Changes in Hypothalamically Mediated Acute-Phase Inflammatory Responses to Lipopolysaccharide in Diet-Induced Obese Rats

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Recent evidence suggests that inflammation may be a common underlying cause of many obesity-associated conditions. To test whether obesity changes the response to inflammation, we investigated its effects on the acute phase of the inflammatory response to an endogenous pathogen, lipopolysaccharide (LPS). Diet-induced obese male Wistar rats exhibited an increased and prolonged fever response to LPS (100 μg/kg) relative to lean rats. LPS-treated obese rats also showed a greater increase in circulating TNF-α, IL-6, and IL-1 receptor antagonist within the first 8 h after LPS injection. LPS induced an increase in circulating leptin only in obese rats with no effect in lean rats. Analysis of expression of pyrogenic signaling in the hypothalamus demonstrated that obese rats show a greater increase in IL-1β peaking at 2 h after LPS injection and suppressor of cytokine signaling 3 and IL-6 peaking at the 8-h time point. LPS-treated obese rats showed a significantly higher expression of IL-1 receptor antagonist in white adipose tissue (WAT) than lean rats, and WAT from obese rats incubated in LPS-supplemented medium (100 ng/ml) secreted a significantly higher level of IL-6. Overall, these results suggest that diet-induced obesity induces changes in the inflammatory response rendering the obese rats more responsive to the effects of LPS. These data also support the hypothesis that qualitative changes in WAT associated with obesity may contribute to these effects. (Endocrinology 150: 4901–4910, 2009)

Obesity is associated with an array of medical conditions including hypertension, type 2 diabetes, and cardiovascular disease (1–4), making the recent increase in its prevalence a serious public health concern (5, 6). Although the relationship between obesity and these diseases is well documented, little is known about the causal pathway mediating this association. Recent evidence suggests that a common underlying cause of many of these conditions is inflammation (7). Support for this hypothesis comes from numerous reports showing that diseases for which obesity represents a major risk factor, such as cardiovascular disease and type 2 diabetes, are themselves exacerbated by the inflammatory state (7).

The recognition that white adipose tissue (WAT) is not merely a passive energy store but constitutes an endocrine organ that is both an important source and target of cytokines (8) has provided one of the strongest mechanistic links between obesity and immune function. In both humans and rodents, WAT secretes cytokines and other inflammatory signals including TNF-α, IL-6, IL-1 receptor antagonist (IL-1ra), and leptin from adipocyte cells and the stromal vascular fraction of the tissue, which is composed mainly of macrophages and neutrophils (9–11). The expansion of WAT that occurs in obesity results in both an increase in size and number of adipose cells and a progressive infiltration of non-WAT-derived macrophages (11–13). Bigger adipocytes acquire a greater capacity for cytokine production (14), and this together with the increase in macrophage (an important source of cytokines) number results in WAT becoming a significant source of circulat-
ing cytokines in obese individuals. Indeed, increases in basal circulating levels of IL-6 have been observed in individuals with a body mass index greater than 40 (15–17). Higher levels of circulating IL-6 have also been found in genetically obese mice (18) and in sedentary, but not exercised, obese Zucker rats (19). The proinflammatory adipokine leptin is also very highly correlated with weight and adipose mass in humans and animals (20, 21). There is less consensus with respect to the relationship between another proinflammatory cytokine, TNF-α, and adiposity. Some studies report elevated levels of this cytokine in the circulation of obese individuals (17, 22, 23), whereas others have found no differences between obese and lean subjects (24–26). Finally, fewer studies have examined the circulating levels of antiinflammatory cytokines such as IL-1ra in obesity, but evidence points toward a higher level of this antiinflammatory cytokine in morbidly obese than in lean individuals (27).

Although there is now strong evidence that obesity is associated with changes in WAT that may, in cases of more severe obesity, lead to increased levels of immune mediators in the circulation, little is known about the effects of obesity on the immune response itself. A number of studies have shown that obese individuals are more susceptible to opportunistic infections such as those seen postoperatively (28) and also have an impaired capacity for wound healing (29–31). Increased susceptibility to experimentally induced infection has also been observed in genetically obese rats (32) and diet-induced obese mice (33). It seems probable therefore that the response to an acute immune challenge is altered in obese organisms. To test this, we compared the effects of a challenge with bacterial lipopolysaccharide (LPS) on the cytokine-mediated fever response. This included measuring levels of circulating pro- and antiinflammatory cytokines as well as cytokine mRNA expression in WAT and the hypothalamus, an important brain region for regulating sickness behaviors including fever, between lean rats and rats made obese by exposure to a calorically dense, highly palatable diet. To investigate the direct contribution of qualitative changes in WAT to the modulation of the inflammatory response in obese rats, we in addition compared the ability of LPS to stimulate cytokine secretion from WAT explants obtained from lean and obese rats.

Materials and Methods

Animals

Adult male Wistar rats (~300 g; Charles River, Saint Constant, Quebec, Canada) were used in all experiments. Rats were group housed (five rats per cage) during the initial phases of the induction of diet-induced obesity and then were housed individ-

ually for the remainder of the experiment. All animals were provided with food and water ad libitum throughout the experimental period and maintained at an ambient temperature of 22 ± 1 °C on a 12-h light, 12-h dark cycle with lights on at 0800 h. All procedures were approved by the Concordia University Animal Care Committee under the guidelines of the Canadian Council of Animal Care.

Diet

At the start of the experiment, rats were assigned to one of two groups, obese and lean, so that the groups had similar means and range of body weight. Rats in the obese group were given ad libitum access to chocolate-flavor Replenish Plus, a liquid diet composed of 15% protein, 24% fat, and 60% carbohydrate with a caloric density 1.51 cal/ml. This diet was presented in a graduated cylinder with a sipper tube. The lean group was given access to laboratory chow (Charles River Autoclavable Rodent Chow). Rats were tested when the average body weight of the obese group exceeded that of the lean group by 15%. Respective diet conditions were maintained during the entire testing period.

Procedure

For all experiments, inflammation was induced by a single ip injection of LPS, 100 μg/kg (extracted from Escherichia coli, 90H4012; Sigma-Aldrich Canada Ltd., Oakville, Ontario, Canada). Rats in the control groups were injected with sterile endotoxin-free physiological saline (1 ml/kg). All injections were administered between 1000 and 1030 h.

Experiment 1: LPS-induced fever in lean and obese rats

To compare LPS-induced changes in core body temperature between lean and obese rats, we used remote radio-biotelemetry, as previously described (34), in saline- and LPS-treated rats in each diet condition. Briefly, rats were anesthetized (5.7 mg ketamine and 0.86 mg xylazine/100 g body weight) and implanted ip with precalibrated temperature-sensitive radio transmitters (TA10TA-F40; Data Sciences, Minneapolis, MN). All rats were given 4 d to recover from surgery before injection. Recovery from surgery was assessed by monitoring basal core body temperature before injection. All rats exhibited a normal basal body temperature for the 16 h of measurement before injection. Transmitter output frequency (hertz) 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 was logged into a peripheral processor (BCM; Data Sciences), which relayed frequency data to a personal computer using Dataquest software (Data Sciences) and the signal converted to a temperature readout in degrees centigrade. Measurements of body temperature were conducted during the light phase of the light-dark cycle, from 0800 h on the day of injection to 1000 h the following day.

Experiment 2: LPS-induced circulating cytokine release

To compare circulating cytokines among LPS- and saline-treated lean and obese rats, blood was collected from a tail nick using a sterile razor blade on the extremity of the tail (1 mm from the tip of the tail) 30 min before injection as well as 1, 2, 4, 8, 12, and 24 h after injection. Blood volume collected was up to 0.6 ml.
per time point depending on the weight of each rat. The blood volume collected from each rat (4.2 ml) over the entire time course did not exceed 10% of the total blood volume. Samples were centrifuged (10,000 x g, 10 min at 4 °C), and serum was collected and stored at −80 °C until assay. Concentrations of serum levels for all blood collection time points of TNF-α, IL-6, and IL-1ra were measured using sandwich ELISA (NIBSC, Potters Bar, UK) as previously described (35). Circulating IL-1β levels were not measured in this experiment, because this cytokine is typically not detectable in the circulation of febrile animals, acting instead at the local site of inflammation (36, 37). Intraassay variation coefficients were maintained within 18%, and the detection limit was between 0.008 and 0.031 ng/ml for all assays.

Experiment 3: LPS-induced cytokine expression in hypothalamus and WAT

Gene expression of markers of inflammation in the hypothalamus were quantified 2, 8, and 24 h after LPS or saline injection by quantitative PCR. In short, rats were deeply anesthetized with a lethal dose of sodium pentobarbital (ip 60 mg/kg body weight) 2, 8, or 24 h after injection and perfused through the heart with autoclaved physiological saline (1 ml/kg). Brains were then removed at each time point and placed on dry ice until frozen. To evaluate whether levels of circulating cytokines are reflected in differences in gene expression in WAT, the epididymal fat was extracted and frozen in the same way as the brains. Brains and WAT were stored at −80 °C until analysis.

The hypothalamic region extending from the organum vasculosum of the lamina terminalis to the arcuate nucleus was microdissected and homogenized in 1 ml TRIzol (Invitrogen, Burlington, Ontario, Canada). Total isolated RNA was dissolved in 40 µl diethylpyrocarbonate-treated water. A piece of approximately 200 mg of epididymal WAT was cut from the frozen fat pad. RNA was extracted after homogenization in 1.5 ml TRIzol according to the manufacturer’s instructions, and total isolated RNA was dissolved in 50 µl DEPC water.

For both tissues, 1 µg total RNA was transcribed by heating in a Gene Amp PCR System 9700 Thermocycler (Applied Bioscience Systems, Streetsville, Ontario, Canada) with 5 µM random hexamers (Applied Bios) and 1 mM dNTPs (Sigma-Aldrich) at 65 °C for 10 min. cDNA synthesis was then performed by incubating with di-thiothreitol (Invitrogen), murine myeloleukemia virus reverse transcriptase (Invitrogen, Burlington, Ontario, Canada) and first-strand buffer (Invitrogen) in the thermocycler (Gene Amp PCR system 9700; Applied Biosciences) at 37 °C for 1 h and then at 90 °C for 5 min in a total volume of 20 µl. The product was then diluted to 200 µl with autoclaved distilled water. cDNA was amplified in duplicate by quantitative real-time PCR using a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA), each sample containing 2 µl prepared cDNA, 1 µl primer/probe mixture (Applied Biosystems), and 10 µl TaqMan PCR master mix (Applied Biosystems) in a volume of 20 µl. Each hypothalamic sample was then assayed for changes in the gene expression of IL-6 and IL-1β, the rate-limiting enzyme for prostaglandin synthesis cyclooxygenase 2 (COX2), and suppressor of cytokine signaling 3 (SOCS3) as an indicator of activation of the signal transducer and activator of transcription 3 (STAT3) pathway. WAT samples were analyzed for IL-6, TNF-α, the antiinflammatory cytokine IL-1ra, and the adipokine leptin. The housekeeping gene 18S was used to normalize levels of cDNA expression for each sample. The final value for each sample represents a fold difference from a randomly selected control sample value taken from the same assay. In this case, it was a sample from a lean saline-treated rat. The identification numbers for each primer are listed here: IL-6 RN99999011_m1; IL-1β, RN00580432_m1; COX2, RN01483828_m1; SOCS3, RN00585674_s1; TNF-α, RN99999017_m1; IL-1ra, RN00573488_m1; leptin, RN00565158_m1; and 18S, 4352640E.

Experiment 4: LPS-induced cytokine secretion from WAT of lean and obese rats

To measure cytokine release from WAT, explant cultures were prepared by collecting epididymal WAT from rats deeply anesthetized with a lethal dose of sodium pentobarbital (ip, 60 mg/kg body weight). Once extracted, the tissue was immediately placed in ice-cold Hanks’ balanced salt solution (Invitrogen) supplemented with HEPES (1.50 ml/100 ml; MP Biomedical, Solon, OH) and antibiotics (penicillin-streptomycin 1/500; Invitrogen). The left fat pad was cultured for half of the rats in each group, and the right fat pad for the other half. Each fat pad was then dissected into three pieces representing the caudal, medial, and rostral portions of the tissue. One tissue fragment of approximately 60 mg from each of the three portions was transferred into 12-well plates (Sarstedt, Solon, OH) and antibiotics (penicillin-streptomycin 1/500; Invitrogen). Each well contained a representative piece from each level of epididymal WAT (rostral, medial, and caudal) to avoid any confounding effects due to tissue heterogeneity. Plates were incubated at 37 °C, 90% humidity, and 5% CO2 for a period of 24 h. The settling medium was removed at the start of treatment and replaced by 2 ml fresh medium containing 100 ng/ml LPS or saline. Medium was collected 24 h after treatment, centrifuged for 3 min at 3000 rpm, aliquoted, and stored at −80 °C until assay using ELISA as described above.

Statistical analysis

Fever data were analyzed with a three-way mixed-measures ANOVA with diet (lean vs. obese) and treatment (saline vs. LPS) as between-subject factors and sampling time points as the within-subject factor followed by two-way repeated-measures ANOVA and an all-pairwise Bonferroni’s multiple-comparison post hoc test. The data are presented in Fig. 1 as hourly measurements of body temperature. The time course for circulating cytokines was analyzed with a three-way mixed-measures ANOVA with diet and treatment as between-subject factors and sampling time points as the within-subject factor, followed by two-way ANOVA for each sampling time point. The time course for hypothalamic gene expression was analyzed with a three-way independent-measures ANOVA with diet, treatment, and time as the between-subject factors, followed by two-way ANOVA for each time point. All remaining data were analyzed with two-way independent-measures ANOVA with diet and treatment as between-subject factors.
Results

The body weight and WAT weight corresponding to the sum of the retroperitoneal and epididymal fat pads for rats in each experiment are presented in Table 1.

Experiment 1: LPS induces a higher and more prolonged fever in obese than in lean rats

In rodent models of inflammation to LPS, the magnitude and duration of the fever response varies according to several parameters including the dose, ambient temperature, and method of injection (38). Typically, however, it is a response with two main phases peaking approximately 3 and 6 h after treatment and may last for up to 12 h. It is generally preceded by a brief increase in core body temperature in both saline- and LPS-treated groups attributable to a stress reaction to the injection (38).

Treatment with LPS (100 µg/kg) induced a significant increase in core body temperature in both lean and obese animals when compared with saline controls (Fig. 1). There were also significant and clear differences in the magnitude of the fever response in the obese animals between 7 and 10 h ($P < 0.05$) and 16 and 24 h ($P < 0.05$) after LPS injection ($n = 4$). Unexpectedly, although the temperature increase in the LPS-treated animals began to decline after the second phase, returning to basal by 11 h, the core body temperature of the obese group showed an additional phase of elevated fever ($P < 0.05$), compared with the lean conspecifics and with saline controls during the dark phase of the light-dark cycle between 16 and 24 h.

Experiment 2: LPS induces higher levels of cytokines in the circulation of obese compared with lean rats

Average serum levels for all groups at each sampling time ($-0.5, 1, 2, 4, 8, 12, \text{and } 24 \text{ h}$) are presented in Fig. 2 for all the cytokines tested in the present study. As can be seen from this figure, LPS induced a significant increase in all of the cytokines tested in the circulation of both obese and lean rats (significant main effects for treatment in all cases $P < 0.05$) with the exception of leptin. The pattern of induction by LPS varied across cytokines, so that levels of TNF-α peaked at 1 h and those of IL-6 and IL-1ra at 2 h after LPS injection. In each case, the magnitude of the response was greater in obese than in lean rats, resulting in significant treatment $\times$ diet $\times$ time interactions ($n = 6–8; P < 0.05$). An increase in circulating leptin after LPS was, however, seen only in obese rats resulting in a significant treatment $\times$ diet $\times$ time interaction ($n = 5–6; P < 0.05$).

Experiment 3: LPS-induced inflammatory signals were higher in hypothalami and epididymal WAT of obese animals

Figure 3 shows the average gene expression in the hypothalamus for all groups at each sampling time. LPS treatment induced a robust increase in IL-6, IL-1β, COX2, and SOCS3 expression (significant main effects of treatment, $P < 0.05$ in all cases). As with circulating cytokine levels, the pattern of expression of these signals varied. Peak levels of IL-1β and COX2 expression were seen 2 h after injection, whereas expression of IL-6 and SOCS3 was highest at the 8-h time point. For IL-6, SOCS3, and IL-1β ($n = 4–6$), the expression was greater in obese than lean rats (significant diet $\times$ treatment interaction, $P < 0.05$). No significant interaction was observed for COX2 ($P > 0.05$).

Figure 4 shows the gene expression after injection in WAT from the same rats in which hypothalamic gene ex-

### Table 1. Body and WAT weights of rats in each experiment

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Lean rats</th>
<th>Obese rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Body weight (g)</td>
<td>WAT weight (g)</td>
</tr>
<tr>
<td>1</td>
<td>503.75 ± 21.46$^a$</td>
<td>18.75 ± 1.59$^b$</td>
</tr>
<tr>
<td>2</td>
<td>504.27 ± 5.56$^b$</td>
<td>14.40 ± 1.81$^b$</td>
</tr>
<tr>
<td>3</td>
<td>496.64 ± 5.82$^b$</td>
<td>15.54 ± 1.53$^b$</td>
</tr>
<tr>
<td>4</td>
<td>543.50 ± 10.63$^b$</td>
<td>20.63 ± 1.41$^b$</td>
</tr>
</tbody>
</table>

Mean body weight and WAT weight corresponding to the sum of both of retroperitoneal and epididymal fat pads at the time of each experiment are shown as mean ± SEM.

$^a^b$ Statistically significant differences between lean and obese rats mean body weights and WAT weights, respectively, are indicated: $^a P < 0.05$ and $^b P < 0.001$. 

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expression was examined at 2 h (n = 5–7). As can be seen from this figure, LPS induced robust increases in IL-6, IL-1ra, and TNF-α expression at this time point (significant main effect of treatment, P < 0.05 in each case). In addition, WAT from obese rats showed a significant increase in IL-1ra expression (diet × treatment interaction, P < 0.05). This effect did not reach statistical significance for IL-6 or TNF-α (P > 0.05). In contrast to the other cytokines measured, LPS treatment had no effect on leptin gene expression at this time point (P > 0.05).

Experiment 4: LPS induces more cytokine release from WAT of obese compared with lean animals

The amount of cytokines secreted into the medium by WAT explants over the 24-h period after LPS or saline treatment is shown in Fig. 5 (n = 5). There was a large increase in IL-6, TNF-α, and IL-1ra secretion from LPS-
treated explants (main effect for treatment, \( P < 0.05 \) in all cases). Overall, more IL-6 was secreted from fat explants obtained from obese than from lean rats (\( P < 0.05 \)). There was no significant main effect of obesity or of diet \( \times \) treatment interaction on secretion of any of the other cytokines examined.

**Discussion**

In the current study, we demonstrated that obese rats showed both a higher fever between 7 and 10 h and more prolonged fever between 16 and 24 h after LPS injection than lean rats. This was associated with increased levels of the proinflammatory cytokines IL-6 and TNF-\( \alpha \) as well as the antiinflammatory cytokine IL-1ra during the first 8 h after treatment. LPS injection also induced an increase in circulating levels of the adipokine leptin, but only in the obese group. The time course of the leptin response was quite different from that observed for the other cytokines such that increases in leptin levels peaked 8 h after injection and remained slightly, although not significantly, elevated at the 24-h time point. The obesity-associated differences in circulating cytokine levels after LPS injection were accompanied by an overall increase in hypothalamic expression of the mRNA for IL-1\( \beta \) as well as significant increases in SOCS3 and IL-6 mRNA at the 8-h time point. Similarly, using an *in vitro* approach, we showed that WAT of obese rats secreted higher levels of IL-6 but not TNF-\( \alpha \) or IL-1ra per gram of fat after exposure to LPS, whereas WAT of obese rats expressed higher mRNA levels of IL-1ra 2 h after LPS injection. Overall, these results demonstrate that diet-induced obesity produces a change in the acute inflammatory response at multiple levels and suggests that a functional change in WAT resulting in an increased capacity for IL-6 secretion may be contributing to this effect. Whether other fat depots such as brown adipose tissue also contribute to the alterations in the immune response in obese organisms remains to be determined, because brown adipose tissue, a tissue involved in thermogenesis, has also been shown to be involved in the development of obesity (39).
To the best of our knowledge, this is the first examination of the effect of diet-induced obesity on a 24-h fever response to LPS. Our current finding of an enhanced (magnitude and duration) fever response in obese rats contrasts with earlier reports using genetic models of obesity. Both Zucker and Koletsky rats (40–42) exhibit an attenuated fever response to LPS at room temperature (22 C) (43, 44) or a normal fever at thermoneutrality (28 C) (43, 45). One instance of higher fever response in obese rats was reported in obese (fa/fa) vs. lean (Fa/Fa) rats after intraventricular microinfusion of IL-1β (46).

As expected, LPS injection produced a robust increase in circulating IL-6, TNF-α, and IL-1ra that peaked at 2 h after injection and, with the exception of IL-1ra, had returned to baseline by approximately 8 h after treatment (47–49). IL-6 and TNF-α along with IL-1β are known as the principal pyrogenic cytokines (50). Unlike IL-1β, which acts mainly as a local inflammatory signal, its endogenously occurring receptor antagonist is readily detectable in the circulation of infected animals where it acts principally to limit the extent of inflammation by attenuating the release of IL-6 into the circulation (47, 51, 52). The levels of this antiinflammatory cytokine are often used as an indicator of local inflammatory actions of IL-1β (52).

In the current study, obese rats showed a greater increase in circulating levels of IL-6, TNF-α, and IL-1ra in the first 8 h after LPS treatment. Of the major pyrogenic cytokines, strong evidence supports IL-6 as the essential circulating cytokine mediating fever. IL-6 rises consistently in response to inflammatory challenge, and its levels correlate highly with the magnitude of the fever response (37, 53–57). Moreover, neutralization of IL-6 with antisera (58) or its absence in IL-6 knockout mice (59) attenuates the fever response to LPS. It is therefore plausible that the higher increase in circulating IL-6 after LPS injection in obese rats was the main driving force behind their accentuated fever response, at least in the earlier phases. The peak circulating IL-6 levels preceded the peak of the fever response presumably because, as we have previously demonstrated, IL-6 acts at the cerebral vasculature where it induces the prostaglandin synthase COX2 and subsequently the production of PGE2, the ultimate mediator of fever (60).

We also observed an increase in circulating leptin after LPS treatment in obese but not in lean rats. This was somewhat surprising, because increases in circulating leptin in nonobese rodents after LPS treatment have been reported (61–67). Whether differences in species, strain, or dose contributed to this discrepancy remains to be determined. In our experiment, leptin levels in obese rats peaked 8 h after LPS. This coincides temporally with the prolonged fever in the obese group, raising the possibility that leptin contributed to this prolongation, especially because leptin can act as a pyrogenic cytokine (68), and neutralizing its actions in vivo attenuates the fever response to LPS in rats (66, 69, 70).

To investigate whether the differences in circulating cytokines are reflected in differences in brain inflammatory signals that form part of the fever pathway, gene expression of members of the pyrogenic cascade were measured in the hypothalamus. LPS-treated obese rats expressed higher levels of IL-1β than lean rats, peaking at 2 h after injection as well as significantly higher levels of SOCS3 and IL-6 peaking at the 8-h time point. The close temporal pattern of expression of IL-1β and COX2 in this experiment is in accordance with previous reports showing that they are highest at 2 and 3 h after LPS (71, 72). Surprisingly, and in contrast to IL-1β, there was no significant difference in hypothalamic COX2 expression between lean and obese rats. It is possible that our use of whole hypothalami, rather than specific nuclei, to measure COX2 mRNA may have diluted the effect of obesity. Of particular interest is the observation that hypothalamic levels of IL-6 mRNA were significantly higher in obese vs. lean counterparts at a relatively late time point (8 h) after LPS. Given that we have recently demonstrated that IL-6 can induce COX2 in the brain directly via a STAT3-dependent mechanism (73), the higher levels observed in obese rats might well have contributed to the increased duration of their fever response. This possibility is supported by our observation that SOCS3, a product of the STAT3 pathway used experimentally as a marker of its brain activation (74) showed a temporal pattern of expression similar to that of IL-6 in the LPS-treated rats.

Finally, we investigated the possibility that WAT contributed to the higher circulating cytokine surge in obese rats. Given that the peak for most circulating cytokines was 2 h after LPS, we used this time point to measure cytokine expression in epididymal WAT. LPS treatment increased IL-6, TNF-α, and IL-1ra expression in both lean and obese rats, as previously reported (75–77). IL-1ra mRNA was significantly higher in the WAT from obese animals than lean rats. No change in LPS-induced leptin expression was observed. Because circulating leptin levels peaked much later than other cytokines (8 h after injection), the time point at which its expression was measured in WAT may have been too early. To measure cytokine secretion, we used a WAT explant culture. Similarly to cytokine expression, LPS increased cytokine secretion in both lean and obese rats with the WAT from obese rats secreting significantly more IL-6 per gram than that from lean rats.

The discrepancies in the effects of obesity on levels of expression and release of cytokines are most likely due to
differences in experimental procedures. mRNA expression was measured in WAT excised from animals 2 h after an LPS injection, whereas the release was assessed in tissue incubated in vitro with LPS for 24 h. It is also possible that the documented extensive posttranscriptional modification of cytokines also contributed to the differences in measures of mRNA and cytokine release (78). Overall, however, our data show that WAT from obese animals exhibits greater sensitivity to LPS, which likely contributes to the higher increase in circulating cytokines and, possibly, to the more pronounced fever response. It remains unclear, however, which cytokines originating from WAT make the greatest contribution to this effect.

It is noteworthy that in contrast to studies of severe obesity and of genetic models of obesity, we did not observe increases in baseline cytokine expression or in the circulation in obese rats. This could reflect the fact that the rats in the current study had been obese for a relatively short period of time. Alternatively, the type of diet might be an important variable. Increased basal cytokine levels in obesity result in part from activation of the innate immune system through stimulation of the Toll-like receptor 4 (79). In fact, diets rich in saturated fatty acids activate whereas unsaturated fatty acid diets inhibit Toll-like receptor 4 signaling (80). The higher proportion of unsaturated vs. saturated fatty acids in our diet may have contributed to preventing basal cytokine increase or at least delaying it.

Taken together, our data demonstrate that diet-induced obesity produces profound changes in the acute-phase response to endotoxin challenge. Obese rats are more responsive to the effects of LPS as exhibited by a greater fever response particularly at later phases in the fever response. Higher levels of circulating cytokines after LPS in obese rats led to more pronounced pyrogenic signaling in the hypothalamus. After LPS, circulating leptin levels increased only in obese rats, suggesting a role for leptin in these effects, especially because leptin is known to possess pyrogenic properties that could have promoted the higher inflammatory response in these rats. Finally, WAT is likely an important contributor to the higher circulating levels of cytokines in obese rats and thus to their more pronounced inflammatory response. Our data suggest that WAT may have undergone a functional change such that it acquired a greater capacity for stimulating cytokine expression and secretion to LPS treatment.

Acknowledgments

We thank Claudia Frate for excellent technical help.

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This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada and the Canadian Institutes of Health Research (CIHR) (to B.W. and G.N.L.). J.P. was the recipient of a training grant from the CIHR Neuroinflammation Training Program at McGill University and subsequently of a doctoral training award from the Fonds de la Recherche en Sante du Quebec, which also supported this work through an infrastructure grant to Groupe de recherche en neurobiologie comportementale.

Disclosure Summary: The authors have nothing to disclose.

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