High-fat diet induces site-specific unresponsiveness to LPS-stimulated STAT3 activation in the hypothalamus

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Borges BC, Rotaro R, Uchoa ET, Marangon P, da Silva GS, de Paula FJ, Branco LS, Antunes-Rodrigues J, Elias LK. High-fat diet induces site-specific unresponsiveness to LPS-stimulated STAT3 activation in the hypothalamus. Am J Physiol Regul Integr Comp Physiol 306: R34–R44, 2014. First published November 13, 2013; doi:10.1152/ajpregu.00147.2013.—Hypophagia induced by inflammation is associated with Janus kinase (JAK)-2/signal transducer and activator of transcription (STAT) 3 signaling pathway, and leptin-mediated hypophagia is also mediated by JAK2-STAT3 pathway. We have previously reported that lipopolysaccharide (LPS) did not reduce food intake in leptin-resistant high-fat diet (HFD) rats but maintained body weight loss. We investigated whether changes in p-STAT3 expression in the hypothalamus and brain stem could account for the desensitization of hypophagia in HFD animals after a low LPS dose (100 μg/kg). Wistar rats fed standard diet (3.95 kcal/g) or HFD (6.3 kcal/g) for 8 wk were assigned into control diet-saline, control diet-LPS, HFD-saline, and HFD-LPS groups. LPS reduced feeding in the control diet but not HFD. This group showed no p-STAT3 expression in the paraventricular nucleus (PVN) and ventromedial hypothalamic nucleus (VMH), but sustained, though lower than control, p-STAT3 in the nucleus of the solitary tract (NTS) and raphe pallidus (RPa). LPS decreased body weight in HFD rats and increased Fos expression in the NTS. LPS increased body temperature, oxygen consumption, and energy expenditure in both control diet and HFD rats, and this response was more pronounced in HFD-LPS group. Brown adipose tissue (BAT) thermogenesis and increased energy expenditure seem to contribute to body weight loss in HFD-LPS. This response might be related with increased brain stem activation. In conclusion, LPS activates STAT3-mediated pathway in the hypothalamus and brain stem, leading to hypophagia, however, LPS effects on food intake, but not body weight loss, are abolished by leptin resistance induced by HFD. The preserved STAT3 phosphorylation in the brain stem suggests that unresponsiveness to LPS on STAT3 activation under HFD might be selective to the hypothalamus.

High-fat diet; lipopolysaccharide; phospho-STAT3; Fos; uncoupling protein-1; body temperature; indirect calorimetry

Obesity, one of the largest public health problems, has been reported to be coupled with chronic low-grade inflammation, which is a causal factor of a range of metabolic disturbances such as diabetes and hypertension (14, 42). In diet-induced experimental obesity, rats fed high-fat diet (HFD) show resistance to leptin and increased inflammatory response, expressing high levels of cytokines in the hypothalamus (38, 59). Lipopolysaccharide (LPS) from the outer lipid bilayer of Gram-negative bacteria cell wall is an important component of inflammation during obesity development in mice, whose plasma LPS levels increase two to three times after 4-wk HFD treatment (10). Kelly and co-workers (34) reported that circulating LPS may transiently increase following energy-rich meals in both healthy and obese human subjects. Experimental approaches with nonobese rodents have shown that LPS acutely leads to hypophagia, cytokine production, and leptin secretion (5, 26, 33, 48, 50).

Leptin participates to some extent in the anorexigenic responses during endotoxemia induced by LPS (6, 50). In hypothalamic cells of the arcuate nucleus (ARC), leptin activates Janus kinase (JAK)-2/signal transducer and activator of transcription (STAT) 3 signaling, stimulating the expression of proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) peptides, known to drive, at least in part, the anorexigenic effect of leptin (12, 23, 31). In addition to the hypothalamus, leptin receptor is also expressed within the brain stem, which is a crucial area for processing both peripheral signals of energy metabolism and the control of satiety (37, 54). Furthermore, brain stem neurons have reciprocal interconnections with the hypothalamic paraventricular nucleus (PVN) (52) known to take part in the anorexigenic effects during endotoxemia.

Examining the interplay between leptin signaling and LPS-induced hypophagia, we reported in a previous study that HFD rats showed no hypophagic response induced by leptin, indicating leptin resistance and acute LPS treatment failed to induce hypophagia in these leptin-resistant rats (6). Although there was no effect on food intake, we observed that LPS reduced body weight in HFD rats (6). Body weight reduction and increasing of energy expenditure in rodents might be related with the expression of uncoupling protein-1 (UCP-1) in brown adipose tissue (BAT), which produces heat by oxidation of fatty acids, preventing the progress of obesity (2, 19, 22). An
overview of the current literature shows increased BAT UCP-1 mRNA and protein content after feeding a HFD in mice and rats. Furthermore, Cannon and co-workers (11) have proposed that BAT thermogenesis is also stimulated by cytokines such as interleukin (IL)-1β and IL-6, which are produced in response to LPS.

From the aforementioned data as indicative of common signaling pathway recruited by leptin and LPS in the hypothalamus to modulate food intake reduction, and because STAT3 phosphorylation is not only a marker of leptin signaling but also an indicator of cytokine activation (4, 8, 61), in the present report we intended to investigate whether STAT3 phosphorylation in response to LPS in the hypothalamus and brain stem could be affected by leptin resistance induced by HFD, leading to desensitization of hypophagia induced by LPS. We also evaluated the neuronal Fos expression in the brain stem to verify whether neuronal activation in HFD-fed rats after LPS treatment could, at least in part, account for body weight loss. To better address the LPS-induced weight loss in HFD-fed rats, we also investigated BAT UCP-1 protein expression, core body temperature, oxygen consumption, and energy expenditure.

MATERIALS AND METHODS

Animals

Male Wistar rats, 100–110 g (4 wk old) (Central Animal Facility of the University of Sao Paulo, Campus Ribeirao Preto), were individually housed in a light/dark- (lights on: 06:00 AM-06:00 PM) and temperature- (23 ± 1°C) controlled room with food and water available ad libitum, unless otherwise stated. Obesity was induced during an 8-wk interval of HFD consumption, whereas the control group received standard diet consumption. Diets were prepared every week based on the American Institute of Nutrition (AIN)-93 guidelines for laboratory rodents’ diets (47). Rats were adapted to the laboratory environment for at least 3 days before the experimental procedures. During this period, rats were daily handled. All experimental protocols were approved by Ethical Committee for Animal Use of the School of Medicine of Ribeirao Preto.

Animal Treatment Protocol

The animals were subjected to intraperitoneal injection of saline (0.15 M NaCl, 1 ml/kg) or LPS from Escherichia coli (100 μg/kg in 1 ml/kg, serotype O26:B6, Sigma) between 04:00 PM and 04:30 PM, 2 h before lights off.

Experimental Procedures

Measurement of food, caloric intake, body weight gain, and BAT UCP-1 content in HFD rats after LPS stimulation. Rats were individually housed and fed for 8 wk with standard diet (3.95 kcal/g; 7% fat) or HFD (6.3 kcal/g; 50% fat). Food/caloric intake by each rat was measured every other day and body weight was measured once a week. After 8 wk of control or HFD, rats were anesthetized with 2.5% tribromoethanol (TBE) for body fat content determination and 2 h after, they were anesthetized with an overdose of 2.5% TBE and transcardially perfused with 200 ml of saline followed by 300 ml of 4% formaldehyde in 0.1 M phosphate buffer (PBS). Brains were collected, postfixed in the same fixative solution for 1 h, placed in PBS containing 30% sucrose, and stored at 4°C for posterior immunohistochemistry procedures.

Analysis of core body temperature, oxygen consumption, RQ, and energy expenditure in HFD-fed rats after LPS stimulation. Rats treated for 8 wk with standard diet or HFD were assigned into the four above-mentioned groups (n = 6–9/group). Seven days before the experiment, anesthetized rats (mixture of ketamine and xylazine, 3:2, in a dose of 60 mg/kg ketamine and 7.5 mg/kg xylazine at a volume of 0.1 ml/100 g) were submitted to paramedian laparotomy for the insertion of a temperature datalogger (SubCue, Calgary, Canada). After surgery, the rats received a prophylactic injection of penicillin (50,000 units im). Each datalogger was programmed to acquire body temperature (Tb) every 5 min, at least 2 days before and throughout the experiments. On the day of the experiment, at 4:00 PM, rats received intraperitoneal injection of saline or LPS (100 μg/kg). Twenty-four hours after the end of the experiments, animals were killed by decapitation, the dataloggers were removed, and the data were downloaded using SubCue software.

Another set of rats treated for 8 wk with standard diet and HFD was submitted to the laparotomy for the insertion of the datalogger, as described above, and the rats were used for measurements of oxygen consumption, respiratory quotient (RQ), and energy expenditure by indirect calorimetry. For these purposes, each animal was placed individually into the chamber for gas measurements, as described below, and allowed to rest for at least 40 min before starting baseline measurement, which was recorded during 45 min. After that, rats were injected with LPS (100 μg/kg ip, at 4:00 PM) and allowed to rest in the chamber for 60 min before post-LPS measurements were started, which were performed during 120 min (from 1 to 3 h post-LPS). Data were collected every minute and averaged over the period of measurement. The temperature of the chamber was monitored during indirect calorimetry measurements. During the experiments of Tb measurements and indirect calorimetry, the room temperature was similar (22–23°C) in all groups.

Indirect Calorimetry

Measurements of oxygen consumption (\(V\)O\(_2\)) and carbon dioxide production (\(V\)CO\(_2\)) were performed in a 5-liter chamber using open respirometry approach. \(V\)O\(_2\) and \(V\)CO\(_2\) were analyzed (Gas analyzer ML206, ADInstruments, NSW, Australia) from the inflow and outflow gas, and \(V\)O\(_2\) and \(V\)CO\(_2\) were computed from the inflow outflow concentration difference of the respective gases, multiplied by the flow, which was maintained steady (1,500 ml/min) and continuously monitored by a flowmeter. \(V\)CO\(_2\) and \(V\)O\(_2\) were expressed as milliliters per minute or as percentage of baseline values. The RQ was obtained by the ratio between \(V\)CO\(_2\) and \(V\)O\(_2\) (\(V\)CO\(_2}/#{V}\)O\(_2\)). Energy expenditure (EE, expressed as kcal/min) was calculated as the product of caloric value of oxygen (CV) and \(V\)O\(_2\), where CV = 3.815 + 1.232(RQ) (15, 18, 54).
Table 1. Food and caloric intake, body weight gain, and percentage of body fat of rats fed with standard diet or HFD for 8 wk

<table>
<thead>
<tr>
<th></th>
<th>Control Diet</th>
<th>HFD</th>
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<tr>
<td>Starting body weight, g</td>
<td>128.7 ± 2.4</td>
<td>132.6 ± 1.39</td>
</tr>
<tr>
<td>Final body weight, g</td>
<td>512.5 ± 14.12</td>
<td>576.9 ± 16.25*</td>
</tr>
<tr>
<td>Change in body weight, g</td>
<td>390.98 ± 8.15</td>
<td>444.35 ± 17.52*</td>
</tr>
<tr>
<td>% Body fat</td>
<td>16.8 ± 1.59</td>
<td>25.32 ± 1.53*</td>
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<tr>
<td>Average food consumption per day, g</td>
<td>26.08 ± 0.53</td>
<td>18.81 ± 0.51*</td>
</tr>
<tr>
<td>Average caloric consumption per day, kcal</td>
<td>103.02 ± 2.09</td>
<td>115.11 ± 3.12*</td>
</tr>
</tbody>
</table>

Data are means ± SE; (n = 8). Control diet: 3.95 kcal/g; high-fat diet (HFD): 6.3 kcal/g. Student’s t-test was performed. *P < 0.05 when compared with the control group.

Immunohistochemistry

Brain coronal sections were cut at 30-μm thickness and preserved in cryoprotectant (ethylene glycol and glycerol) at −20°C. One of every fourth section was immunostained for p-STAT3 and Fos/tyrosine hydroxylase (TH). Immunohistochemistry procedures were carried out as previously described (6, 49). Briefly, sections were rinsed with buffer and incubated for 24 h at room temperature with rabbit anti-Fos antibody (Ab-5, 1:10,000, Calbiochem) or for 48 h at 4°C with rabbit anti-p-STAT3 antibody (1:1,000, Cell Signaling). After rinsing was completed, sections were incubated for 1 h with secondary biotinylated antibody goat anti-rabbit (1:200, Vector) and processed using the Vectastain Elite avidin-biotin immunoperoxidase method (Vector Laboratories). Solutions of diaminobenzidine (DAB), nickel sulfate, and H2O2 were used to generate blue-black immunolabeling. For double labeling, sections processed for Fos were incubated for 48 h at 4°C with monoclonal anti-TH antibody (anti-TH), raised in mouse (1:1,000, Chemicon), to identify catecholaminergic neurons. After incubation, sections were rinsed and submitted to the same protocol described above, using biotinylated antibody antimouse (1:200, Vector) followed by avidin-biotin-peroxidase complex. The brown cytoplasmic color was detected using nonintensified DAB solution. Finally, sections were mounted on gelatinized slides, air-dried overnight, dehydrated, cleared in xylene, and placed under a coverslip with Entellan.

Immunopositive cells were identified in hypothalamic regions according to coordinates from the rat brain atlas of Paxinos and Watson (43): PVN: −0.92 mm to −2.12 mm from bregma; ARC and ventromedial hypothalamic nucleus (VMH): −2.3 mm to −3.5 mm from bregma; and in the brainstem rostral raphe pallidus nucleus (RPa): −12.30–14.30 from bregma, and medial nucleus of the solitary tract (NTS): −13.68 mm from bregma. Photomicrographs were captured with a Leica microscope equipped with a DC 200 digital camera, attached to a contrast enhancement device. The number of immunoreactive-positive cells of all the sections in the series (8–18 sections) per rat were obtained by counting black (nuclear) staining or black (nuclear)/brown (cytoplasmic) staining from a constant area of the PVN, ARC, VMH, RPa, and NTS, using Image J software (Version 1.38, NIH).

Immunoblot Analysis

Total BAT protein was extracted using ice-cold sucrose buffer (300 mM sucrose, 2 mM EDTA, 10 mM Trizma base, 1% Triton; pH 7.2). Tissue samples (0.1 g/100 ml) were homogenized, and the homogenate was centrifuged at 4°C, 2,500 rpm for 10 min. The supernatants were...
Differences were accepted as significant at muscle, bone, and fat mass after control diet and HFD treatment. We used Student’s t-test to analyze differences between muscle, bone, and fat mass after control diet and HFD treatment. Two-way ANOVA, followed by Newman-Keuls post hoc test was performed. Data are expressed as means ± SE. (n = 5–6). *P < 0.05.

RESULTS

Measurement of Food, Caloric Intake, and Body Weight Gain in HFD-Fed Rats After LPS Stimulation

Food and caloric intake, body weight gain, and percentage of body fat of rats fed with standard diet or HFD for 8 wk are presented in Table 1. Rats fed with control diet ingested higher amount of food (g) than rats fed with HFD (P < 0.05). Despite the lower food consumption, rats from the HFD group showed higher (P < 0.05) daily average caloric intake (kcal).

The body weight gain increased similarly in both control diet and HFD group, and it had a significant divergence in HFD rats compared with control rats from the eighth week on. We found higher (P < 0.05) fat mass and percentage of body fat in rats fed with HFD compared with control diet-fed rats, with no changes in lean and bone mass (Fig. 1, A–C), indicating the development of obesity.

LPS reduced (P < 0.05) food intake 2 and 24 h after injection only in the animals from the control diet group, with no effect on feeding of HFD animals (Fig. 1D). Interestingly, LPS reduced (P < 0.05) body weight gain, in both the control diet and HFD animals (Fig. 1E).

Analysis of STAT3 Phosphorylation, Fos, and Fos/TH Expression After LPS Stimulation in HFD-Fed Rats

HFD increased the number of p-STAT3-positive cells (P < 0.05) in the ARC compared with the control diet (Fig. 2). In control diet-fed rats, LPS increased (P < 0.05) the STAT3 phosphorylation in the ARC (Fig. 2), PVN (Fig. 3), and VMH (Fig. 3), but it had no additional effect in HFD animals.

Similar to control diet group, HFD treatment did not induce STAT3 phosphorylation in the brain stem NTS and RPa neurons (Fig. 4). On the other hand, LPS increased (P < 0.05) the number of p-STAT3-positive cells in both control diet and HFD-fed rats in these nuclei. Nevertheless, LPS-induced p-STAT3 expression in the NTS and RPa of HFD group was significantly reduced (P < 0.05) when compared with control diet group.

To investigate the activity of noncatecholaminergic and catecholaminergic neurons in the brain stem, we performed Fos and Fos/TH double labeling. We observed that HFD treatment did not induce Fos and Fos/TH expression in the NTS (Fig. 5). LPS, in turn, increased (P < 0.05) the number of Fos and Fos/TH-expressing neurons in both control diet and HFD-fed animals. Interestingly, in HFD-fed rats LPS promoted an additional Fos and Fos/TH expression in the NTS. In control diet-fed rats 13.3% of TH neurons were activated by LPS, whereas in HFD-fed rats 33.3% of TH neurons were activated, suggesting that in diet-induced obese rats, LPS exacerbates the

![Fig. 2. Representative photomicrographs of p-STAT3-positive cells (dark points) in the arcuate nucleus of the hypothalamus (ARC) (boundaries delineated with dotted line) and graph showing the number of p-STAT3-positive cells in the ARC (right) of animals assigned at the groups: control diet-saline, control diet-LPS, HFD-saline and HFD-LPS. 3V, third ventricle limits. Scale bar: 100 μm. Two-way ANOVA, followed by Newman-Keuls post hoc test was performed. Data are expressed as means ± SE. (n = 5–6). *P < 0.05.](http://ajpregu.physiology.org/ by 10.1152/ajpregu.00147.2013 • www.ajpregu.org)
brain stem catecholaminergic neuronal activation, as well as noncatecholaminergic activation. We did not observe Fos expression in RPa nuclei in our experimental protocol.

Measurement of BAT UCP-1 Content in HFD-Fed Rats After LPS Stimulation

As expected, BAT UCP-1 expression was clearly increased \((P < 0.05)\) in HFD animals (Fig. 6). In rats fed with control diet, LPS significantly increased \((P < 0.05)\) BAT UCP-1 expression, but curiously, LPS had no additional effect on UCP-1 expression in HFD group.

Effects of LPS on Core Body Temperature in HFD-Fed Rats

Room temperature was similar (at 22–23°C) in all groups and did not change during the experiments. The core body temperature in rats injected with LPS increased 60 min postinjection and remained higher than the saline groups during the following 6 h (Fig. 7A). Interestingly, HFD-LPS rats showed a more pronounced increase in body temperature and increased control diet-LPS group (Fig. 7, A and B). HFD group showed increased basal thermal index when compared with the control diet group.

Effects of LPS on Oxygen Consumption, RQ, and Energy Expenditure in HFD-Fed Rats

Before LPS injection, \(\dot{V}O_2\) was \(7.7 \pm 0.5\) ml/min in control diet-fed rats and significantly higher \((P < 0.05)\) in HFD group \((10.5 \pm 0.8\) ml/min). One hour after LPS injection, \(\dot{V}O_2\) significantly increased in both control diet \((9.5 \pm 0.6\) ml/min) and HFD \((12.5 \pm 1.0\) ml/min) rats (Fig. 8A); however, the HFD-LPS group presented a higher \(\dot{V}O_2\) when compared with the control diet-LPS group. \(\dot{V}O_2\) enhanced around 20% from baseline after LPS injection in both control diet and HFD groups (Fig. 8B). In basal condition the RQ, which reflects the balance of substrate oxidation, was \(1.00 \pm 0.02\) in control diet and it was significantly lower \((P < 0.05)\) in HFD \((0.92 \pm 0.00)\), suggesting that HFD-fed rats oxidized less carbohydrate than control diet-fed rats. LPS did not change the RQ in control diet \((0.98 \pm 0.02)\) and HFD-fed rats \((0.93 \pm 0.00)\) (Fig. 8C).
In basal condition, HFD rats showed an increased EE when compared with control diet. Accordingly, rats injected with LPS showed a higher EE, compared with basal conditions, in both control diet and HFD groups; however, HFD-LPS showed a more pronounced EE than control diet-LPS group (Fig. 8D).

**DISCUSSION**

The present data demonstrate that HFD abolishes the hypophagic effect of acute low-dose LPS stimulus in rats, and this effect is associated in part with reduced STAT3 phosphorylation in the hypothalamus. Additionally, our results show for the first time that, in contrast to the failure to activate STAT3 in the hypothalamus, this response is preserved, to some extent, in the NTS and RPa. Furthermore, the preserved sympathetic autonomic responsiveness and the increase in thermogenesis and energy expenditure could account for the body weight loss after acute dose of LPS despite the failure of LPS to reduce food intake in HFD rats.

Diet-induced obesity (DIO) is characterized by elevated serum leptin and reduced response to exogenous leptin, usually caused by defects in leptin signaling (53). In 2004, Münzberg et al. (41) mapped leptin-responsive cells in brains from DIO mice using p-STAT3 immunohistochemistry and reported a careful description that ARC is the major site of leptin resistance, in contrast to other hypothalamic and extrahypothalamic nuclei, which maintain the responsiveness to leptin. Leptin resistance also emerges after increased inflammatory response in the hypothalamus, which contributes to the altered food intake both in genetic or dietary fat-induced obesity (38, 56). In our study, obesity induced by HFD for 8 wk led to enhanced STAT3 phosphorylation in the ARC but not in the PVN and VMH. p-STAT3 expression in the ARC of HFD group might be due to increased hypothalamic inflammation (15, 56), given that STAT3 can be also phosphorylated by local action of cytokines (45). This finding is in accordance with previous report from Martin et al. (36) that described elevated...
basal STAT3 phosphorylation in the arcuate nucleus of DIO mice. We could not rule out that endogenous metabolic endotoxemia could induce STAT3 phosphorylation. Anorectic action of leptin depends on its ability to phosphorylate STAT3 (8). Mice specifically lacking the STAT3-binding site in the leptin receptor (4) and mice with reduced levels of STAT3 proteins selectively in the central nervous system (24) are hyperphagic and obese. We found that cumulative food intake (g) and daily average food consumption (g) of control diet-fed rats were higher than HFD. In contrast, considering all amount of food consumed during the 8 wk of treatment, the average of caloric intake in HFD was higher than control diet-fed rats (Table 1). However, at the end of 8 wk HFD-fed rats injected with saline showed reduced caloric intake compared with control diet-fed rats. Previous reports showed that hyperphagic response in HFD rodents depends on the composition of the diet and may be transient with initial increased followed by decreased food intake (46).

LPS reduced food intake in control diet-fed rats but not in HFD. Our data give support to the hypothesis that LPS-induced hypophagia also depends on the intact STAT3 signaling, as we previously demonstrated that control diet-fed rats challenged with LPS showed hypophagia and high p-STAT3 expression in the hypothalamus (6). Moreover, the present data expand this notion showing that STAT3 activation in the PVN and VMH is important to reduce food intake, since STAT3 activation only in the ARC was not sufficient to induce hypophagia after LPS in HFD group. We cannot exclude the contributions of other hypothalamic and extrahypothalamic sites, such as lateral hypothalamus, dorsomedial nucleus of the hypothalamus, cortex, amygdala, and hippocampus, not examined in our study, in the hypophagic effect of LPS.

In contrast to our results, Lawrence and co-workers (35) found that in mice fed with HFD for a longer period (20 wk), LPS (100 µg/kg) caused a greater and more prolonged hypophagic effect compared with control mice. They also found that LPS induced Fos expression in several brain regions of control mice, but, in contrast to our findings, they found fewer Fos-positive cells observed in the brains of obese mice. These mice exhibited an altered inflammatory response to LPS, evidencing that obesity impairs the ability of the immune system to appropriately respond to bacterial infection. The divergent hypophagic and Fos response, observed between our results and the above-mentioned report, could be due to the different species studied (mice vs. rat) and different period of HFD feeding (20 wk vs. 8 wk). Of note, the rats used in our study were younger (4 wk old) than the mice used in the cited study (8 wk old), suggesting that the age of subjects during the LPS treatment could also be an important factor to affect LPS responsiveness in obese rodents.

Immunohistochemical studies demonstrate rapid activation of STAT3 by leptin in extrahypothalamic brain regions, such as the periaqueductal gray and the dorsal raphe (DR) in the midbrain and the parabrachial nucleus and NTS in the brain.
food intake, with no resistance on leptin effect on sympathetic autonomic activity (55).

Despite the unchanged food intake in obese HFD rats after single LPS injection, as reported earlier (6), we observed that endotoxin had a prominent effect on body weight reduction, both in control diet and HFD group. Furthermore, we observed increased Fos and Fos/TH double-labeled neurons in the medial NTS after LPS, which were significantly higher in HFD than in control diet group. The lower number of double-labeled Fos/TH neurons in the NTS indicates that besides catecholaminergic neurons other neuronal phenotypes are involved in the activation of NTS in response to acute LPS stimulation. Activation of peripheral vagal afferents directly by cytokines may induce Fos expression in the NTS (27). Pohl et al. (44) showed an increase in hypothalamic expression of the mRNA for IL-1β, IL-6, and SOCS-3 and higher levels of IL-6 in the white adipose tissue after exposure to LPS in DIO mice. Taken together, these results demonstrate that diet-induced obesity produces an alternation in the acute inflammatory response at multiple levels, and could explain, in part, the enhanced activity of brain stem neurons of HFD-fed rats after LPS injection. Furthermore, treatment with the same low LPS dose (100 μg/kg) was shown to induce a significant increase in core body temperature in both lean (control diet) and obese (HFD) rats (41), contributing to the body weight loss. Accordingly, in our study we observed that HFD-fed rats showed increased core body temperature and increased energy expenditure compared with control diet-fed rats stimulated with LPS.

Fig. 6. Percentage uncoupling protein-1 (UCP-1) expression in the brown adipose tissue (BAT) of animals assigned at the groups: control diet-saline, control diet-LPS, HFD-saline, and HFD-LPS. Two-way ANOVA, followed by Newman-Keuls post hoc test was performed. Data are expressed as means ± SE. (n = 5–6). *P < 0.05.

Similarly to leptin, LPS also induces STAT3 activation in brain stem areas. In our study, we observed an intense p-STAT3 expression in the NTS and RPa after LPS injection both in control diet- and HFD-fed rats. However, in HFD-fed rats, p-STAT3 expression was significantly lower than in controls. The massive p-STAT3 expression observed in control diet group after LPS may be explained, in part, by the presence of higher levels of endogenous leptin stimulated by the endotoxin (6), in addition to p-STAT3 induction by LPS-stimulated cytokines. Therefore, the present results show that inability to activate STAT3 in response to LPS in HFD is site specific, with unresponsiveness in the hypothalamus but maintenance of brain stem responsiveness. These results are in accordance with the recent evidence showing that resistance to leptin appear to be specific for the p-STAT3 expression in the hypothalamus (16, 41) and consequent effect on the control of

Fig. 7. Twelve hours core body temperature (Tb) time course (A) and thermal index (calculated from area under the curve) (B) of animals assigned at the groups: control diet-saline, control diet-LPS, HFD-saline, and HFD-LPS. Two-way ANOVA, followed by Newman-Keuls post hoc test was performed. Data are expressed as means ± SE. (n = 7–9). *P < 0.05 LPS vs. saline; #P < 0.05 HFD-LPS vs. control diet-LPS.
Brain stem and parasympathetic and sympathetic preganglionic cells in the spinal cord, which innervate BAT, are densely innervated by projections from the PVN (11, 32). Leptin triggers the synthesis of UCP-1 in BAT mitochondria by the release of norepinephrine from its sympathetic nerve terminals, leading to thermogenesis (19). To clarify the body weight loss in HFD obese rats during endotoxemia, we tested the hypothesis that increased BAT UCP-1 expression induced by LPS in HFD group might be, at least in part, responsible for the weight loss by the enhancement of thermogenesis and energy expenditure. In our observations, as expected, fat-rich diet clearly increased BAT UCP-1 expression. Accordingly, Fromme and Klingenspor (22) reported a compilation of several reports on mice and rats fed with different types of fat-rich diets, showing a thermoregulatory effect mediated by UCP-1. In our study, control diet-fed rats showed increased UCP-1 expression after LPS injection, and these data are in agreement with previous report (11) showing that cytokines produced in response to LPS stimulate BAT thermogenesis. However, LPS had no additional effect on UCP-1 expression in HFD-fed group, suggesting that changes in UCP-1 could be limited as an indicator of BAT thermogenesis during endotoxemia.

Activity of neurons in the rostral RPa in the brain stem is predominantly involved in determining the level of sympathetic nerve discharge to target tissues involved in metabolism and thermoregulation. RPa contains premotor neurons that innervate the intermedio-lateral cell column containing sympathetic preganglionic neurons that control BAT functions (9). In the present study Fos expression was not observed after LPS in the RPa, both in control and HFD group. Although there was no Fos expression, STAT3 phosphorylation induced by LPS in the RPa is maintained in HFD group, but to a lesser extent than in control diet group, showing that in obese rats the brain stem areas remain sensitive to the circulating cytokines stimulated by the endotoxin.

Despite unchanged UCP-1 expression, increased BAT thermogenesis in fact may contribute, at least in part, to body weight loss in HFD rats challenged with LPS. Jepson et al. (32) demonstrated that below thermoneutral conditions the febrile response developed by animals challenged with LPS depends, in part, on sympathetic activation of BAT thermogenesis. Accordingly, we observed that control diet and HFD LPS-treated rats, placed below thermoneutral conditions, showed increased thermal index (calculated from area under the curve) and increased body temperature. Interestingly, as observed previously by Pohl et al. (44), fever response was higher in HFD-LPS than in control diet-LPS group.

We also observed an enhancement of $V_{O2}$ induced by LPS in both control diet and HFD rats, with an exacerbated $V_{O2}$ in HFD-LPS rats compared with control diet-LPS. In the literature, studies have shown an evident positive correlation between thermogenesis and $V_{O2}$ (21, 25). Horan and co-workers (28) first reported increases in $V_{O2}$ and body temperature in adult endotoxemic rats. As expected, the enhancement of the $V_{O2}$ during 2 h coincided with the increased body temperature observed in LPS-treated groups. We found that HFD rats showed higher $V_{O2}$ and EE in basal condition compared with control diet group, which could be due to the higher UCP-1 expression and, at least in part, to the higher basal thermal index observed in HFD animals. This is in line with previous data showing that HFD increased $V_{O2}$, heat production, and EE (1, 13, 39). In our study, HFD rats showed lower RQ (RQ), as indicative of lower utilization of carbohydrate as fuel compared with control diet rats (25). LPS injection had no effect on RQ. We observed that EE increased after LPS injection in both control diet-and HFD-fed rats, being more prominent in the HFD group. It is important to point out that below the thermoneutral zone an organism must expend energy to generate heat (58).

In conclusion, our data indicate that LPS activates STAT3-mediated pathway in the hypothalamus and brain stem, leading to hypophagia; however, acute effects of LPS on food intake, but not body weight loss, is abolished in HFD lepin-resistant rats. Despite the absence of hypophagic effect, body weight loss induced by LPS is sustained and this response is associated with higher brain stem neuron activation and increased ther-
mogenesis and energy expenditure. Thus resistance to acute LPS effects under HFD seems to be selective to food intake control.

Perspectives and Significance

The prevalence of overweight and obesity is increasing worldwide and it makes the investigation of mechanisms underlying the unbalance of energy homeostasis crucial for the understanding of the pathophysiology of obesity. In this context, leptin resistance is a hallmark of obesity, which has been shown to be associated with metabolic endotoxicemia. Our data give support to the notion that leptin resistance has an effect on endotoxicemia-induced hypophagia but is unlikely to alter thermogenesis and EE. The comprehension of the common mechanisms between leptin and LPS signaling pathways will contribute to the development of strategies to fight against obesity.

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GRANTS

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