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Research Article

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Endotoxin and Cytokines Induce Expression of Leptin, the *ob* Gene Product, in Hamsters

A Role for Leptin in the Anorexia of Infection

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Abstract

The expression of leptin, the *ob* gene product, is increased in adipose tissue in response to feeding and energy repletion, while leptin expression decreases during fasting. Infusion of leptin decreases food intake. Because adipose tissue gene expression is regulated by cytokines induced during infection and because infection is associated with anorexia, we tested whether induction of leptin might occur during the host response to infection. Administration of endotoxin (LPS), a model for gram negative infections, induces profound anorexia and weight loss in hamsters. In fasted animals, LPS increased the expression of leptin mRNA in adipose tissue to levels similar to fed control animals. There is a strong inverse correlation between mRNA levels of leptin and subsequent food intake. TNF and IL-1, mediators of the host response to LPS, also induced anorexia and increased levels of leptin mRNA in adipose tissue. As assessed by immunoprecipitation and Western blotting, circulating leptin protein is regulated by LPS and cytokines in parallel to regulation of adipose tissue leptin mRNA. Induction of leptin during the host response to infection may contribute to the anorexia of infection. (*J. Clin. Invest.* 1996. 97:2152–2157.) Key words: leptin • LPS • TNF • IL-1 • anorexia

Introduction

Anorexia and weight loss frequently accompany infection. Despite the presence of multiple metabolic changes during infection, recent evidence indicates that decreased caloric intake plays a major role in infection-induced weight loss. For example, in AIDS, a state characterized by increased resting energy expenditure and disturbances in lipid metabolism (1), weight loss only occurs in the presence of decreased caloric intake (2, 3). Cytokine administration mimics the metabolic changes of infection (4). Several studies indicate that cytokine-induced weight loss is also tightly linked to anorexia (5–7). Therefore, understanding the mechanisms by which anorexia occurs during infection could allow for the development of interventions to prevent or ameliorate the weight loss of infection.

The mechanisms by which infection induces anorexia are unknown. Several cytokines including TNF, IL-1, IL-6, IL-8, and IFN-γ have been shown to induce anorexia and cachexia (5–15). Cytokines released in response to infection and/or inflammation may have direct effects on the brain, such as inducing anorexia (8, 13). However, data from other laboratories suggest that cytokines act on peripheral tissues (14, 15). Several hormones known to regulate appetite, including corticotropin releasing hormone, cholecystokinin, prostaglandins, glucagon, insulin, and corticosteroids are induced by LPS and cytokines (8, 16–21).

Recent studies indicate that food intake and body weight are regulated by leptin (also known as the *ob* gene product), a protein whose synthesis is localized in adipose tissue (22). Defects in this gene lead to overeating and are the genetic abnormality accounting for the obesity seen in *ob/ob* mice (22). Leptin levels increase in the fed state, when triglyceride is being stored in adipose cells, and decrease during nutritional deprivation, when adipose triglyceride is being depleted (23–26). Infusion of leptin decreases food intake and weight in both obese and normal mice (27–29). Intracerebral infusion of leptin is even more effective at decreasing food intake than peripheral infusion (29). Infusion of leptin decreases hypothalamic levels of neuropeptide Y (30), a neurotransmitter that increases food intake (31, 32). These data suggest that leptin produced by repleted adipose tissue plays a key role in signaling the brain that adequate caloric stores are present, inducing satiety.

LPS and the cytokines that mediate the host response to infection are well known to regulate adipose tissue metabolism. LPS, TNF, IL-1, the interferons, and leukemia inhibitor factor decrease the activity of lipoprotein lipase, decrease de novo fatty acid synthesis, and increase lipolysis in adipose cells (4, 33–35). Many of these effects are due to regulation of adipose tissue mRNA. More recently we have shown that LPS, TNF, or IL-1 decrease circulating levels of cholesterol ester transfer protein, by decreasing its mRNA in hamster adipose tissue (36); adipose tissue is thought to be the major source of circulating CETP in hamsters.

Therefore, we hypothesized that LPS and cytokines might

increase leptin mRNA in adipose tissue, thereby increasing circulating levels of leptin. Increased circulating leptin levels during infection could decrease food intake. We now report that LPS or cytokine treatment of fasted hamsters increase levels of leptin protein in the circulation and leptin mRNA in adipose tissue. The increase in leptin levels correlates with decreased food intake, suggesting that leptin may contribute to the anorexia of infection.

Methods

Materials. [α-^{32}P]dCTP (3,000 Ci/mmol) was purchased from New England Nuclear (Boston, MA); LPS (*Escherichia coli* 055:B5) was from Difco Laboratories (Detroit, MI); human TNF with a sp act of 5×10^7 U/mg was kindly provided by Genentech Inc. (So. San Francisco, CA); recombinant human IL-1β with a sp act of 10^9 U/mg was kindly provided by Immunex Corp. (Seattle, WA); Multiprime DNA labeling system kits were purchased from Amersham Life Sciences International (Arlington Heights, IL); Minispin sephadex G50 columns were from Worthington Biochemical Corporation (Freehold, NJ); Nytran+ nylon membranes were from Schleicher and Schuell (Keene, NH). A mouse epididymal fat pad cDNA library, oligo (dT)-primed and random-primed in λ gt11, was obtained from Clontech Inc. (Palo Alto, CA). Oligonucleotide primers were synthesized at the UCSF Biomolecular Resource Center (San Francisco, CA). LPS was freshly prepared in pyrogen free 0.9% saline (McGraw Inc., Irvine, CA). Cytokines were freshly prepared in 0.1% HSA (Alpha Therapeutic Corp., Los Angeles, CA).

Animal procedures. Male Syrian hamsters ($\sim$ 100–120 g) were purchased from Simosen Laboratories (Gilroy, CA). The animals were maintained in a normal light cycle (6:00 a.m.–6:00 p.m. light, 6:00 p.m.–6:00 a.m. dark) and were provided with standard rodent chow (Simonsen Laboratories) and water as indicated in the text. For intraperitoneal injection, animals were anesthetized with isofluorane then given saline, LPS, TNF, and/or IL-1 as indicated in the text.

For studies of food intake, animals were placed in individual metabolic cages (Nalgene Co., Rochester, NY), which allow for quantification of food intake. The animals were adapted to the cages for several d before the experiments began. At 4:00 p.m. on the first day, hamsters were weighed, anesthetized with isofluorane, and given LPS, normal saline, 0.1% HSA, TNF, and/or IL-1 as indicated in the text. Food intake was measured over the next 18 h, at which point the animals were weighed again. Subsequently, food intake and weight were monitored every 24 h for 3 more days.

Cloning of leptin cDNA. Two primers (5′-GGAGGAAAATGT-GCTGGAGACC-3′, sense, and 5′-CTTCAGCATTCAGGGCTA-ACATCC-3′, antisense) spanning nucleotides 108–621 of the murine *ob* gene sequence (22) were used to clone a full length cDNA from the murine epididymal fat pad library.

Isolation of RNA Northern blotting. Total RNA was isolated from adipose tissue by modification of the method of Chomczynski and Sacchi (37); lipid extraction with CHCl$_3$ was performed immediately after homogenization; the aqueous phase was then acidified and subjected to the standard acid/phenol/chloroform extraction. Gel electrophoresis, transfer, Northern blotting, and densitometry were performed as previously described (38). Because we and others have shown that LPS and cytokines increase levels of mRNA for actin and cyclophilin, two mRNAs commonly used for normalizing data, we loaded equal amounts of total RNA (10 μg determined by spectrophotometry) and assessed uniformity of sample application by ultraviolet visualization of the acridine orange—stained ribosomal RNAs in the gel before electrophoretic transfer. Multiple studies previously reported by our laboratory show that the effects of LPS or cytokines are specific for individual mRNAs in individual tissues both in terms of the direction and the order of magnitude of change. For example, treatment of hamsters with LPS or cytokines decreases the level of CETP mRNA in adipose tissue (36). In liver, specific mRNAs may be
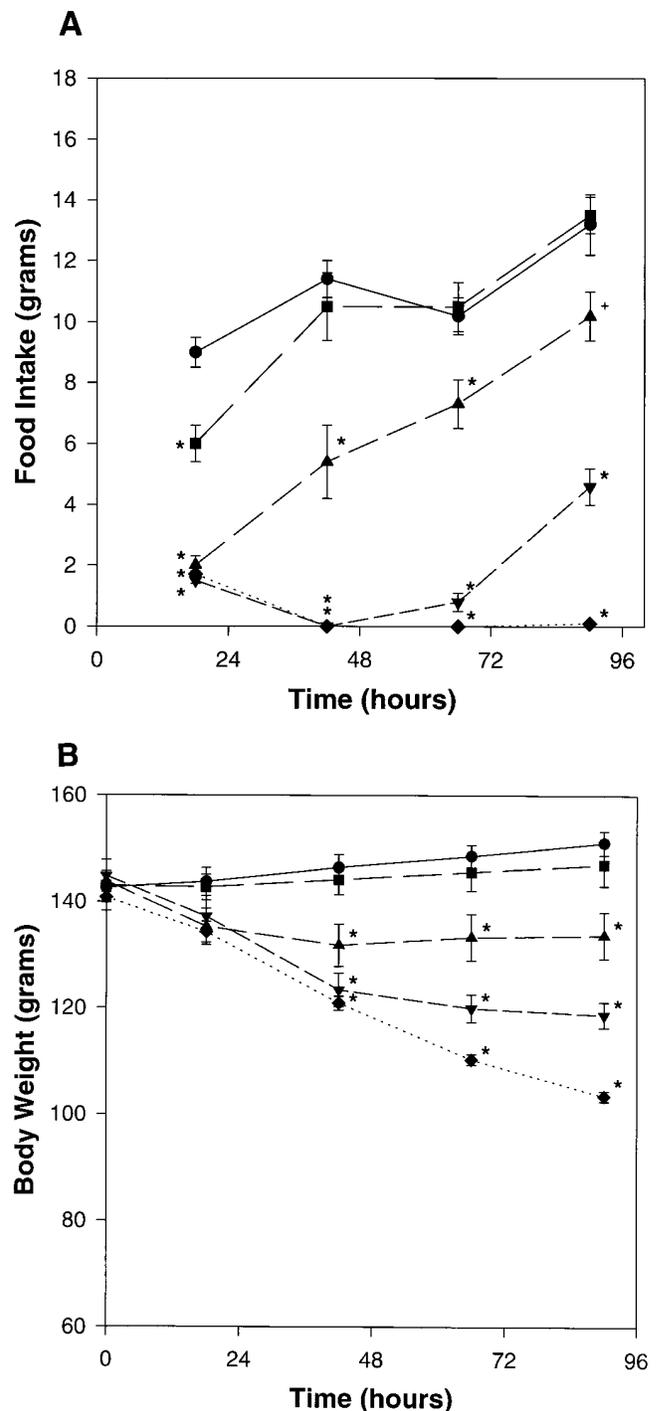
Figure 1. The effect of LPS on food intake and body weight in hamsters. At 4:00 p.m. hamsters were given either normal saline (*circles*) or 0.1 μg (*squares*), 1 μg (*triangles*), 10 μg (*inverted triangles*), or 100 μg (*diamonds*)/100 g BW of LPS. Animals were maintained in individual Nalgene metabolic cages. Food intake (*A*) and weight (*B*) were measured at the times indicated. $n = 5$ for each group. Values are mean$\pm$SEM. *$P < 0.01$ vs. control. ***$P < 0.05$ vs. control.

increased or decreased to varying degrees ranging from 30-fold increases to 95% decreases (39, 40).

Measurement of circulating leptin protein. Leptin levels were measured by a semiquantitative method described in detail elsewhere (24). In brief, 100 μl serum were immunoprecipitated with affinity-purified polyclonal antibody to recombinant leptin. Where indicated,
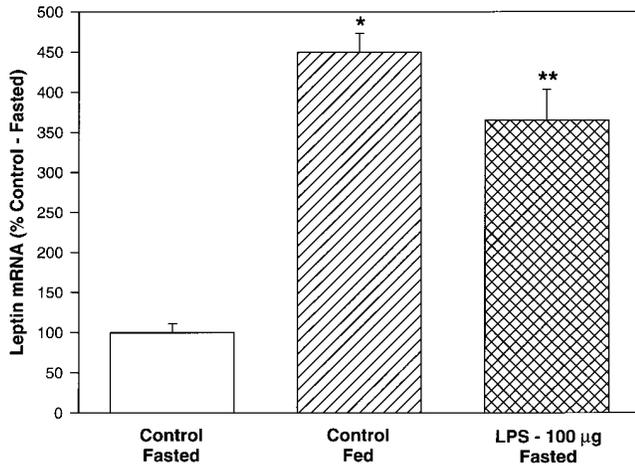
Figure 2. Comparison of leptin mRNA levels in adipose tissue from fasted, fed, and fasted LPS-treated hamsters. Control animals were given normal saline and fasted or fed *ad libitum* for 24 h. LPS-treated animals were given 100 μg LPS/100 g BW and then fasted for 24 h in parallel with the controls. Animals were anesthetized, epididymal adipose tissue was removed, and leptin mRNA prepared as described in Methods. $n = 5$ for each group. Values are mean±SEM. *$P < 0.001$ vs. fasted controls; **$P < 0.001$ vs. fasted controls, $P < 0.05$ vs. fed controls.

serum from *ob/ob* mice was spiked with recombinant leptin and immunoprecipitated in parallel. The precipitates were subjected to SDS polyacrylamide electrophoresis, transferred to nitrocellulose, and analyzed by Western blotting.

Statistics. For comparison among several groups, statistical significance was determined using ANOVA with Newman-Keul's as the post hoc test. For dose response curves, statistical significance was calculated using ANOVA with Dunnet's as the post hoc test.

Results

To quantify the effect of LPS on food intake and weight, we administered varying doses of LPS to hamsters housed in individual metabolic cages. LPS or saline was given at 4:00 p.m. (time 0), then weight and food intake were measured over the next 18 h and every 24 h thereafter for 3 more d. The lowest dose of LPS tested (0.1 μg/100 g body wt [BW][1]) induced a 44% decrease in food intake during the first 18 h, then food intake returned towards normal (Fig. 1 *A*). Doses of LPS between 1–100 μg/100 g BW induced a 90% decrease in the food intake over the first 18 h. Animals administered 1 μg LPS showed recovery of food intake over the next 3 d. Hamsters treated with 10 or 100 μg LPS/100 g BW had no food intake over the subsequent 24 h. Thereafter, hamsters given 10 μg LPS/100 g BW began to increase their food intake whereas those given 100 μg LPS/100 g BW remained anorectic over the entire 90 h of study.

Control animals treated with normal saline progressively gained weight during the experiment (Fig. 1 *B*). Weight gain tended to be blunted in hamsters treated with 0.1 μg LPS/100 g BW, but this did not reach significance. Hamsters given higher doses of LPS progressively lost weight over the first 24 h. Sub-
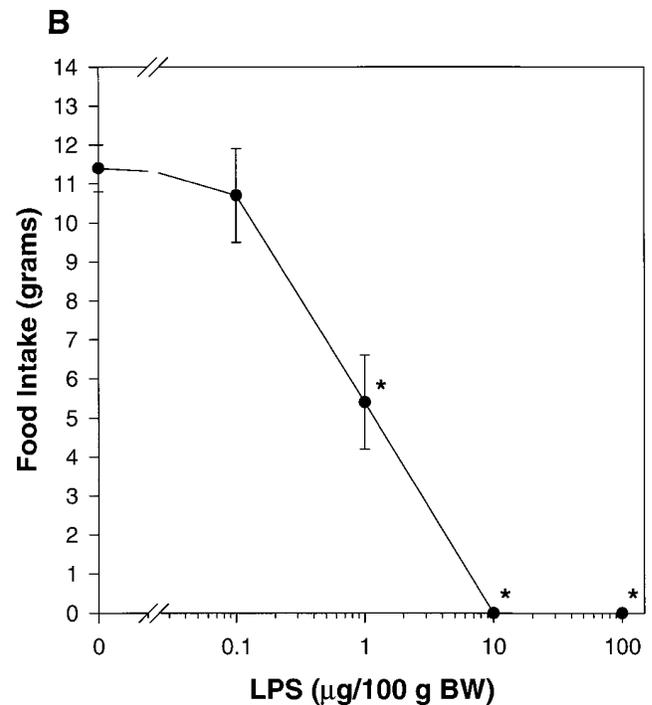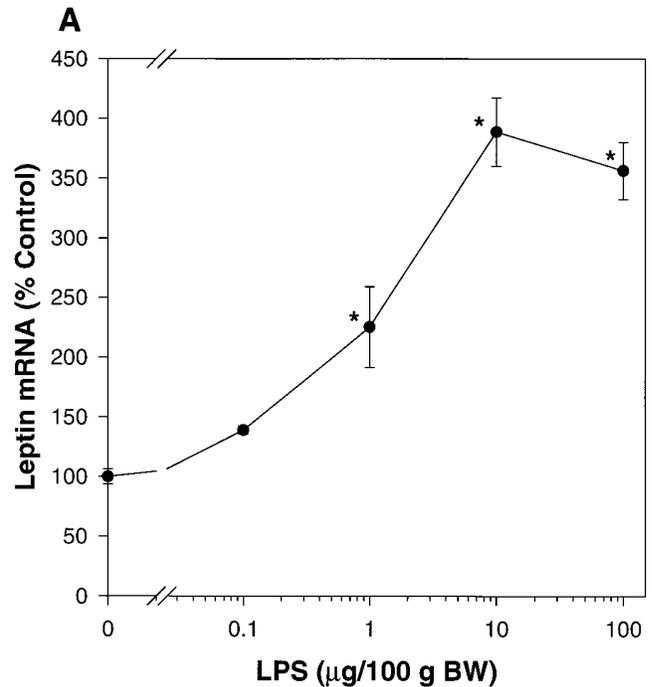
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1. *Abbreviation used in this paper:* BW, body weight.

Figure 3. LPS dose response curve. (*A*) Hamsters were administered saline or the dose of LPS indicated on the abscissa, then epididymal adipose tissue was removed at 16 h and leptin mRNA levels measured as described in Methods. All animals were fasted after injection to control for food intake. $n = 5$ for each group. *$P < 0.01$ vs. saline. (*B*) The data on food intake for the period from 18 to 42 h from Fig. 1 are plotted as a function of the dose of LPS administered. $n = 5$ for each group. Values are mean±SEM. *$P < 0.01$ vs. control.

sequently, those treated with 1 μg/100 g BW began to regain weight, while those treated with 10 or 100 μg LPS/100 g BW continued to lose weight (Fig. 1 *B*).

Similar to what has been reported by others (23, 24), we

A

B

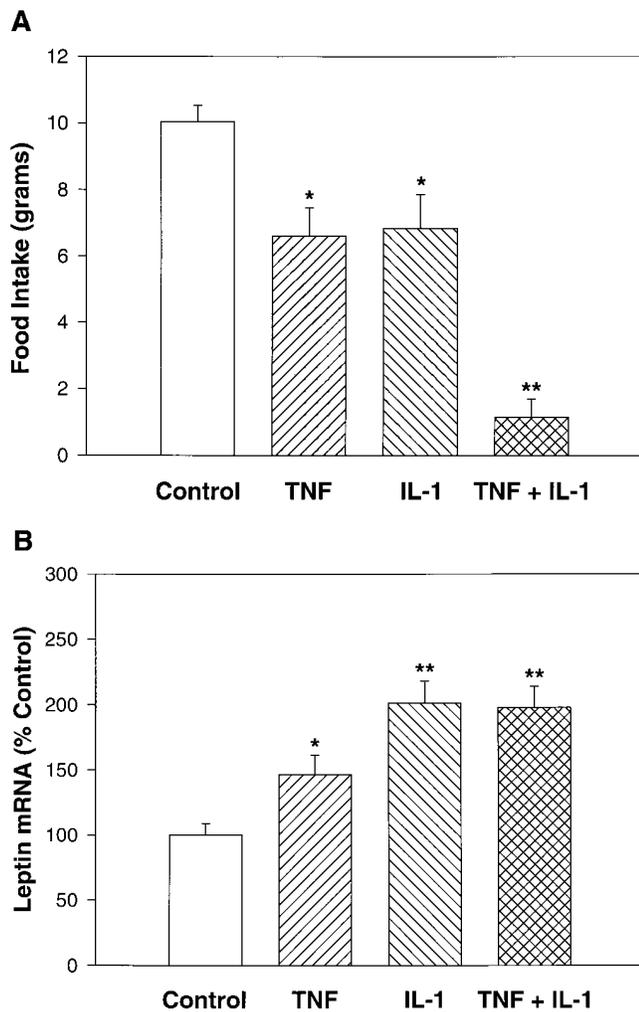*Figure 4.* Effect of cytokines on leptin mRNA and food intake. Hamsters were treated with 0.1% HSA (*control*), TNF (17 μg/100 g BW), IL-1 (1 μg/100 g BW), or TNF plus IL-1 as indicated. $n = 5$ for each group. Values are mean±SEM. (*A*) One set of animals was treated and allowed to eat ad libitum; food intake was measured in the first 18 h after 0.1% HSA or cytokine administration. *$P < 0.05$ vs. control; **$P < 0.001$ vs. control, TNF, and IL-1. (*B*) A second set of animals were treated in parallel, but food was withdrawn. At 8 h, animals were killed and epididymal adipose tissue processed for leptin mRNA levels. These animals were fasted after injection. *$P < 0.05$ vs. control; **$P < 0.001$ vs. control, $P < 0.05$ vs. TNF.

found that expression of leptin mRNA in epididymal adipose tissue (Fig. 2) is decreased in control hamsters that have been fasted for 24 h compared to fed hamsters. To explore whether leptin induction occurs during the anorexia of infection, we tested the effect of an anorexia-inducing dose of LPS on the levels of leptin mRNA in adipose tissue in animals that were subsequently fasted for 24 h. Despite fasting, LPS was able to induce levels of leptin mRNA that were the equivalent of the fed or satiated state (Fig. 2).

We next examined the dose response curve for LPS induction of leptin mRNA at 16 h in hamster epididymal adipose tissue (Fig. 3 *A*). The half maximal stimulatory dose was 1 μg LPS/100 g BW, with maximal stimulation occurring at 10 μg LPS/100 g BW. Fig. 3 *B* shows the dose response curve for food intake over the period from 18 to 42 h after LPS using
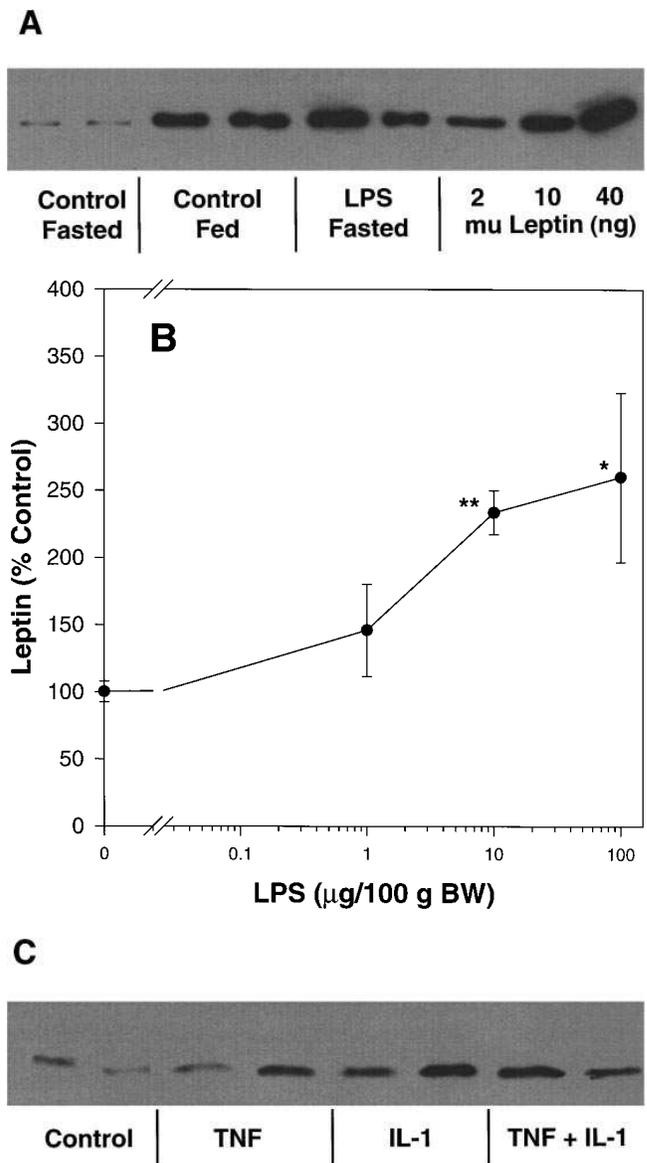
A

B

C

Figure 5. Effect of LPS on leptin protein in serum. Hamsters were treated with LPS, normal saline, cytokines, or 0.1% HSA, and then serum was obtained at the time indicated. Leptin was immunoprecipitated, subjected to gel electrophoresis, and Western blotted as described in Methods. (*A*) Control animals were given saline and either fasted or fed. LPS treated (100 μg/100 g BW) animals were fasted after treatment. Serum was obtained 24 h later. For comparison, serum from *ob/ob* mice was spiked with the indicated quantity of recombinant murine leptin per milliliter and analyzed in parallel. (*B*) Hamsters were administered normal saline or the indicated doses of LPS, then food was withdrawn. Serum was obtained 16 h later. Control serum contained 13.0±1.0 ng equivalent of murine leptin (hamster standard is not available). $n = 5$ for each point. *$P < 0.05$, **$P < 0.005$ vs. control. (*C*) Hamsters were given 0.1% HSA, TNF (17 μg/ 100 gm BW), IL-1 (1 μg/100 gm BW), or TNF plus IL-1, and then food was withdrawn. 8 h later, serum was obtained.

data from the experiment in Fig. 1 *A*. The half maximal dose for inhibition of food intake is 0.85 μg LPS/100 g BW, with maximal anorexia induced at 10 μ LPS/100 g BW. There is a strong inverse correlation between leptin mRNA expression and food intake. In separate experiments, we found that

16 h after LPS, leptin mRNA was also increased in omental (219±25%), subcutaneous (175±16%), and renal (149±17%) adipose tissue (data not shown).

Because cytokines mediate many of the immunological and metabolic effects of LPS, we tested the ability of the cytokines TNF and IL-1 to induce anorexia and increase leptin mRNA levels in adipose tissue. TNF or IL-1, given individually, induced a similar degree of anorexia over 18 h (Fig. 4 *A*). When TNF and IL-1 were given together, greater anorexia was induced (Fig. 4 *A*). 8 h after administering TNF, IL-1, or the combination of TNF plus IL-1, leptin mRNA levels were increased in epididymal adipose tissue (Fig. 4 *B*).

A quantitative assay for leptin protein from hamsters does not exist. Therefore, we used a polyclonal antibody to mouse leptin to immunoprecipitate leptin from hamster sera; the immunoprecipitates were run on SDS gels, transferred, and analyzed by Western blotting (24). Recombinant leptin standard was precipitated from serum of *ob/ob* mice and handled in parallel. As reported previously for mice (24), in hamsters, fasting is accompanied by low levels of leptin, while feeding induces higher levels of leptin in serum (Fig. 5 *A*). LPS administration to hamsters that were subsequently fasted increased leptin to levels similar to those seen in fed animals. A dose response for induction of leptin 16 h after LPS administration is shown in Fig. 5 *B*. There was a good correlation between the dose response for leptin protein levels (Fig. 5 *B*) and levels of leptin mRNA in adipose tissue (Fig. 3 *A*). These doses of LPS induced a 2.5-fold increase in serum leptin levels. Furthermore, cytokine treatment led to an increase in serum leptin (Fig. 5 *C*) that was similar to the change in leptin mRNA found in adipose tissue (Fig. 4 *B*).

Discussion

Many studies indicate that adipose tissue is the source of a circulating factor that regulates food intake, whose levels are increased when fat cell stores are replete (41). Recent studies have identified this factor as the hormone leptin, which is defective in the *ob/ob* mouse. When control animals or humans are calorically restricted and adipose tissue stores decrease, leptin mRNA and protein levels decrease, stimulating eating (23–26). Conversely, leptin mRNA levels are increased in adipose tissue in obesity (22, 42–45). Administration of leptin to normal and *ob/ob* mice leads to decreased food intake (27–29).

LPS administration, a well characterized model of infection, induces severe decreases in food intake and weight. In this paper we demonstrate that, in parallel, LPS led to a rapid increase of serum leptin levels and leptin mRNA in adipose tissue compared to fasted control animals. The level of leptin in the fasted LPS-treated animals approached that of fed controls, whereas in fasted controls, leptin levels are markedly suppressed. Additionally, the induction of leptin protein and mRNA at 16 h after LPS was inversely proportional to the food intake over the subsequent 24-h period.

The host response to infection is primarily mediated by cytokines of the immune system, in particular, TNF and IL-1. Here we confirm in the hamster the findings that cytokines induce anorexia. In parallel, we also demonstrate that TNF and/or IL-1 can increase leptin mRNA levels in adipose tissue and leptin protein in the circulation. Again, despite the decrease in food intake, which would normally suppress leptin expression, these cytokines increase leptin expression.

The studies presented here suggest that leptin induced by LPS and cytokines directly contributes to the anorexia seen during infection. Alternatively, the induction of leptin may prevent the body's normal compensatory mechanisms in the face of decreased food intake. The decrease in leptin levels that is normally seen in fasted animals compared to fed animals is a stimulus for eating and other associated behaviours. LPS, by preventing the decrease in leptin levels that would normally be induced by decreased food intake, would bypass this homeostatic response, eliminating a signal to eat.

Multiple factors are involved in mediating food intake (31). Therefore it is likely that multiple factors are involved in mediating the anorexia of infection. Previous data indicate that cytokines increase hypothalamic levels of CRH, a central nervous system neurotransmitter that suppresses food intake, and suppress the firing of glucose-sensitive neurons, which would also decrease food intake (8, 32). A role for prostaglandins in mediating the anorectic effects of LPS and cytokines has also been proposed (16, 17). In the periphery, LPS and cytokines have been shown to decrease gastric emptying and to regulate hormones such as CCK, glucagon, insulin, and corticosteroids, all of which can influence food intake (8, 32). Insulin and corticosteroids have been shown to regulate leptin mRNA (23, 46). The finding that LPS and cytokines stimulate leptin production suggests that neuropeptide Y may also be involved in the anorexia of infection. Neuropeptide Y increases food intake and leptin decreases hypothalamic levels of neuropeptide Y (30). Further studies will be needed to determine which of these factors are involved in cytokine induction of leptin, which are induced by leptin, and which act in conjunction with leptin to induce anorexia.

In addition to regulating food intake, leptin regulates activity. For example, leptin infusion into *ob/ob* mice restores depressed stereotypic behavior activity and increases metabolic rate to normal (27–29). Whether leptin mediates some of the hypermetabolism induced by LPS or plays a role in the physical responses to LPS remains to be determined.

In summary, we have shown that LPS, TNF, and IL-1 induce leptin even in the face of anorexia. The decrease in food intake is inversely proportional to the increase in leptin. These data suggest a role for leptin in the anorexia of infection. Given the prominent role that anorexia plays in the wasting syndrome, it is possible that development of leptin antagonists may play a useful role in ameliorating the anorexia and wasting of infections such as AIDS.

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