss.

*Sminthopsis crassicaudata* (the Fat-tailed Dunnart) is an Australian nocturnal marsupial (10–20 g) with a lifespan in captivity of ~2 yr. One of the unique features of this marsupial is that it stores ~25% of total adipose tissue in the tail (24). We have previously shown that both tail width and leptin mRNA expression relate directly to total adiposity (22), and, after removal of the tail, fat reaccumulates in subcutaneous adipose tissue depots (24).

*S. crassicaudata* prefer a diet of mealworms, which have a higher caloric density compared with the standard laboratory diet (22). When offered a choice of mealworms or laboratory diet, mealworms are preferentially consumed, and adiposity increases (22). In animals fed standard laboratory diet, exogenously administered human methionyl recombinant leptin (2.5 mg/kg ip two times daily) results in a substantial decrease in fat mass, which is secondary to a decrease in energy intake and increase in energy expenditure. In contrast, when the animals are fem a mealworm diet, there is resistance to the actions of this dose of exogenously administered leptin, and fat mass progressively increases (21).

The aims of this study were to determine in *S. crassicaudata* the effect of leptin on food intake, body weight, tail width, and leptin mRNA expression after diet-induced weight loss followed by ad libitum feeding with either a standard laboratory diet or their preferred increased-fat, high-calorie diet.

**MATERIALS AND METHODS**

The experimental protocol was approved by the Animal Ethics Committee of the University of Adelaide.

**Animals, Diets, and Drugs**

Adult male *S. crassicaudata* (aged 8–12 mo) were purchased from a breeding colony maintained by the University of Adelaide, Animal Services. Animals were placed in single cages 1 wk before commencement of experiments and housed in a constant-temperature environment.
The light regime was set on long days (16 h light, 8 h dark; lights off 1600). A laboratory diet (1.01 kcal/g, containing ~70% water, and 20% fat, 25% protein, 35% carbohydrate by dry weight) consisting of a mixture of Woofs dog food (Ridley Agri Products), Whiskas cat food (Uncle Ben’s, New South Wales, Australia), and water was available ad libitum, except where stated. Live mealworms (Tenebrio molitor larvae; 2.99 kcal/g, containing ~55% water, and 30% fat, 60% protein, 10% carbohydrate by dry weight) were provided ad libitum where stated. Purified r-met human leptin (5 mg/ml; Amgen) was stored at −70°C and diluted in sterile PBS, pH 7.2, to the required concentration before use. PBS or leptin (2.5 mg/kg) was administered intraperitoneally two times daily at 0830 and at 1600. Sterile 29-gauge needles were used, and injection volume did not exceed 350 μl.

**Experimental Protocol**

*S. crassicaudata* (n = 32) were initially fed laboratory diet ad libitum for 3 days, and food intake was measured. For the next 7 days (days 4–10), each animal received laboratory diet at 75% of their previous average intake. They were then divided into two groups that had comparable initial body weight, tail width, and food intake (n = 16); both groups were fed ad libitum with either laboratory diet or a choice of laboratory diet and mealworms for 14 days (days 11–25). During this time, one-half of the animals (n = 8) in each group was injected intraperitoneally with either PBS or purified r-met human leptin in a dose of 2.5 mg/kg two times daily. Body weight, tail width, and food intake were measured daily at 1530. On day 25, animals were injected at 0830 and given their allocated diet; at 5–7 h postinjection of drug or placebo, the animals were killed by rapid decapitation, and trunk blood was collected. All visible adipose tissue was removed and snap-frozen in liquid nitrogen before storage at −70°C.

**Measurements**

*Food intake.* Daily intake of laboratory diet was determined by weighing plastic bowls containing food immediately before and 24 h after feeding. To correct for weight loss through evaporation, bowls of food were placed in empty cages and weighed as above. The mealworms were presented in a small jar, and intake was recorded by weighing the jar at each time point (21). Data for food intake are expressed as kilocalories eaten.

Tail width (mm) was measured at the widest point by drawing the tail through circular holes of known diameter until an exact fit was obtained (24).

*Plasma glucose and free fatty acids.* The trunk blood collected at death was spun immediately, and the plasma was frozen at −70°C for the subsequent measurement of glucose and free fatty acids using a Cobas Bio centrifugal analyzer (Roche Diagnostica, Basel, Switzerland) and standard Roche enzymatic kits (Roche Diagnostica; see Ref. 19).

**RNA Extractions and Agarose Gel Electrophoresis**

Total RNA was extracted from subcutaneous white adipose tissue (WAT) by a modification of the method described by Chirgwin et al. (12). Briefly WAT was homogenized in guanidium thiocyanate, and the resulting homogenates were layered on cushions of 5.7 M cesium chloride and centrifuged at 65,000 rpm for 18 h at 20°C. Pellets were resuspended in diethyl pyrocarbonate-treated water and ethanol precipitated at −80°C overnight. The RNA concentrations of the extracts were determined from the absorbance at 260 nm. Total RNA (15 μg/lane) was electrophoresed on a 1.4% agarose formaldehyde gel. Ethidium bromide staining was used to determine RNA loading. RNA were then transferred overnight to a Hybond-N+ nylon transfer membrane (Amersham, New South Wales, Australia) by capillary blotting and fixed to the membrane by cross-linking with ultraviolet light.

**[32P]cDNA Probes**

A 1.8-kb cDNA for *S. crassicaudata* leptin was isolated from a cDNA library made from WAT (23). The full-length cDNA, labeled with α-32P to a specific radioactivity of ~1 × 10⁶ dpm/μg DNA using a Gigaprime DNA labeling kit (Bresatec), was used to hybridize the RNA blots. Prehybridization was performed for 30 min at 65°C in QuickHyb hybridization solution (Stratagene Integrated Sciences, New South Wales, Australia). RNA blots were hybridized for 4 h at 65°C containing probe at 1.25 × 10⁶ counts/ml. Posthybridization washes were performed at 50°C and consisted of two 5-min washes in a solution of 2× saline-sodium citrate (SSC)-0.1% SDS and one 15-min wash in 0.1× SSC-0.1% SDS. Blots were then exposed to Kodak X-Omat AR film (Integrated Sciences) for 2–14 days at −80°C.

**Quantitation of Gene Expression After Northern Blotting**

The intensity of the bands was determined using Sigma Scan Image (Jandel Scientific). Loadings were corrected for by the measurement of 18S ribosomal RNA band intensity.

**Statistical Analyses**

Statistical significance was determined by two-way ANOVA with repeated measures and paired or unpaired Student’s t-tests where appropriate. Changes during the time period of caloric restriction were analyzed separately (the animals had been prerandomized to their subsequent treatment groups). The factors were either drug or diet with time as a repeated measure. The time points at baseline and at the end of the caloric restriction phase were used as covariates. Data were analyzed with the SPSS 10.0 for windows software package (SPSS). Body weight and tail width are expressed as change from baseline. Results are shown as means ± SE. A P value of <0.05 was considered significant in all analyses.

**RESULTS**

**Body Weight**

At baseline, body weights (g) were comparable in each group (17.1 ± 0.5, 17.6 ± 0.1, 16.8 ± 0.7, and 17.4 ± 0.6, in groups A–D, respectively). In response to caloric restriction (between days 4 and 10), body weight decreased [effect of time F(7,154) = 56.42, P < 0.001] similarly in all groups of animals (Fig. 1, A and B). The response to ad libitum feeding (days 11–25), however, differed between the two dietary groups.

**Animals fed laboratory diet.** In the PBS-treated animals, body weight returned to baseline and remained stable thereafter, whereas in the leptin-treated animals, body weight increased transiently and then progressively decreased [effect of drug F(1,165) = 18.68, P = 0.001; drug × time interaction F(15,165) = 0.74, P = 0.048; time F(15,165) = 8.92, P < 0.001; Fig. 1A].

**Animals fed both laboratory diet and mealworms.** In the PBS-treated animals, body weight increased above baseline, whereas in the leptin-treated animals, the increase on body weight was less [effect of drug F(1,165) = 17.98, P = 0.001; drug × time interaction F(15,165) = 4.83, P = 0.014; time F(15,165) = 16.90, P < 0.001; Fig. 1B].
Fig. 1. Body weight (mean ± SE) expressed as a change in weight from baseline in *Sminthopsis crassicaudata* in response to ip PBS or leptin (2.5 mg/kg) two times daily after diet-induced weight loss followed by ad libitum feeding with either laboratory diet (LabD; A; 1.01 kcal/g, 20% fat) or a choice of laboratory diet and mealworms (MW; B; 2.99 kcal/g, 30% fat). After caloric restriction, body weight decreased (*P* < 0.05). After refeeding, laboratory diet (A) body weight returned to baseline in the PBS group, whereas in the leptin-treated animals body weight decreased from day 1 of leptin treatment (*P* < 0.001). In the groups fed both laboratory diet and mealworms (B), body weight increased above baseline in the PBS-treated animals (*P* < 0.001) but returned to baseline in the leptin-treated animals from day 1 (*P* = 0.04). *P* < 0.05. Arrows represent beginning and end of caloric restriction.

**Tail Width**

At baseline, tail widths (mm) were similar in each group (4.1 ± 0.2, 4.3 ± 0.2, 4.0 ± 0.1, and 3.9 ± 0.2 in the groups A-D, respectively). In response to caloric restriction (between days 4 and 10), the reductions in tail width were comparable [effect of time *F*(7,154) = 17.89, *P* < 0.001] in each group of animals (Fig. 2, A and B). The response to ad libitum feeding (days 11–25) differed between the two dietary groups.

**Laboratory diet-fed animals.** In the PBS-treated animals, tail width returned to baseline and remained stable thereafter, whereas in the leptin-treated animals a transient increase in tail width was followed by a progressive decrease [effect of drug × time interaction, *F*(15,165) = 12.97 *P* < 0.001; time *F*(15,165) = 6.633, *P* = 0.001; Fig. 2A].

**Animals fed both laboratory diet and mealworms.** In the PBS-treated animals, tail width increased above baseline, whereas in the leptin-treated animals tail width returned to baseline but did not increase further [effect of drug *F*(1,165) = 23.90, *P* < 0.001; drug × time interaction *F*(15,165) = from day 3 7.841, *P* = 0.006; time *F*(15,165) = 19.44, *P* < 0.001; Fig. 2B].

**Food Intake**

At baseline, food intake (kcal/day) was comparable in each group (16.6 ± 1.4, 14.9 ± 1.5, 17.2 ± 1.8, and 17.6 ± 1.6, in groups A-D, respectively Fig. 3). In response to caloric restriction (between days 4 and 10), caloric intake decreased similarly in each group [effect of time *F*(7,154) = 65.80, *P* < 0.001; Fig. 3]. On return to ad libitum feeding (days 11–25), there was a transient increase in food intake in all groups, although the magnitude of this effect differed in different groups [effect of time *F*(15,330) = 38.12, *P* < 0.001; group × time *F*(45,330) = 4.42, *P* < 0.001]. Thereafter, food intake decreased in all groups, with differences between the groups as detailed below (Fig. 3).

**Animals fed laboratory diet.** In the first 24 h after ad libitum feeding, food intake increased significantly in the PBS-treated
libitum feeding, daily food intake increased transiently in all groups (P < 0.001). In the laboratory diet-fed animals, food intake increased in the drug, leptin-treated group (P < 0.05) but by 72 h had returned to baseline in the leptin-treated group (effect of drug, P < 0.05). Thereafter, food intake returned to that of baseline for both groups of laboratory diet/meal worm-fed animals. *P < 0.05. Arrows represent beginning and end of caloric restriction.

Fig. 3. Food intake (mean ± SE), expressed in kcal, at baseline after diet-induced weight loss and in response to ip PBS or leptin (2.5 mg/kg) two times daily after refeeding with either laboratory diet (1.01 kcal/g, 20% fat) or a choice of laboratory diet and mealworms (2.99 kcal/g, 30% fat). Food intake was initially similar in all four groups. Food intake decreased in all animals (P < 0.001) when laboratory diet was fed at 75% of basal intake. After ad libitum feeding, daily food intake increased transiently in all groups (P < 0.001). In the laboratory diet-fed animals, food intake increased in the PBS-treated (∗) but not in the leptin-treated group (●; P < 0.05) after 24 h, but by 48 h food intake had returned to baseline in both groups. From 72–96 h, energy intake in the leptin-treated laboratory diet group was below baseline (P < 0.001). Thereafter, in the laboratory diet-fed animals, there was no difference in food intake from baseline, in either the leptin or PBS-treated animals. In the laboratory diet/meal worm-fed animals, food intake increased during the first 48 h for both PBS-treated (■) and leptin-treated groups (□; P < 0.05) but by 72 h had returned to baseline in the leptin-treated group (effect of drug, P < 0.05). Thereafter, food intake returned to that of baseline for both groups of laboratory diet/meal worm-fed animals. *P < 0.05. Arrows represent beginning and end of caloric restriction.

Group (P < 0.05) but not in the leptin-treated animals (P = 0.176). By 48 h postrefeeding, the groups did not differ from baseline. From 72 to 96 h, however, energy intakes in the leptin-treated animals were significantly below baseline (P < 0.001). Thereafter, there were no differences between the groups, with food intake returning to baseline and no effect of drug over the remaining time.

Animals fed both laboratory diet and mealworms. In the first 48 h after ad libitum feeding, food intake increased similarly in both the PBS and the leptin-treated groups (P < 0.01). By 72 h postrefeeding, food intake remained increased in the PBS-treated group (P < 0.05) but had returned to baseline for the leptin-treated group; there was a significant effect of drug (P < 0.05). From 96 h, the groups did not differ from baseline, and there was no effect of drug at that and over the remaining time points (Fig. 3).

Leptin mRNA Expression

Laboratory diet-fed animals. There was no significant difference in leptin mRNA expression between the PBS and leptin-treated animals (Table 1).

Animals fed both laboratory diet and mealworms. The expression of leptin mRNA was fourfold greater in the PBS-fed animals compared with the leptin-treated animals (P < 0.05; Table 1).

Metabolic Parameters

Laboratory diet-fed animals. Plasma free fatty acids were lower in the leptin-treated compared with the PBS-treated animals (P = 0.046). Plasma glucose and liver glycogen were similar in the leptin and PBS-treated animals (Table 2).

Animals fed both laboratory diet and mealworms. There were no differences in plasma free fatty acids between the leptin and PBS-treated animals. Plasma glucose levels were higher in the leptin-treated than PBS-treated animals (P = 0.023), but liver glycogen levels were similar in both groups (Table 2).

DISCUSSION

We have previously shown that the exogenous administration of leptin results in a decrease in fat mass in the marsupial S. crassicaudata and that ad libitum administration of a high-fat high-calorie diet leads to an increase in fat mass that is not prevented by exogenous leptin administration (21). The current study establishes that, after diet-induced weight loss, the exogenous administration of leptin 1) decreases fat mass in laboratory diet-fed animals and 2) attenuates the development of obesity in animals fed a highly palatable, calorie-dense, preferred diet.

The dose and duration of leptin administration in this study was similar to our previous study in ad libitum laboratory diet-and mealworm-fed animals (21). The magnitude of weight loss (~1.5 g) and the decrease in tail width (~0.7 mm) in the laboratory diet-fed, leptin-treated animals were similar in the two studies. The most interesting observations, therefore, relate to the effect of leptin on fat mass in response to a high-fat, high-calorie diet after diet-induced weight loss, which suggest that either prior caloric restriction or weight loss modifies the

Table 1. Effect of leptin (2.5 mg/kg ip) two times daily after diet-induced weight loss and refeeding ad libitum with either LabD or a choice of LabD or MW, on fat leptin mRNA, of Sminthopsis crassicaudata

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Leptin mRNA expression, relative units</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Leptin + LabD</td>
<td>1.44 ± 0.12</td>
</tr>
<tr>
<td>B</td>
<td>Leptin + LabD/MW</td>
<td>1.71 ± 0.35*</td>
</tr>
<tr>
<td>C</td>
<td>PBS + LabD</td>
<td>1.55 ± 0.25</td>
</tr>
<tr>
<td>D</td>
<td>PBS + LabD/MW</td>
<td>7.20 ± 1.43*</td>
</tr>
</tbody>
</table>

Values are means ± SE. LabD, laboratory diet, 1.01 kcal/g, 20% fat; MW, meal worms, 2.99 kcal/g, 30% fat. There was a significantly higher fat leptin mRNA in the PBS than leptin-treated LabD/MW group. *P < 0.05.

Table 2. Effect of leptin (2.5 mg/kg ip) two times daily after diet-induced weight loss and refeeding ad libitum with either LabD or a choice of LabD or MW, on plasma FFA and glucose, and liver glycogen of S. crassicaudata

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Plasma FFA, mmol/l</th>
<th>Plasma Glucose, mmol/l</th>
<th>Liver Glycogen, µmol</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Leptin + LabD</td>
<td>0.31 ± 0.06*</td>
<td>4.18 ± 0.61</td>
<td>47.5 ± 13.2</td>
</tr>
<tr>
<td>B</td>
<td>Leptin + LabD/MW</td>
<td>0.31 ± 0.02</td>
<td>5.89 ± 0.43*</td>
<td>96.7 ± 25.3</td>
</tr>
<tr>
<td>C</td>
<td>PBS + LabD</td>
<td>0.50 ± 0.09*</td>
<td>5.01 ± 0.40</td>
<td>51.8 ± 11.7</td>
</tr>
<tr>
<td>D</td>
<td>PBS + LabD/MW</td>
<td>0.33 ± 0.04</td>
<td>4.69 ± 0.32*</td>
<td>213.5 ± 55.3</td>
</tr>
</tbody>
</table>

Values are means ± SE. FFA, free fatty acids. There was a significantly lower plasma FFA in the leptin than PBS-treated LabD-fed group. There was significantly higher plasma glucose in the leptin-treated LabD/MW group when compared with those receiving PBS. There were no differences in liver glycogen resulting from leptin treatment within dietary groups. *P < 0.05.
sensitivity to exogenous leptin. Nevertheless, the effect of leptin remains diet dependent, since the laboratory diet-fed animals continued to lose weight while the body weights of the animals fed laboratory diet plus mealworms returned to baseline.

On day 25, tail width, which is an indirect measure of fat mass (23, 24), and leptin mRNA expression were similar in the PBS-treated laboratory diet-fed animals and the leptin-treated mealworm-fed animals, but greater in the PBS-treated mealworm-fed animals. This suggests that the effect of leptin was, as expected, predominantly on fat mass.

Leptin appears to function primarily as an “anti-starvation” hormone, since in both humans (6, 32) and animals (2), calorie restriction is associated with a rapid reduction in leptin levels before the occurrence of weight loss. Furthermore the sensitivity to increasing leptin levels seems less (compared with when levels decrease); in the setting of an increased intake of a high-fat, high-calorie diet, this permits fat stores to be increased to a genetically predetermined set point (17, 25). At some point, however, leptin must be effective because there is a progressive increase in fat mass and abnormal feeding behavior only occurs in the setting of leptin deficiency (10, 17, 25).

Leptin administration reduces fat mass by decreasing food intake and increasing energy expenditure (10, 17, 18, 19). Consistent with our previous study (21), the overall difference in energy intake between the leptin- and placebo-treated mealworm-fed animals was not significant. There was, however, a trend for energy intake to be lower in the leptin-treated animals. The reason for this discrepancy is uncertain, but it may relate to effects of leptin on meal preference under certain circumstances. In rodents, leptin also has limited ability to restrain intake of highly palatable, high-fat diets that are normally associated with an increase in adiposity (31, 34). The effects of leptin on energy expenditure may be more important than the effects on food intake. We have observed that leptin administration increases physical activity in S. crassicaudata (21). The effects of leptin on energy intake and energy expenditure may be dissociated (29), and presumably the threshold at which each occurs may have been modified as a consequence of the prior caloric restriction in the current study. Although not measured in this study, we have reported, in animals fed laboratory diet, that leptin prevents a decrease in oxygen consumption despite substantial weight loss (21).

Studies in mice suggest that defects downstream of the leptin receptor in the hypothalamus may account for leptin resistance (5). The observation that elevated plasma leptin is not associated with an increase in cerebrospinal fluid levels of leptin has led to the hypothesis that the leptin transport system may be defective or saturated in obese humans (11, 30). Consistent with this concept, C57BL/6 and AKR diet-induced obese mice have preserved sensitivity to centrally administered leptin, despite resistance to peripherally administered leptin (31). This does not, however, appear to account for the apparent resistance to leptin that occurs in obese animals (16, 17), the resistance to exogenously administered leptin that occurs in association with a highly palatable high-fat diet (21, 31, 34), or the current observation that prior caloric restriction affects the sensitivity to leptin. The hypothesis that there may be plasticity in sensitivity to leptin is supported by data showing variations in sensitivity to leptin in relation to changes in fat mass associated with alterations in photoperiod (1), as well as the observation that in rodents susceptible to diet-induced obesity, once obesity occurs, the new body weight is defended and the animals are resistant to attempts at weight loss (26). The data from the current study are consistent with those obtained by Buisson et al. (8), who showed that after 7 days of exogenous leptin administration the increase in fat mass in postobese rats fed a high-fat diet was less than in control animals. Although we cannot generalize our observations in male animals to female animals, because of variations in estradiol, which has been shown to affect fat mass and leptin sensitivity (4), similar findings to those observed in this study have been shown in female rodents (8).

Liver glycogen stores were higher in the mealworm-fed animals than the laboratory diet-fed animals, and this was not modified by the administration of leptin. In contrast, plasma glucose was higher in the leptin-treated laboratory diet plus mealworm-fed animals, but not the other groups, in whom the plasma glucose levels were comparable. In the ob/ob mouse, leptin has been shown to increase both hepatic glycogen synthesis and, at least acutely, hepatic glucose production (9, 13). Observations in a profoundly leptin-deficient animal cannot, necessarily, be extrapolated to the current situation. The effects of leptin on carbohydrate metabolism are complex, tissue-specific, and dependent on nutritional state (3, 9). Leptin maintains steady-state energy stores by decreasing lipid synthesis and increasing fat mobilization, with decreased glucose oxidation as a result of increased fatty acid oxidation (7). By day 25 both body weight and tail width in the laboratory diet plus mealworm-fed leptin-treated animals were stable, and it seems reasonable to assume that energy balance was at steady state. Accordingly, the increase in plasma glucose levels is consistent with the known physiological actions of leptin. In contrast, in the leptin-treated laboratory diet-fed mice, liver glycogen stores were lower, fat stores were depleted, and consequently glucose utilization increased.

Plasma free fatty acid levels were lower in the mealworm-fed than the laboratory diet-fed animals. There are a number of mechanisms by which leptin might account for this situation. Chronic leptin administration decreases de novo synthesis of free fatty acids by the liver (13). In normal adipocytes, leptin decreases the expression of fatty acid synthase, increases peroxisome proliferator-activated receptor-α and the enzymes of free fatty acid oxidation, and stimulates a novel form of lipolysis in which glyceral is released without a proportional release of free fatty acids (33). Plasma free fatty acid levels were similar in both the PBS- and leptin-treated laboratory diet plus mealworm-fed animals.

Therefore, we conclude that, after weight loss induced by diet, leptin permits restoration, but not an increase above baseline, of fat mass in response to consumption of a high-fat, high-calorie diet, previously shown to induce obesity and leptin resistance. This suggests that the therapeutic use of leptin should be directed at the prevention of an increase in fat mass after successful diet-induced weight loss, rather than obesity per se. The distinction between the responses to leptin after diet-induced weight loss in an animal with prior obesity compared with a never obese animal remains to be determined.
REFERENCES


