Acute starvation alters lipopolysaccharide-induced fever in leptin-dependent and -independent mechanisms in rats

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Inoue W, Luheshi GN. Acute starvation alters lipopolysaccharide-induced fever in leptin-dependent and -independent mechanisms in rats. Am J Physiol Regul Integr Comp Physiol 299: R1709–R1719, 2010. First published October 13, 2010; doi:10.1152/ajpregu.00567.2010.—A decrease in leptin levels with the onset of starvation triggers a myriad of physiological responses including immunosuppression and hypometabolism/hypothermia, both of which can counteract the fever response to pathogens. Here we examined the role of leptin in LPS-induced fever in rats that were fasted for 48 h prior to inflammation with or without leptin replacement (12 μg/day). The preinflammation fasting alone caused a progressive hypothermia that was almost completely reversed by leptin replacement. The LPS (100 μg/kg)-induced elevation in core body temperature ($T_{core}$) was attenuated in the fasted animals at 2–6 h after the injection, an effect that was not reversed by leptin replacement. Increasing the LPS dose to 1,000 μg/kg caused a long-lasting fever that remained unabated for up to 36 h after the injection in the fed rats. This sustained response was strongly attenuated in the fasted rats whose $T_{core}$ started to decrease by 18 h after the injection. Leptin replacement almost completely restored the prolonged fever. The attenuation of the prolonged fever in the fasted animals was accompanied by the diminution of proinflammatory PGE$_2$ in the cerebrospinal fluid and mRNA of proopiomelanocortin (POMC) in the hypothalamus. Leptin replacement prevented the fasting-induced reduction of POMC but not PGE$_2$. Moreover, the leptin-dependent fever maintenance correlated closely with hypothalamic POMC levels ($r = 0.77, P < 0.001$). These results suggest that reduced leptin levels during starvation attenuate the sustained fever response by lowering hypothalamic POMC tone but not PGE$_2$ synthesis.

Fever is a common response to various types of infection and constitutes an important component of an adaptive strategy for fighting disease (18, 29). The development of fever is a finely tuned, complex event that involves both the immune and the central nervous systems through which a series of inflammatory, thermoregulatory, and metabolic processes are regulated (46). The sum of the changes required to mount an effective fever response is an energy-demanding process that can be influenced by the energy status of the host. This was clearly shown by a number of studies reporting that malnutrition prior to the induction of inflammation compromises the fever response in various species of experimental animals (20, 21, 24, 28, 30, 53, 54, 60). The suggested explanations for this observation include suppression of inflammatory responses (20, 21, 53), an alteration of the thermoregulatory response (24, 30), and an attenuation of thermogenic effector activity (28, 54). However, it remains unclear how the signal relating to the reduced energy status of the host is relayed to the aforementioned fever-related functions and subsequently alters the overall response.

Among the multiple metabolic and hormonal changes that occur during malnutrition is the reduction in leptin levels (1, 6). This adipose tissue-derived hormone was originally identified as an important satiety factor that prevents excessive weight gain by signaling the presence of sufficient energy stores (i.e., fat mass) (17). However, it is now well established that leptin also signals the transition from energy balance sufficiency to insufficiency. The levels of this hormone decrease rapidly with the onset of caloric deprivation and the prevention of this reduction by administering exogenous leptin blunts a number of physiological changes known to occur during starvation (2, 15). The reported roles of leptin in the adaptation to starvation are broad, affecting a wide variety of functions including neuroendocrine (2), immune (22, 33), behavioral (11, 19), and metabolic (8, 41) adaptations. All of these functions serve as important prerequisites for an effective fever response during inflammation. For example, a decrease in leptin levels is one of the major contributors to immunosuppression during starvation (22, 33). Administration of exogenous leptin in food-deprived mice improves the host defense response against bacterial infection, in part by recovering the impaired phagocytic activity of macrophages, neutrophil mobilization, and cytokine production (36, 48). In the brain, leptin regulates expression and release of various neuropeptides involved in energy balance regulation (25). The reduction of leptin signaling shifts the balance between catabolic [corticotrophin-releasing factor (CRF), α-melanocyte-stimulating hormone (MSH), β-endorphin, and cocaine and amphetamine-related transcript (CART)] and anabolic [neuropeptide Y (NPY) and agouti-related peptide (AGRP)] neuropeptide signaling toward the latter (25). Although, such a shift in neuropeptide balance has been repeatedly linked to the enhanced appetite of starved animals, it can also account for the occurrence of a regulated hypometabolism/hypothermia associated with starvation (39, 47, 50, 59, 64). Collectively, the available data indicate that leptin serves as a permissive signal for the immune, thermoregulatory, and metabolic functions when energy balance is sufficient. Despite the strong functional link between leptin and the aforementioned important prerequisites for an effective fever response, the exact role of this hormone in starvation-induced fever alteration has never been tested. The aim of the present study was to address whether and how the decline in leptin levels affects the fever response resulting from a single systemic injection of LPS in rats that have been food deprived for 48 h.
MATERIALS AND METHODS

Animals

All experimental procedures were approved by the Animal Care Committee of McGill University pursuant to the Canadian Council of Animal Care guidelines. Adult female Sprague-Dawley rats with initial body weights of 200–225 g (Charles River, Saint Constant, QC, Canada) were used in all experiments. They were housed individually in a controlled environment at an ambient temperature of 21 ± 1.5°C on a 12:12-h light-dark cycle (lights on from 0800 h to 2000 h), with free access to water and standard laboratory chow (rat chow, cat. no. 5012; Purina, St. Louis, MO) unless otherwise indicated.

Experimental Design

Experiments were performed over 5 days; 1 day for baseline measurement (day 0) followed by 4 days of feeding manipulation (days 1–4). Animals were weighed daily between 0930 h and 1030 h. Rats were initially divided into four weight-matched feeding × drug groups (fed-vehicle, fed-leptin, fasted-vehicle, and fasted-leptin). On day 1, Alzet miniosmotic pumps (0.5 μl/h, model 1007D; Durect, Vacaville, CA) containing either saline or recombinant rat leptin (1 μg/μl, 12 μg/day; PeproTec, Rocky Hill, NJ) were implanted subcutaneously in the dorsal midline caudal to the scapulae of the animals under isoflurane anesthesia. Lidocaine (2%, 25–50 μl) was applied to the area of incision. Surgeries were performed between 1000 h and 1200 h, and thereafter food was removed from the fasted groups. All groups of rats had free access to water throughout the experimental period. On day 3, each group was subdivided into three treatment groups (n = 6–8 per group) receiving a single intraperitoneal injection of either 100 or 1,000 μg/kg of LPS (Escherichia coli O111:B4, lot 42k4120; Sigma, St. Louis, MO) or 1 ml/kg saline as a control between 0930 h and 1030 h (−48 h after the start of fasting/drug infusion).

Measurement of Body Temperature Using Remote Biotelemetry

Precalibrated temperature-sensitive radio transmitters (model TA10TA-F40; Data Sciences, St Paul, MN) were implanted via midline incision into the abdominal cavity of anesthetized rats (intramuscular; 50 mg/kg ketamine hydrochloride, 5 mg/kg xylazine hydrochloride, 0.5 mg/kg acepromazine maleate; total volume: 1 ml/kg). The level of anesthesia was assessed by the withdrawal reflex to a toe pinch. Lidocaine (2%, 25–50 μl) was applied topically to the area of incision. Animals were allowed to recover for at least 7 days prior to experimentation. Transmitter output frequency (Hz) was monitored at 10 min intervals by an antenna mounted in a receiver board situated beneath the cage of each animal. The output data from each transmitter were transformed into degrees centigrade using Dataquest A.R.T. software (Data Sciences).

Sample Collection

To assess the levels of PGs in the cerebrospinal fluid (CSF) and to measure the expression levels of inflammatory and neuropeptide genes in the hypothalamus, CSF and brains were collected from the fed and fasted rats treated with either saline (1 ml/kg ip) or LPS (1,000 μg/kg ip) (n = 5–6 per group). Animals were killed 30 h after the injection under deep anesthesia with a terminal dose of pentobarbital sodium (60 mg/kg ip). CSF was sampled from the cisterna magna with a 27-gauge needle connected to a microsyringe (250 μl) via polyethylene-20 tubing. The sampled CSF was immediately frozen on dry ice and kept at −80°C. Blood was collected from the same animals via cardiac puncture by using sterile heparinized syringes, and plasma samples were prepared for cytokine assay. Animals were then perfused with ice-cold saline, the brains removed, and the hypothalami dissected, frozen on dry ice, and kept at −80°C until use.

Cytokine and Prostaglandin Measurement

Sandwich ELISAs for cytokines (NIBSC, Potters Bar, UK) were performed as previously described (24). Plasma samples were diluted 1:10 for leptin and 1:5 for TNF, IL-1β, and IL-6. Intra-assay and interassay variations were > 10%. The sensitivity of the assay was 15.6 pg/ml for leptin and 7.8 pg/ml for TNF, IL-1β, and IL-6. All samples were assayed in duplicate. PGE2 and 15-deoxy-D12,14-PGJ2 (15-0-PGJ2) levels in CSF were measured using an enzyme immunoassay (EIA) kit (PGE2; EIA kit, monocolonal; Cayman Chemical, Ann Arbor, MI; 15-deoxy-D12,14-PGJ2; EIA kit, Assay Designs, Ann Arbor, MI) according to the manufacturer’s protocol. The CSF samples were diluted 1:5 in an assay buffer provided as part of the kit. Intra-assay and interassay variations were < 10%. The sensitivity of the assay for PGE2 and 15-0-PGJ2 was 7.8 pg/ml and 36.8 pg/ml, respectively. All samples were assayed in duplicate.

RNA Extraction and Quantitative RT-PCR

Total RNA was extracted from the hypothalamus in 1 ml of Trizol (Invitrogen, Burlington, Ontario, Canada) according to the manufacturer’s protocol. The first-strand cDNA was synthesized from 1 μg of total RNA using 200 units of Moloney murine leukemia virus reverse transcriptase (Invitrogen), 5 μM of random hexamers (Applied Bioscience, Streetsville, ON, Canada), and 1 μM of dNTP mix (Sigma) in a total reaction volume of 20 μl. The cDNA product was diluted with autoclaved distilled water for a final volume of 200 μl and stored at −20°C until used. Quantitative RT-PCR was carried out in duplicate using preoptimized primer/probe mixture (TaqMan Gene Expression Assays; Applied Biosystems, ON, Canada) and TaqMan universal PCR master mix (Applied Biosystems) on a 7500 real-time PCR System (Applied Biosystems). The cDNA quantities between different reactions were normalized by using a housekeeping gene β-actin (cat. no. 4352640E; Applied Biosystems) as a reference. The sample values represent X-fold differences from a control sample (given a designated value of 1) within the same experiment. The assay identification for each gene are as follows: IL-1β (Rn00850432_m1); cyclooxygenase (COX)-2 (Rn01483828_m1); microsomal PGE synthase (mPGE2) (Rn00572047_m1); macrophage inflammatory protein (MIP)-β (Rn00587286_m1); endothelin-1 (Rn00561129_m1); nitric oxide synthase-1 (NOS-1) (Rn00583793_m1); NOS-2 (Rn00561646_m1); NOS-3 (Rn02132634_s1); NPY (Rn01410145_m1); proopiocortin (POMC) (Rn00595020_m1); AGRP (Rn01431703_g1); CART (Rn00567382_m1); CRF (Rn01462137_m1).

Measurement of Body Temperature

Two measures have been used to assess the fever response: absolute core body temperature (Tcore) and the rise in Tcore (ΔTcore: ΔTcore = Tcore − preinjection Tcore). However, these two measures are not interchangeable when animals have different preinjection Tcore (e.g., day/night variation and different feeding conditions as in the present study). Feng et al. (14) demonstrated that absolute Tcore rather than ΔTcore provides a better representation of the physiological characteristic of fever because the former is solely determined by the dose of pyrogen (PGE2) and is largely unaffected by day/night variation in preinjection Tcore. The validity of this definition was supported by several other studies investigating the fever response in animals with different preinjection Tcore (26, 27, 35). Therefore, in the present study we used absolute Tcore rather than ΔTcore to assess the fever response.

To evaluate the overall change in Tcore by pyrogenic stimuli in the fed and fasted animals, preinjection Tcore and postinjection Tcore were calculated. The preinjection Tcore was defined as the average Tcore during the 1 h prior to the injection. The postinjection Tcore was defined as the average Tcore 2–8 h after the injection for the rising phase and 26–32 h for the sustained phase.
Statistics

All data are presented as mean values ± SE and were analyzed using Sigma Stat software (SPCC, Chicago, IL). Two-way repeated-measures ANOVA (time × treatment) was used to analyze the data from body weight change and temperature expression studies. Because of nonnormal distribution of the mRNA expression levels of some of the genes examined, quantitative PCR data were transformed with the natural logarithm prior to analysis. Association between Tcore and biochemical variables during LPS-induced inflammation was calculated by Pearson’s correlation coefficient (r) among LPS-treated animals (excluding the saline-treated control). Two-way between-factors ANOVA (feeding × leptin) was used to analyze the data among LPS-treated animals. Newman-Keuls multiple comparison test was performed as post hoc analysis when applicable. The effects of LPS within-fed-vehicle animals were tested with Student’s t-test. In all cases, P values < 0.05 were deemed statistically significant.

RESULTS

Changes in Body Weight During Fasting, Leptin Infusion, and LPS-Induced Inflammation

Table 1 shows the body weight changes of the animals in all treatment groups. The fed and fasted rats were implanted with an osmotic pump for vehicle or leptin (12 µg/day) infusion on day 1, and thereafter the rats in the fasted groups were deprived of food until the end of the experiment. The initial body weight on day 1 was similar across all groups and decreased significantly following food deprivation. Leptin infusion significantly decreased the body weight under ad libitum-fed conditions compared with vehicle infusion, verifying the physiological effect of the dose of leptin used in the present study. In the fasted groups, however, leptin caused no additional body weight loss compared with the fasted-vehicle counterpart. On day 3, the animals received a single intraperitoneal injection of saline or two different doses of LPS (100 or 1,000 µg/kg) at a time point ~48 h after the start of drug infusion/feeding manipulation. LPS injection resulted in a dose-dependent reduction of body weight 24 h after treatment in the fed groups. In contrast, neither dose of LPS injection in the fasted groups caused further loss of body weight compared with the saline-treated counterpart. Leptin infusion did not cause additional body weight loss following LPS injection regardless of the feeding conditions.

Effect of Leptin on Basal Body Temperature

Figure 1 shows the daily changes of Tcore of the animals treated with saline on day 3 as an example of the circadian Tcore cycles under noninflammatory conditions. The animals in all treatment groups displayed clear day/night variations of Tcore that were higher in the active (dark) and lower in the inactive (light) phase. Leptin infusion at a physiological dose (12 µg/day) had no effect on either the baseline Tcore or its daily rhythm under ad libitum-fed conditions (Fig. 1A), a result consistent with a previous study using a similar dose of leptin (8). In the fasted groups, however, leptin infusion prevented the development of hypothermia that was evident in vehicle-infused rats on days 2 and 3 (Fig. 1B), confirming previous reports that the fasting-induced hypothermia is largely mediated by the reduction of leptin in rats (59) and mice (8). After the Tcore recording around 1200 on day 4 (30 h after saline injection), all animals were killed and blood leptin levels verified (Fig. 1C). As expected, fasting significantly reduced the plasma levels of leptin and leptin infusion increased the hormone levels.

Role of Leptin in the Altered Fever Response Following Food Deprivation

Early phase. We examined whether the repletion of leptin during fasting affects the fever response. The animals received a single intraperitoneal injection of saline or two different doses of LPS (100 or 1,000 µg/kg) between 1000 h and 1030 h on day 3, a time point ~48 h after the start of drug infusion/feeding manipulation. As described above, leptin repletion effectively prevented the fasting-induced hypothermia in saline-injected groups, reversing the lowered Tcore to levels similar to those of the fed animals (Fig. 2A). All groups of rats transiently elevated their Tcore in response to saline injection, presumably due to the stress associated with the handling. Interestingly, the stress-induced hyperthermia of the hypothalamic fasted-vehicle rats reached a level similar to the normothermic groups, implying that the thermogenic capacity of the fasted rats remains relatively intact. Following the injection of the lower dose of LPS (100 µg/kg), the Tcore of the fasted rats was consistently lower than those of the fed counterparts (Fig. 2B; 60–340 min, P < 0.05 for fed-vehicle vs. fasted-vehicle), confirming our recent study using the same protocol (24). However, leptin repletion in the fasted rats did not reverse the reduction in Tcore. In the fed rats, leptin infusion slightly blunted the elevation of Tcore that reached statistical significance at a few time points (140 and 260 min, P < 0.05 for fed-vehicle vs. fed-leptin). Because the leptin system is often implicated in severe systemic inflammation (13, 57), we also examined the fever response to a higher dose of LPS (1,000

Table 1. Body weight changes

<table>
<thead>
<tr>
<th></th>
<th>Saline 100 g/kg LPS</th>
<th>1000 g/kg LPS</th>
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<tbody>
<tr>
<td><strong>Fed</strong></td>
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<tr>
<td>Day 1 baseline body weight, g</td>
<td>273.7 ± 17.8</td>
<td>269.4 ± 14.3</td>
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<tr>
<td>Day 3 body weight at injection, g</td>
<td>273.5 ± 15.7</td>
<td>267.3 ± 14.1*</td>
</tr>
<tr>
<td>24-h body weight change after injection, %</td>
<td>0.2 ± 2.1</td>
<td>0.1 ± 1.3</td>
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<tr>
<td><strong>Fasted</strong></td>
<td></td>
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</tr>
<tr>
<td>Day 1 baseline body weight, g</td>
<td>258.5 ± 8.5</td>
<td>257.3 ± 12.3</td>
</tr>
<tr>
<td>Day 3 body weight at injection, g</td>
<td>221.3 ± 10*</td>
<td>223 ± 9.9*</td>
</tr>
<tr>
<td>24-h body weight change after injection, %</td>
<td>-5.8 ± 2.2</td>
<td>-5.7 ± 1.5</td>
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</table>

Values are means ± SE; numbers in parenthesis are number of animals. *Two-way ANOVA (leptin × time); P < 0.01 for drug × time; post hoc, P < 0.05 within leptin, body weight at injection vs. baseline; †two-way ANOVA (leptin × time); P < 0.001 for time; ‡two-way ANOVA (drug × injection); P < 0.001 for injection; post hoc P < 0.001 vs. saline; §two-way ANOVA (drug × injection); P < 0.001 for injection; post hoc P < 0.01 vs. 100 g/kg LPS.
μg/kg). Fasting likewise attenuated the increase in $T_{core}$, but this effect was less prominent than with the lower dose (Fig. 2C). Specifically, the $T_{core}$ of the fasted rats was significantly attenuated (relative to fed rats) only at the very early phase of the fever response (60–160 min, $P < 0.05$, fed-vehicle vs. fasted-vehicle); thereafter the $T_{core}$ increased to a level similar to those of fed counterparts. Leptin treatment in the fasted rats had no effect on the $T_{core}$ at any time during the fever response. In the fed rats, leptin infusion exaggerated the initial hypothermia (80 min, $P < 0.05$ for fed-vehicle vs. fed-leptin), but thereafter had no effect on the elevation of $T_{core}$.

Fig. 2. $T_{core}$ change after LPS injection: the rising phase. $T_{core}$ course of rats treated with an intraperitoneal injection of saline (A), 100 μg/kg LPS (B), or 1,000 μg/kg LPS (C) at time point 0 as indicated by a thick arrow. $T_{core}$ is plotted every 20 min. Values are means ± SE for 6–8 rats. Two-way repeated-measures (treatment × time) ANOVA, $P < 0.001$ for interaction, $P < 0.001$ for feeding, and $P < 0.05$ for leptin. DL, detection limit (0.15 ng/ml).
Late phase. Previous studies (including our own) reporting an altered fever response in fasted animals all focused their observation on the rising phase of fever, typically up to 3–8 h following pyrogen administration (24, 28, 30, 53, 54, 60). Less is known about the effects of the fasting and the accompanying reduction of leptin on the fever response beyond the early phase. In the present study, we found that fasting strongly attenuated the fever response at a later stage and that this effect was leptin dependent, which was more prominent with the higher dose of LPS. In response to the lower LPS dose (100 μg/kg) the fed rats maintained a moderate but significant elevation of $T_{\text{core}}$ well beyond the early phase ($\sim 36$ h; Fig. 3A). Leptin infusion in the fed rats did not affect the $T_{\text{core}}$ during this sustained fever phase. On the other hand, the $T_{\text{core}}$ of the fasted rats progressively decreased and deviated from that of the fed counterparts (13–24 h and 26–36 h, $P < 0.05$ for fed-vehicle vs. fasted-vehicle). Leptin repletion in the fasted group partially, yet significantly, prevented the decline of $T_{\text{core}}$ (28–36 h, $P < 0.05$ for fasted-vehicle vs. fasted-leptin). Figure 3B shows the average $T_{\text{core}}$ of the early (2–8 h, the period shown in Fig. 2) and the late (26–32 h, 24 h after the early period) phase of the fever. This result clarifies the following points. First, fasting decreased the $T_{\text{core}}$ both during the early and late phase, whereas leptin repletion reversed the $T_{\text{core}}$ decline only in the late but not in the early phase. Second, the average $T_{\text{core}}$ was lower in the late than the early phase in all treatment groups, indicating that the lower LPS dose-fever started to resolve in the late period.

The maintenance of fever and the effect of leptin on $T_{\text{core}}$ in the late phase were more evident with the higher dose of LPS (1,000 μg/kg). The fever of the fed group remained unabated until the end of the recording period ($\sim 36$ h; Fig. 3C), whereas the $T_{\text{core}}$ of the fasted rats decreased from that of the fed counterparts (17–36 h, $P < 0.05$ for fed-vehicle vs. fasted-vehicle). Leptin repletion in the fasted group almost completely prevented the decline of $T_{\text{core}}$ (20–27 h and 29–36 h, $P < 0.05$ for fasted-vehicle vs. fasted-leptin); whereas leptin infusion had no effect in the fed group. Figure 3D clarifies that the fed group maintained the elevated $T_{\text{core}}$ during the late phase to a level similar to the early phase ($P > 0.05$, early phase vs. late phase). By contrast, the fasted rats failed to sustain the febrile $T_{\text{core}}$; the late phase $T_{\text{core}}$ was significantly lower than the early phase ($P < 0.01$, early phase vs. late phase). Leptin infusion in the fasted rats prevented the decline of the sustained fever ($P > 0.05$, early phase vs. late phase); the $T_{\text{core}}$ was recovered to a level similar to the fed group ($P > 0.05$, fed-vehicle vs. fasted-leptin). These results indicate that fasting diminishes the sustained phase of fever through a leptin-dependent mechanism, and that this phenomenon is more evident in a longer-lasting fever induced by a relatively more severe inflammation.

Fig. 3. $T_{\text{core}}$ change after LPS injection: the sustained phase. $T_{\text{core}}$ course of rat over 36 h after an intraperitoneal injection of 100 μg/kg LPS (A) and 1,000 μg/kg LPS (C) at time point 0 as indicated by an thick arrow. A thin arrow indicates the time when animals are handled for body weight measurement. $T_{\text{core}}$ is plotted every 1 h. *$P < 0.05$, fed-vehicle vs. fed-leptin; †$P < 0.05$, fed-vehicle vs. fasted-vehicle; ‡$P < 0.05$, fasted-vehicle vs. fasted-leptin. The average pre- and postinjection $T_{\text{core}}$ of rats treated with 100 μg/kg LPS (B) and 1,000 μg/kg LPS (D). ##$P < 0.01$, ###$P < 0.001$. All values are means ± SE for 6–8 rats.
Mechanisms of Leptin-Mediated Maintenance of the Fever Response

In the second set of experiments, we sought to understand how lowered leptin levels during fasting compromise the sustained $T_{core}$ elevation during the late fever phase. To this end, we focused on the higher dose of LPS and examined the following variables during the sustained phase of LPS-induced fever: 1) circulating levels of pyrogenic cytokines, 2) febrigenic inflammatory response in the brain, and 3) the levels of neuropeptides involved in metabolism/thermoregulation in the hypothalamus. Four groups of rats [(fed or fasted) × (vehicle or leptin)] were injected with LPS (1,000 μg/kg ip), and an additional fed-vehicle group received an intraperitoneal saline injection as a control. All animals were killed 30 h after injection, a time point when leptin repletion significantly reversed fever attenuation in the fasted rats. The leptin-dependent alteration of fever during fasting was confirmed in this cohort of animals, and predicted changes in plasma leptin levels were verified (Table 2).

Febrigenic Inflammatory Responses in the Periphery and the Brain

Plasma levels of TNF, IL-1β, and IL-6, all well-recognized pyrogenic cytokines (34), were not elevated above the detection limit of the assay (39 pg/ml for all cytokines), indicating that peripheral inflammatory response were resolved by this time point (30 h).

We next examined whether the fever alteration was attributable to the changes in febrigenic inflammatory response at the level of the brain (Table 3 and Fig. 4). When compared among fed groups, LPS treatment significantly increased the concentration of PGE₂ in the CSF ($P < 0.001$, saline vs. LPS) and mRNA levels of the genes involved in PGE₂ synthesis ($P < 0.05$ for COX-2 and mPGES-1, $P < 0.001$ for IL-1β) in the hypothalamus, confirming that the central febrigenic inflammatory response was still sustained at this time point. Among the LPS-injected animals, CSF PGE₂ levels, as well as mRNA levels of COX-2, mPGES-1, and IL-1β were all positively associated with the magnitude of sustained fever as

### Table 2. Pre- and postinjection core body temperature ($T_{core}$) and plasma leptin level 30 h after injection

<table>
<thead>
<tr>
<th></th>
<th>Fed-Saline</th>
<th>Fed-LPS</th>
<th>Fasted-LPS</th>
<th>Linear Fit</th>
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<tr>
<td></td>
<td>Vehicle (5)</td>
<td>Vehicle (6)</td>
<td>Leptin (6)</td>
<td>Leptin (6)</td>
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<tr>
<td>$T_{core}$, °C</td>
<td></td>
<td></td>
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<tr>
<td>Preinjection</td>
<td>37.0 ± 0.16</td>
<td>37.11 ± 0.12</td>
<td>37.03 ± 0.08</td>
<td>36.26 ± 0.14$^b$</td>
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<td>Postinjection</td>
<td>37.1 ± 0.11</td>
<td>38.25 ± 0.06$^a$</td>
<td>38.13 ± 0.05$^c$</td>
<td>37.32 ± 0.18$^b$</td>
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<td>Plasma leptin, ng/ml</td>
<td>0.84 ± 0.42</td>
<td>0.94 ± 0.34</td>
<td>2.91 ± 0.35</td>
<td>&lt;0.15</td>
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Values are means ± SE; numbers in parenthesis are number of animals. $^aP < 0.001$, t-test (saline vs. LPS); $^bP < 0.001$ for feeding effect, 2-way ANOVA (feeding × leptin); $^cP < 0.05$ for leptin effect, 2-way ANOVA (feeding × leptin); $^dP < 0.05$, $^eP < 0.001$ for post hoc test vehicle vs. leptin within fasted-LPS.

### Table 3. Biochemical variables in the brain at 30 h after injection

<table>
<thead>
<tr>
<th></th>
<th>Fed-Saline</th>
<th>Fed-LPS</th>
<th>Fasted-LPS</th>
<th>Linear Fit</th>
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<td>Vehicle (5)</td>
<td>Vehicle (6)</td>
<td>Leptin (6)</td>
<td>Leptin (6)</td>
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<td>CSF PGs, pg/ml</td>
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<tr>
<td>PGE₂</td>
<td>110.56 ± 31.38</td>
<td>774.52 ± 124.59$^b$</td>
<td>799.04 ± 195.45</td>
<td>441.33 ± 143.28$^a$</td>
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<tr>
<td>15-deoxy-PGE₂</td>
<td>666.7 ± 198.24</td>
<td>406.44 ± 79.14</td>
<td>628.5 ± 115.71</td>
<td>684.52 ± 134.78</td>
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<td>Hypothalamus inflammation-related mRNA, ln fold change</td>
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<td>COX-2</td>
<td>0.03 ± 0.04</td>
<td>0.36 ± 0.11$^a$</td>
<td>0.29 ± 0.07</td>
<td>0.17 ± 0.09</td>
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<tr>
<td>mPGES-1</td>
<td>−0.04 ± 0.03</td>
<td>0.23 ± 0.09$^a$</td>
<td>0.19 ± 0.05</td>
<td>0.06 ± 0.06</td>
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<td>IL-1</td>
<td>−0.53 ± 0.15</td>
<td>1.93 ± 0.12$^b$</td>
<td>1.6 ± 0.21</td>
<td>1.25 ± 0.26$^d$</td>
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<tr>
<td>MIP-1</td>
<td>−0.91 ± 0.23</td>
<td>0.51 ± 0.19$^a$</td>
<td>0.46 ± 0.14</td>
<td>0.21 ± 0.19</td>
</tr>
<tr>
<td>Endothelin-1</td>
<td>0.12 ± 0.07</td>
<td>−0.03 ± 0.09</td>
<td>−0.09 ± 0.15</td>
<td>−0.17 ± 0.16</td>
</tr>
<tr>
<td>NOS-1</td>
<td>0.29 ± 0.12</td>
<td>−0.06 ± 0.07$^a$</td>
<td>0.06 ± 0.08</td>
<td>−0.01 ± 0.08</td>
</tr>
<tr>
<td>NOS-2</td>
<td>0.04 ± 0.04</td>
<td>2.43 ± 0.31$^a$</td>
<td>2.46 ± 0.28</td>
<td>2.5 ± 0.68</td>
</tr>
<tr>
<td>NOS-3</td>
<td>0.12 ± 0.13</td>
<td>0.05 ± 0.12</td>
<td>0.1 ± 0.08</td>
<td>0.13 ± 0.13</td>
</tr>
<tr>
<td>Hypothalamus metabolism-related mRNA, ln fold change</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRF</td>
<td>0.09 ± 0.04</td>
<td>0.04 ± 0.08</td>
<td>0.13 ± 0.07</td>
<td>−0.13 ± 0.07$^c$</td>
</tr>
<tr>
<td>CART</td>
<td>−0.14 ± 0.09</td>
<td>−0.24 ± 0.07</td>
<td>−0.19 ± 0.03</td>
<td>−0.31 ± 0.06</td>
</tr>
<tr>
<td>NPY</td>
<td>−0.02 ± 0.06</td>
<td>0.04 ± 0.08</td>
<td>−0.19 ± 0.06</td>
<td>0.09 ± 0.12$^d$</td>
</tr>
<tr>
<td>AGRP</td>
<td>0 ± 0.03</td>
<td>0.49 ± 0.08$^b$</td>
<td>0.32 ± 0.07</td>
<td>0.68 ± 0.09$^e$</td>
</tr>
<tr>
<td>POMC</td>
<td>0.05 ± 0.06</td>
<td>0.03 ± 0.08</td>
<td>−0.03 ± 0.04</td>
<td>−0.41 ± 0.07$^f$</td>
</tr>
<tr>
<td>AVP</td>
<td>−0.19 ± 0.13</td>
<td>−0.38 ± 0.2</td>
<td>0.06 ± 0.2</td>
<td>−0.02 ± 0.16</td>
</tr>
</tbody>
</table>

Values are means ± SE; numbers in parenthesis are number of animals. CSF, cerebrospinal fluid; COX, cyclooxygenase; mPGES, microsomal PGE synthase; MAP, macrophage inflammatory protein; NO, nitric oxide synthase; CRF, crotrophin-releasing factor; CART, cocaine and amphetamine-related transcript; NPY, neuropeptide Y; AGRP, agouti-related peptide; POMC, proopiomelanocortin; AVP, arginine vasopressin; NS, not significant. $^aP < 0.05$; $^bP < 0.001$, t-test (saline vs. LPS); $^cP < 0.05$; $^dP < 0.01$, $^eP < 0.005$, $^fP < 0.001$, $^gP = 0.09$ for feeding effect, 2-way ANOVA (feeding × leptin); $^hP < 0.05$, $^iP < 0.001$, $^jP < 0.001$, $^kP = 0.09$ for post hoc test vehicle vs. leptin within fasted-LPS.
measured by Pearson’s correlation coefficients (Table 3 and Fig. 4). Thus, the degree of central PGE2 synthesis may partly account for the variability of the sustained fever response among animals with different nutritional status and leptin levels. A two-way (feeding × leptin) ANOVA among LPS-treated groups revealed that fasting significantly reduced the CSF PGE2 concentration \(^*(P < 0.05)\) and marginally attenuated IL-1\(\beta\) mRNA levels \(^*(P = 0.09)\); however, leptin treatment (leptin or interaction) did not affect the levels of these variables (Fig. 4). These results indicate that while the reduced fever response on fasting is partly attributable to an attenuated central PGE2 production, the leptin-mediated recovery of fever is not. We also examined the levels of other inflammatory variables that were reported to contribute to the fever response independent of PGE2 synthesis (3, 7, 12, 37, 38). Neither the levels of CSF 15-\(\alpha\)-PGJ2, MIP-1\(\beta\), endothelin-1, NOS-1, NOS-2, nor NOS-3 mRNA was associated with the degree of sustained fever (Table 3). The levels of these inflammatory mediators were not affected by fasting or by leptin replacement.

**Catabolic and Anabolic Neuropeptides Levels**

Among the hypothalamic neuropeptides known to be catabolic (POMC, CRF, and CART) and anabolic (NPY and AGRP), POMC mRNA levels were strongly associated with the magnitude of fever maintenance (Table 3 and Fig. 5). A two-way (feeding × leptin) ANOVA found a significant interaction in the POMC mRNA levels where fasting attenuated and leptin replenishment reversed its expression (Fig. 5C). Thus these results suggest that POMC (derived peptides) may play a role in leptin-dependent recovery of the prolonged fever response in the fasted animals. In addition, NPY and AGRP mRNA levels were inversely associated with the fever magnitude (Table 3 and Fig. 5), an observation in line with their anabolic properties. Although fasting significantly enhanced the expression of both NPY (Fig. 5D) and AGRP (Table 3) as determined by a two-way ANOVA, their levels were not significantly affected by leptin replenishment, negating their contribution to the leptin-mediated fever recovery. Because \(T_{core}\) during fever is most likely affected by multiple factors, we separated the contributions of potential variables with a multiple regression analysis, which showed that the variability of \(T_{core}\) during the late phase was best predicted by a linear combination of POMC, NPY, IL-1\(\beta\), and PGE2 \((r = 0.87, P < 0.001)\). However, the best-fit multiple regression model was significantly affected only by POMC \((P < 0.001)\) and only marginally by IL-1\(\beta\) \((P = 0.07)\) and PGE2 \((P = 0.06)\), indicating the major influence of POMC on the \(T_{core}\) change.
DISCUSSION

The main finding of the present study is that the reduction in circulating leptin is responsible for the attenuation of the long-lasting fever in the fasted animals. In response to the higher dose of LPS (1,000 μg/kg), the ad libitum-fed rats demonstrated a sustained fever response that remained unabated until the end of the recording period (~36 h). Although the fasted rats initially mounted a fever response that was more or less equivalent to the fed rats, they failed to sustain Tcore elevation, which progressively deviated from those of the fed counterparts. Leptin levels dramatically decreased in the fasted rats, and the repletion with exogenous leptin almost completely reversed the fever attenuation in these animals. To the best of our knowledge, the present study is the first to report that food deprivation compromises a prolonged fever response during relatively severe inflammation and the critical role of leptin in this process. Because exogenous leptin infusion effectively recovered the sustained fever response, this fact indicates that the diminution of the prolonged fever is a regulated process, rather than a result of a mere depletion of energy stores. Such regulations would be of fundamental importance to the survival of organisms given the substantial impact of fever (especially when it is long lasting) on energy balance.

In contrast to the sustained phase, leptin repletion did not reverse the fasting-induced fever alteration during the rising phase. This result indicates that starvation-related factor(s) other than leptin are responsible for the observed fever alteration, as fasting causes numerous hormonal and metabolic changes in addition to lowered leptin (6). Along the same line, the fever alteration is not due to a simple additive effect of fasting-induced hypothermia on febrigenic hyperthermia, because the former was almost completely recovered by leptin infusion (Fig. 2A). In this regard, Krall et al. (30) recently reported an intriguing finding that food deprivation in rats enhances cryogenic (as opposed to febrigenic) inflammatory responses to LPS and that this effect is mediated by PGD2, a PG known to induce hypothermia (62). Therefore, it appears that the fever alteration involves the modulations of a process or processes specific to febrigenic/cryogenic signaling (e.g., inflammatory response, thermoregulatory response, or both) during the early phase.

The inability of leptin to recover the rising phase of fever in fasted rats seemingly contradicts previous observations made in ad libitum-fed rats. Lack of leptin signaling due to genetic mutations (defective leptin receptor) or pharmacological blockade (administration of antileptin antiserum) blunts the rising
phase of LPS-induced fever (26, 45, 49, 57). In addition, the idea that leptin as a classic cytokine-like pyrogen has recently been challenged (59), and the role(s) of this hormone in febrigenic processes remain to be clarified. One explanation that accommodates the current (fasted condition) and previous (fed condition) data would be that leptin is essential, but on its own is not sufficient to drive the rising phase of fever and that food deprivation affects multiple factors essential for a normal fever response. We reported previously that the fasting-induced fever alteration during the rising phase was independent of PGE2 synthesis in the brain (i.e., lack of leptin signaling on fasting does not compromise PGE2 production in the brain), but accompanied by reduced cytokine levels (e.g., TNF) in the circulation (24). Conversely, our recent study showed that diet-induced obesity in rats enhances both the fever response to LPS and the accompanying elevation of circulating cytokine levels (including TNF). Interestingly, obesity did not significantly alter central PGE2 production (estimated by hypothalamic COX-2 induction), at least at the time points tested (42). Combined, these results imply the contribution of peripheral cytokines independent of brain PGE2. One potential scenario would be that altered cytokine levels affect peripheral PGE2 production, such as in the lung and liver, which in turn significantly contributes to the early phase of the fever response (58). The impact of food deprivation on peripheral PGE2 synthesis remains to be determined.

To understand how fasting compromises, and leptin reverses the sustained fever response, we examined the brain mechanisms related to febrigenic inflammatory signaling and thermoregulation, two important prerequisites for fever response. Among various biochemical variables examined, hypothalamic POMC mRNA levels most closely associated with the febrile $T_{\text{core}}$ elevation with a Pearson’s correlation of $r = 0.77$ ($P < 0.001$). Thus, the fever attenuation during fasting (and lowered leptin level) was proportional to the reduction of POMC mRNA in the hypothalamus. We further found that food deprivation strongly downregulated, and leptin repletion significantly restored, the POMC expression during LPS inflammation. This observation is in line with our current knowledge that this hormone acts directly on POMC-expressing neurons in the arcuate nucleus of the hypothalamus and stimulates its expression (9, 52). Although the present correlational evidence implicates POMC in the leptin-mediated maintenance of the fever response, functional studies are needed to test whether and how this peptide influences the late phase of fever. POMC peptide is a precursor for several biologically active neuropeptides including $\alpha$-MSH, $\beta$-endorphin, and ACTH (40). Of particular interest to the current study is $\beta$-endorphin as blocker of $\mu$-opioid receptor, one of the receptors for $\beta$-endorphin either by specific antagonists or genetic mutation abrogates the fever response induced by classical pyrogens including LPS (4, 5, 16, 44, 61) via several different mechanisms, including a central hypothymic action, peripheral vasodilation, and peripheral antipyretic activity (44).

We also found that the sustained $T_{\text{core}}$ elevation of LPS-treated animals significantly associated with the CSF concentration of PGE2, a key inflammatory febrigenic signal (51), and with the mRNA levels of the genes involved in its synthesis (IL-1$\beta$, COX-2, and mPGES-1) (10, 23, 31, 63). The fasting significantly suppressed the CSF PGE2 and marginally depressed the hypothalamic IL-1$\beta$ mRNA ($P = 0.09$), indicating that acute starvation may affect the fever maintenance at multiple levels (i.e., PGE2 and POMC). In line with this idea, multiple regression analysis showed that $T_{\text{core}}$ change during the late fever phase was best predicted by a linear combination of POMC, NPY, IL-1$\beta$, and PGE2 ($r = 0.87$, $P < 0.001$). Nevertheless, leptin repletion in fasted rats failed to increase the PGE2 levels, despite the reversal of the $T_{\text{core}}$ elevation. These results do not support a scenario that leptin recovers the fever maintenance via PGE2 synthesis in the fasted rats. Likewise, leptin repletion did not affect the levels of MIP-1$\beta$, endothelin-1, and NOS-1–3 mRNA and CSF 15-n-PGJ2, inflammatory mediators that were reported to modulate fever independently of PGE2 (3, 32, 37, 38). Taken together, we concluded that fasting attenuates the late phase of fever partly through the depression of inflammation, but the leptin-mediated fever recovery is dissociated from the pyrogenic inflammatory responses in the brain.

Although it remains undetermined how leptin contributes to the late phase of fever, the present study sheds light on some potential mechanisms involved. Because leptin’s effect on fever was specific to the fasted rats with no additive pyrogenic effect in the fed rats (Fig. 3), it appears that leptin serves as a permissive signal for the otherwise inhibited fever in fasted rats. A similar disinhibitory role of leptin on energy expenditure has been reported in food-restricted mice (8) and suckling age rats (55, 56) under noninflammatory conditions. Combined with the strong correlation between the leptin-mediated fever recovery with the hypothalamic expression levels of neuropeptides related to metabolism and thermoregulation, available data favor a scenario that leptin contributes to the late phase of fever via a disinhibition of metabolic thermogenesis. With this in mind, it is worth mentioning that our current studies were conducted below thermoneutrality (21°C) and that the leptin-mediated recovery of fever (and the fasting-induced attenuation) would have been less prominent in a higher ambient temperature that requires less metabolic thermogenesis to maintain fever.

In summary, the present study demonstrated that acute starvation altered the fever response to LPS by attenuating the $T_{\text{core}}$ during the entire course of fever. The leptin system, which signals energy sufficiency, was found to be critical for maintaining the increase in $T_{\text{core}}$ induced by a relatively high dose of LPS. This observation highlights the importance of leptin for energy balance regulation not only under normal conditions but also during inflammation. The leptin-dependent maintenance of fever was independent of febrigenic inflammatory responses in the brain, but it was closely associated with changes in levels of neuropeptides known to be involved in metabolism/thermoregulation.

**Perspectives and Significance**

The present study highlights the complexity of the central mechanisms controlling the thermoregulatory response to systemic inflammation. Of interest is the fact that the impact of food deprivation on the fever response differs at different stages of the fever trajectory, since the early-rising fever phase is relatively resistant to the calorific challenge, while the late-sustained phase is more susceptible. Such a difference in turn implies distinct regulatory mechanisms responsible for the respective stages of the thermoregulatory response to inflam-
variation. This hypothesis is supported by our current data that the fasting-induced fever alteration during the rising phase is leptin-independent, while the alteration during the sustained phase is leptin-dependent. In view of the adaptive value of fever (to actively fight against infection), its development appears to be paramount at the onset, even with preexisting caloric deficiency. At the late stage, on the other hand, the priority shifts toward energy saving over the potential benefit of the elevated body temperature, depending on the energy status of the host (43). We are beginning to uncover more complex mechanisms of thermoregulatory response to inflammation beyond the acute febrigenic response (30). The mechanisms of regulating the late phase of the fever response and its interaction with energy balance warrants further investigation.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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