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Chronic exposure to Low dose bacterial lipopolysaccharide inhibits leptin signaling in vagal afferent neurons ☆

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HIGHLIGHTS

- Continuous low-dose LPS infusion induced leptin resistance in vagal afferent neurons
- Chronic low-dose LPS promoted expression of proteins with orexigenic profiles
- After 5 weeks of chronic low-dose LPS treatment rats became hyperphagic
- Chronic LPS treatment attenuates CCK-induced inhibition of food intake

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ABSTRACT

Bacterially derived factors are implicated in the causation and persistence of obesity. Ingestion of a high fat diet in rodents and obesity in human subjects is associated with chronic elevation of low plasma levels of lipopolysaccharide (LPS), a breakdown product of Gram-negative bacteria. The terminals of vagal afferent neurons are positioned within the gut mucosa to convey information from the gut to the brain to regulate food intake and are responsive to LPS. We hypothesized that chronic elevation of LPS could alter vagal afferent signaling. We surgically implanted osmotic mini-pumps that delivered a constant, low-dose of LPS into the intraperitoneal cavity of rats (12.5 μg/kg/hr for 6 weeks). LPS-treated rats developed hyperphagia and showed marked changes in vagal afferent neuron function. Chronic LPS treatment reduced vagal afferent leptin signaling, characterized by a decrease in leptin-induced STAT3 phosphorylation. In addition, LPS treatment decreased cholecystokinin-induced satiety. There was no alteration in leptin signaling in the hypothalamus. These findings offer a mechanism by which a change in gut microflora can promote hyperphagia, possibly leading to obesity.

1. Introduction

Recent studies have underlined a role for the gastrointestinal (GI) microflora in the development and progression of obesity. Obesity in humans and rodents is associated with significant modifications in microflora composition when compared to lean controls [1–3]. Similar changes can be induced by increasing dietary fat consumption in both rodent models and humans [4,5]. A causal role for the GI microflora in the development of obesity has been suggested from studies where colonization of germ-free mice with GI microflora from either an obese or lean donor resulted in recapitulation of the donor phenotype [2,5]; however, the pathways and mechanisms by which microflora composition affects food intake and adiposity are not fully understood.

One possible mechanism is modulation of host physiology by bacterially-derived factors. Genetic and high fat (HF) diet-induced obese mice display a constant increase in low plasma levels of the bacterial breakdown product lipopolysaccharide (LPS), referred to as "metabolic endotoxemia" [6–8]. Metabolic endotoxemia has also been described in humans fed a western type diet for four weeks [9]. We have previously shown that in rats prone to HF diet-induced obesity, weight gain is associated with small but significant increases in plasma levels of LPS compared to lean littermates [10]. Furthermore, knocking out the LPS receptor (Toll-like receptor 4, TLR4) or the TLR4 adapter protein, CD14, confers resistance to HF diet-induced obesity [6,11]. One report has shown that chronic administration of a low-dose of LPS in mice can increase adiposity, but the mechanism was not identified [6].

The majority of previous work has focused on effects of HF diets and TLR4 on inflammatory responses and alteration in neuronal signaling in

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the hypothalamus, yet other neuronal populations involved in regulation of food intake and body weight, including vagal afferent neurons, express TLR4. The peripheral terminals of vagal afferent neurons are positioned within the gut mucosa to respond to mediators released from gut epithelial cells, such as gut hormones, or to factors that cross the epithelium from the gut lumen, like LPS. Acutely, high levels of LPS such as those seen in sepsis activates vagal afferent neurons resulting in hypophagia and loss of body weight [12]. However, no studies have been performed to assess the effect of low, chronic elevation of plasma LPS, such as seen in obesity and in HF-feeding, on vagal afferent neurons. We have previously shown in cultured vagal afferent neurons that LPS upregulates expression of suppressor of cytokine 3 (SOCS3), and inhibits leptin-induced phosphorylation of signal transducer and activator of transcription 3, STAT3. Moreover, leptin resistance develops in vagal afferent neurons in response to 6 weeks of HF diet resulting in reduced cholecystokinin (CCK) signaling and satiation [13]. Recently, we have shown that deletion of leptin receptors in vagal afferent neurons is sufficient to induce obesity in chow fed mice.[14]

In the present study, we tested the hypothesis that chronic low-dose treatment with LPS would induce leptin-resistance in vagal afferent neurons. We aimed to determine the effects of chronic 6-week administration of low-doses of LPS on vagal afferent nerve function, and on food intake and body weight. Chronic LPS treatment activates TLR4 in vagal afferent neurons, up-regulates expression of SOCS3 and inhibits leptin-induced STAT3 phosphorylation. LPS treatment prevented CCK-induced inhibition of food intake and changes in neuropeptide expression. These changes occurred in the absence of increased circulating leptin, and in the absence of changes in leptin signaling in the arcuate nucleus of the hypothalamus. Taken together, these data suggest that bacterially-derived LPS may influence the function of vagal afferent neurons leading to alterations in signaling of satiety from the gut to the brain.

2. Methods and methods

2.1. Animals

Four week study: Male Wistar rats (initial BW = 237 ± 9.4 g) were singly housed in a temperature controlled room with 12 hrs light/dark cycle. Animals were fed a low fat (LF) diet (Research Diets D12450B). A subset of animals were implanted with an osmotic pump (# 2004, Alzet, Cupertino, CA, USA) delivering either saline (n = 5) or chronic low-dose LPS (12.5 μg/hr/day; IP, n = 6). Body weight and food intake were recorded 3 times a week.

Six week study: Male Wistar rats (initial BW = 324.4 ± 18.7 g) were singly housed in a temperature controlled room with 12 hrs light/dark cycle. Animals were fed a LF diet (Research Diets D12450B). Half of the animals (n = 9) were implanted with an osmotic pump (# 2006, Alzet, Cupertino, CA, USA) delivering chronic low-dose LPS (12.5 μg/hr/day; IP). Body weight and food intake were recorded 3 times a week.

All experiments were performed in accordance with protocols reviewed and approved by the Institutional Animal Care and Use Committee, UC Davis.

2.2. CCK feeding study

After 5 weeks of treatment, animals were fasted for 12 hrs on wire bottom cages and injected 1 hr into the dark cycle with either CCK8s (0.3 μg/kg, IP, Bachem, Torrance, CA, USA) or saline (400μl; vehicle). Food intake was recorded for 2 hrs following injection.

2.3. Tissue collection

After 6 weeks of treatment, animals either fed ad libitum (n = 3/group) or fasted for 12 hr (n = 6/group) were deeply anesthetized with a mixture of sodium phenytoin and sodium pentobarbital (0.2 ml/100 g, Beuthanasia-D Special C-III, Shering, Kenilworth, NJ, USA). Fasted rats received leptin (80 μg/kg, IP; n = 3; Sigma, St Louis, MO, USA) or saline (400ul, n = 3) 1 hr prior to euthanasia. Blood was collected by cardiac puncture; serum was stored at −80 °C. Epididymal, mesenteric and retroperitoneal fat tissues were weighed and collected. Left and right nodose ganglia were dissected; for each rat, one ganglion was fixed for immunohistochemistry (4% paraformaldehyde, 2 hrs) and stored in 25% sucrose at 4 °C, the other was directly stored at −80 °C for protein analysis. All other tissue was flash frozen and stored at −80 °C.

2.4. Quantification of serum leptin and LPS

Circulating leptin levels were measured in serum by ELISA according to the manufacturer intructions (#22-LEP-06, ALPCO Diagnostics, Salem, NH, USA). LPS in serum was measured with a Pyrochrome Lysate Mix, a quantitative chromogenic reagent, (Associate of Cape Cod, East Falmouth, MA, USA) diluted in Glucashield buffer (Associate of Cape Cod, East Falmouth, MA, USA) which inhibits cross-reactivity with (1 → 3)-β-D-Glucans. Briefly, serum samples were diluted 1:6 in Pyrogen free water (Lonza, Basel, Switzerland) and heated for 10 min at 70 °C. Samples and reactive solution were incubated at 37 °C for 80 min and absorbance was read at 405 nm.

2.5. Immunohistochemistry

Cryostat sections of fixed nodose ganglia (5 μm) were mounted on polysine-coated slides (Polysine; MenzelGlaser, Braunschweig, Germany). Slides were incubated with primary antibodies (CART 55–102, Phoenix Laboratories, San Antonio, TX, USA; pro-MCH, C-20, Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight at 4 °C, and incubated with secondary antibodies conjugated to AlexaFluor 488 (Invitrogen, Carlsbad, CA, USA) for 1 hr at 37 °C. Images were collected using an Olympus spinning disc confocal microscope (BX61 System, Olympus, Melville, NY, USA).

2.6. Western blot

Tissues were homogenized in Mammalian protein extraction reagent (M-PER, ThermoScientific, Waltham, MA, USA). Western blots were run as previously described [13]. Briefly 10 μg of protein was run on a 10% BisTris gel. Primary antibodies were incubated overnight (phosphoSTAT3 #9145, and SOCS3 #2923 Cell signaling, Danvers, MA, USA; TNF-alpha (ab6671) and MyD88 (ab2064) Abcam, Cambridge, MA, USA; TLR4 (img-579A) Imgenex San Diego CA, USA; TLR4-MD2 (14-9924-81) eBioscience San Diego CA, USA). GAPDH was used as a loading control (#2118, Cell Signaling). Secondary antibody was incubated 1 hour (Anti-rabbit IgG HRP-linked, Cell Signaling). The membrane was exposed to film for 5–45 minutes and developed in a 100 Plus automatic X-Ray film Processor (All Pro Imaging, Hicksville, NJ, USA). The film was analyzed by Imagequant v 5.1 software (Biosciences, Amersham, UK).

2.7. Myeloperoxidase assay

MPO activity was determined as previously described [15]. Absorbance was read at 450 nm at 12 min. Activity was expressed as the difference between the absorbance after a 12 min reaction time and the baseline absorbance per ml of supernatant and per mg of tissues.

2.8. Statistical analysis

Statistical analysis was performed using Prism software (Prism 6.0, GraphPad Software Inc., La Jolla, CA, USA). For the four week experiment, one way ANOVA with LF, Saline or LPS treatment as variable was used (Fisher comparison post-hoc). For the six week experiment,

two-tailed T test with LF or LPS treatment as the variable. Paired T-test was used to analyze food intake experiment following IP injection of CCK. When tested for normality (Kolmogorov-Smirnov test), the data for the plasma LPS distribution was not Gaussian; therefore, the Mann Whitney test was used. Differences were considered significant if $p < 0.05$. Data are mean ± SEM. * represents $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3. Results

3.1. Chronic low-dose LPS treatment increases body weight gain and energy intake

In the first study, we determined the effect of chronic LPS exposure for four weeks on weight gain and energy intake. Both saline- and LPS-treated rats lost weight in the immediate post-surgical period compared to un-operated, LF fed controls; however, LPS-treated rats had a greater post-surgery weight reduction compared to saline-treated rats (Fig. 1A; post-surgery day 2, $p < 0.01$). Ten days post-surgery, there was no difference in weight between the LPS and saline groups, and by 21 days there was no difference between the LF, saline and LPS groups (Fig. 1**A**). During the last week of the experiment, LPS-treated animals consumed significantly more kcal than the LF group ($p < 0.05$, Fig. 1**B**); there was no difference in food intake between the LF and saline-treated groups. Saline-treated rats consumed less kcal than LPS-treated animals although the difference did not reach statistical significance ($p = 0.06$). The results from this preliminary study indicated that animals recovered from the acute effects of surgery and initial exposure to LPS in 10 days and that a trend towards increased food intake developed at week 4. In order to determine the effects of longer term LPS treatment, we repeated the study using pumps that chronically released LPS for six weeks and used a LF-fed group as control.

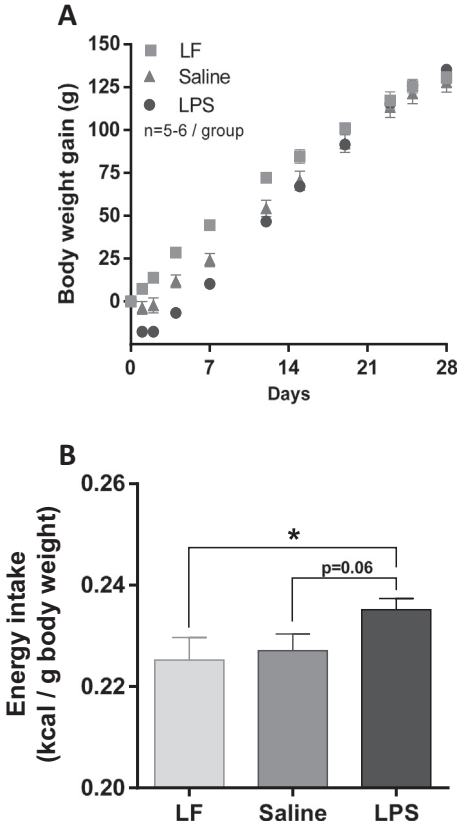

Fig. 1. Four weeks of chronic LPS treatment leaded to an increase in food intake. (A) Weight gain in grams. Surgical implantation of minipumps reduced initial weight gain. LPS treatment reduced weight gain further than saline treatment in the first few days ; however after four weeks of treatment all groups gained similar weight. (No surgery controls [LF] weigh more than Saline controls day 2–15 $p < 0.001$; LF weigh more than LPS days 2–15 $p < 0.001$; Saline weigh more than LPS days 1–7 $p < 0.01$; two-way anova). (B) By week 4, LPS treated rats consumed significantly more kcal than LF animals ($p < 0.05$, one-way anova) Saline animals intake was not different from LF rats and was lower than LPS treated rats although it did not reach significance ($p = 0.06$). Data are mean ± SEM. * represents $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

LPS-treated rats lost a significant amount of body weight in the immediate post-surgical period (<7 days) (Fig. 2**A**), which was associated with hypophagia (Fig. 2**D**). After 15 days, there was no statistical difference in body weight observed between LF-fed and LPS-treated rats ($p > 0.05$; Fig. 2**A**) and no difference in food intake between LF-fed and LPS-treated rats, suggesting full recovery from surgery and any acute anorectic effects of LPS treatment. Four weeks post-implantation, LPS-treated rats consumed significantly more kcal than LF-fed rats ($p < 0.05$) and remained hyperphagic for the rest of the experiment (week 5, $p < 0.001$, week 6, $p < 0.01$). During the same period, LPS-treated rats gained more weight than LF fed controls; this difference in weight gain reach significance in week 6 (19.5 ± 0.9 vs. 14.6 ± 2.3 g, $p < 0.05$) (Fig. 2**B**), although there was no significant difference in final body weight between LPS-treated and LF-fed rats. Chronic LPS treatment led to a redistribution of adiposity characterized by a decrease in epididymal fat (8.8 ± 0.2 vs. 10.7 ± 0.8 g, $p < 0.05$) and an increase in mesenteric fat (8.3 ± 0.4 vs. 7.0 ± 0.2 g, $p < 0.05$) (Fig. 2**C**).

Determination of circulating LPS confirmed that the pump implantation led to a significant increase in circulating LPS (0.2 ± 0.1 vs. 0.07 ± 0.02 EU, $p < 0.01$). There was no significant difference in fasted plasma levels of leptin between LF-fed rats and LPS-treated rats (1.1 ± 0.3 vs. 1.2 ± 0.2 ng/ml, ns).

3.2. Chronic low-dose LPS treatment induces leptin resistance in vagal afferent neurons

Protein expression of TLR4 in the cell bodies of vagal afferent neurons was unaltered by LPS treatment (ns; Fig. 3**A**). However, the active form of TLR4 bound to MD2 was significantly increased in vagal afferent neurons of LPS-treated animals compared to LF-fed rats ($p < 0.01$; Fig. 3**B**). This was associated with an increased abundance of the TLR4 signaling molecule, MyD88 ($p < 0.01$; Fig. 3**C**).

Expression of SOCS3 protein was increased in vagal afferent neurons of LPS-treated rats compared to LF-fed rats ($p < 0.001$; Fig. 3**D**). STAT3 phosphorylation in vagal afferent neurons induced by acute leptin administration (80ug/kg; ip.) was significantly reduced in LPS-treated compared to LF-fed rats (LPS vs LF $p < 0.01$, Fig. 3**E**), indicative of leptin-resistance. However, despite clear evidence for leptin-resistance in vagal afferent neurons in response to chronic low-dose treatment with LPS, there was no evidence of leptin resistance in the arcuate nucleus of the hypothalamus in LPS-treated rats. There was no significant reduction in phosphorylated STAT3 in response to leptin (80ug/kg, IP) in the arcuate nucleus of LPS-treated compared to control rats (ns, Fig. 3**F**).

3.3. Chronic low-dose LPS treatment inhibits CCK-induced signaling and satiation

We previously demonstrated that leptin resistance in vagal afferent neurons reduces CCK-induced signaling in vagal afferent neurons and CCK-induced satiation [13]. Administration of CCK8S (0.3 μg/kg, IP) significantly reduced food intake in LF-fed rats ($p < 0.05$) while there was no decrease in food intake in response to CCK8S administration in LPS-treated rats (ns; Fig. 4**A**). We previously reported that in vagal afferent neurons, feeding or administration of CCK to fasted rats up-regulates the expression of the putative anorectic neuropeptide, cocaine and amphetamine regulated transcript (CART) and down-regulates the expression of the putative orexigenic neuropeptide, melanin concentrating hormone (MCH) expression [16–18]. In the present study, feeding failed

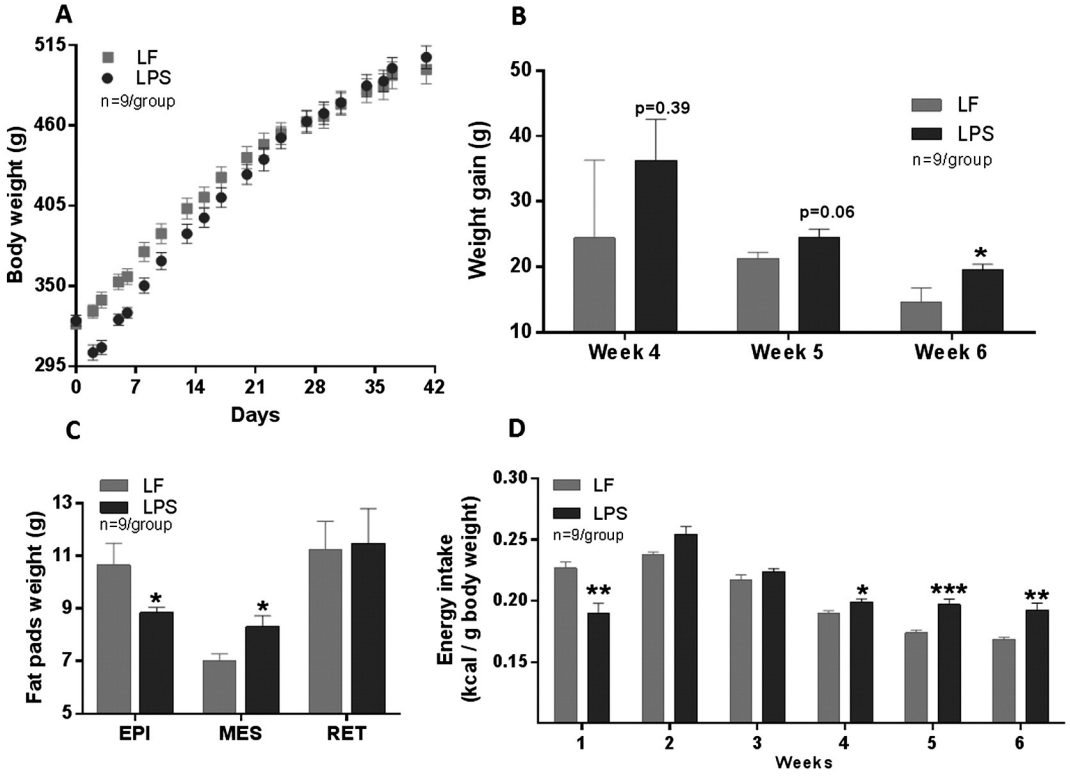

Fig. 2. Chronic low-dose LPS treatment increases body weight gain and energy intake. (A) Weight in grams. Pump implantation initially significantly reduced weight (LPS vs. LF day 1–8, $p < 0.01$), there was no significant differences in body weight throughout the rest of the study. There was a trend towards a higher body weight in the LPS group day 28–42. (B) Body weight gain (g) during the last three weeks of the study. By week 6, LPS treated rats were gaining more weight than LF control ($p < 0.05$). (C) Weight (g) of epidydimal (EPI), mesenteric (MES) and retroperitoneal (RET) fat depots after 6 weeks of treatment. Chronic LPS treatment leaded to a decrease in epidydimal fat ($p < 0.05$) and an increase in mesenteric fat ($p < 0.05$). (D) Average weekly energy intake per 100 g of body weight. Following pump implantation LPS treated animals were hyperphagic ($p < 0.01$). During the last three weeks of the study they consumed more kcal than LF rats (week 4 $p < 0.05$, week 5 $p < 0.001$, week 6 $p < 0.01$). Data are mean ± SEM. * represents $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

to increase CART expression (Fig. 4**B**), while MCH expression remained constitutively high (Fig. 4**C**) in rats treated with low-dose chronic LPS. This findings are consistent with the loss of plasticity seen in a rat model of diet-induced obesity [13}.

3.4. Chronic low-dose LPS treatment had no effect on inflammatory mediators

We have previously shown that HF feeding induces inflammation in the ileal mucosa. In the present study, chronic low-dose LPS treatment had no effect on myeloperoxidase (MPO) activity, a marker of neutrophils, suggesting an absence of inflammation in the ileum (MPO: 1.1 ± 0.1 vs. 1.3 ± 0.2 ΔAbs/ml/mg, ns). LPS treatment led to a small but non-significant increase in circulating TNF-α (2.0 ± 0.7 vs. 2.7 ± 0.3 pg/ml; ns) and no difference was observed in TNF-α protein levels in the arcuate nucleus of the hypothalamus (0.01 ± 0.005 vs. 0.01 ± 0.002 n-fold GAPDH, ns) or in mesenteric fat (0.03 ± 0.008 vs. 0.05 ± 0.02,ns).

4. Discussion

Obesity and models of obesity induced by ingestion of HF-diet in rodents are associated with chronically elevated circulating levels of LPS; however, the consequences of this metabolic endotoxemia on pathways that regulate food intake and body weight are not fully understood. Here we show for the first time that chronic low-dose administration of LPS induces leptin-resistance in vagal afferent neurons and abolition of CCK-induced inhibition of food intake. The vagal afferent pathway is the major neuronal pathway involved in transmission of information from the gut to the brain and plays a significant role in the control of food intake. The terminals of vagal afferent neurons sense signals released from the gut in response to ingested nutrients; the gut hormone CCK, acts via a vagal afferent pathway to prompt meal termination. The close proximity of vagal afferent fibers to the basement membrane and expression of TLR4 [19] make vagal afferent neurons a possible target of LPS action. HF fat feeding has been shown to enhance gastrointestinal permeability promoting the translocation of LPS to the circulation [8,15]. We have previously reported that treatment of cultured vagal afferent neurons with LPS leads to an increase in SOCS3 expression [20]. SOCS3 is a negative regulator of leptin signaling and we have shown in HF diet-induced obese rats that SOCS3 is an important mechanism by which leptin resistance develops in vagal afferent neurons and coincides with the onset of hyperphagia [13]. We have recently shown that leptin signaling in vagal afferent neurons is both necessary and sufficient to induce hyperphagia and prevent the onset of HF diet induced obesity [14]. Taken together, the data provides a mechanism linking changes in gut microbiota induced by ingestion of HF diets to dysregulation of food intake and body weight.

Chronic low-dose LPS treatment induced TLR4 activation and MyD88 signaling in vagal afferent neurons, associated with increased SOCS3 expression and reduced leptin-signaling, characterized by the absence of leptin-induced pSTAT3. Importantly, we found no evidence of leptin resistance in the arcuate nucleus of LPS-treated rats, strongly suggesting that the site of action of LPS is vagal afferent neurons. We observed a significant increase in energy intake in the LPS-treated rats during the last weeks of treatment. We demonstrate that this chronic low dose LPS is sufficient to induce leptin–resistance in vagal afferent neurons, reduced sensitivity to the satiating effects of CCK, and loss of vagal afferent plasticity. Similar changes in vagal afferent neurons occur in response to long term ingestion of a HF diet [13], which has previously shown to increase plasma levels of LPS [21,22]. Together with recent evidence that loss of leptin signaling in vagal afferent neurons

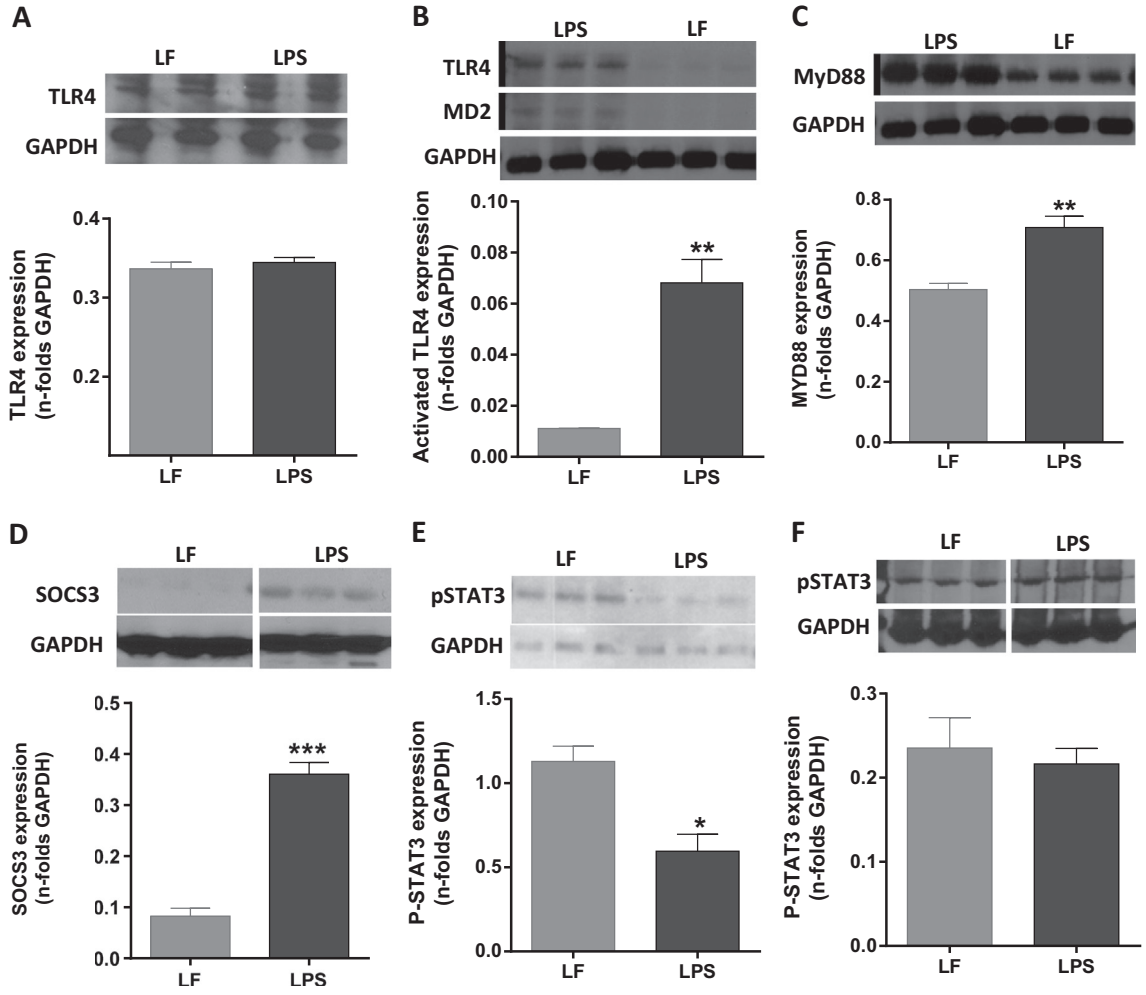

Fig. 3. Chronic low-dose LPS treatment induces leptin resistance in VAN. Western blot analysis was performed in nodose ganglia of rats (A-E) and arcuate nucleus of the hypothalamus (F) after 6 weeks of LPS treatment. (A) TLR4 protein expression was unchanged in nodose ganglia; however (B) nodose ganglia of LPS rats had significantly increased active TLR4 bound to MD2 ($p < 0.01$). (C) MyD88, a downstream signaling molecule of TLR4 was also elevated in nodose ganglia of LPS-treated rats ($p < 0.01$). Results from (B) and (C) were gathered from the same immunoblot. (D) SOCS3 was elevated in the nodose ganglia of LPS-treated rats ($p < 0.001$), and this was associated with (E) a decreased expression of leptin (80ug/kg, ip)-induced phosphorylated STAT-3 protein expression ($p < 0.05$). However, (F) no change in leptin (80ug/kg, ip)-induced phosphorylated STAT-3 protein expression in the arcuate nucleus of the hypothalamus was observed in LPS-treated rats. GAPDH was used as a loading control. Data are mean ± SEM* represents $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

drives hyperphagia and obesity [14], it suggests that the increase in food intake and body weight we observed at week 6 in the LPS treated rats may be caused by LPS-induced leptin resistance.

Recent evidence has shown that food intake, and gut hormones such as CCK, not only activate vagal afferent neurons but also induce changes in neuronal phenotype [13,16–18,23]; for example, expression of the neuropeptide transmitter CART in vagal afferent neurons is low during fasting but increased by feeding or peripheral administration of CCK [18]. In contrast, expression of MCH is high in fasting and reduced by feeding or CCK administration [16,17]. One consequence of leptin-resistance in vagal afferent neurons induced by HF feeding is inhibition of this alteration in phenotype; expression of MCH remains high and CART low in vagal afferent neurons of HF fed rats, regardless of fed status [24]. A similar result was found in the present study in response to LPS treatment. LPS-induced vagal leptin resistance may be, at least partially, responsible for the alteration in vagal signaling and associated hyperphagia observed in HF diet-induced obesity. These data correlate with human studies in which endotoxemia has been shown to correlate with energy intake [25].

Cani et al. showed that chronic LPS treatment of mice for four weeks increased body weight [6]. In our study, LPS-treated rats did not show a significant increase in overall body weight when compared to LF-fed controls; however, we observed a significant increase in food intake after 4 weeks of LPS treatment. In the last week of the study, we observed a significant increase in weight gain in the LPS- treated rats, presumably resulting from increased calorie intake. Due to technical limitations, LPS treatment cannot readily be prolonged as this would involve further surgical intervention and reimplantation of fresh minipumps. In addition, because there was no difference between controls and saline infusion in the first study, we did not include a saline infusion control in the 6 week study, which is a potential limitation.

Cani et al. found that chronic LPS treatment of mice for four weeks increased subcutaneous fat [6], while we found an increase in mesenteric fat in LPS-treated rats. This difference could be due to species differences (mice vs rats) or to the site of LPS release. Cani et al. [6] implanted minipumps subcutaneously which would release LPS directly onto subcutaneous fat, whereas we implanted the minipumps in the intraperitoneal space which would release LPS in close proximity to mesenteric fat, which may suggest that chronic low dose LPS promotes adipogenesis of local fat pads. The increase in mesenteric fat observed in LPS treated rats in this study correlates with our previous observation of increased mesenteric adipose tissue in rats prone to obesity [26]. Visceral fat is believed to be a source of inflammatory mediators [27]; thus, mesenteric fat has been suggested to propagate systemic inflammation in response to HF diets. Over the six weeks of the study using the dose of LPS that we used we observed no elevation in TNF-α protein levels in

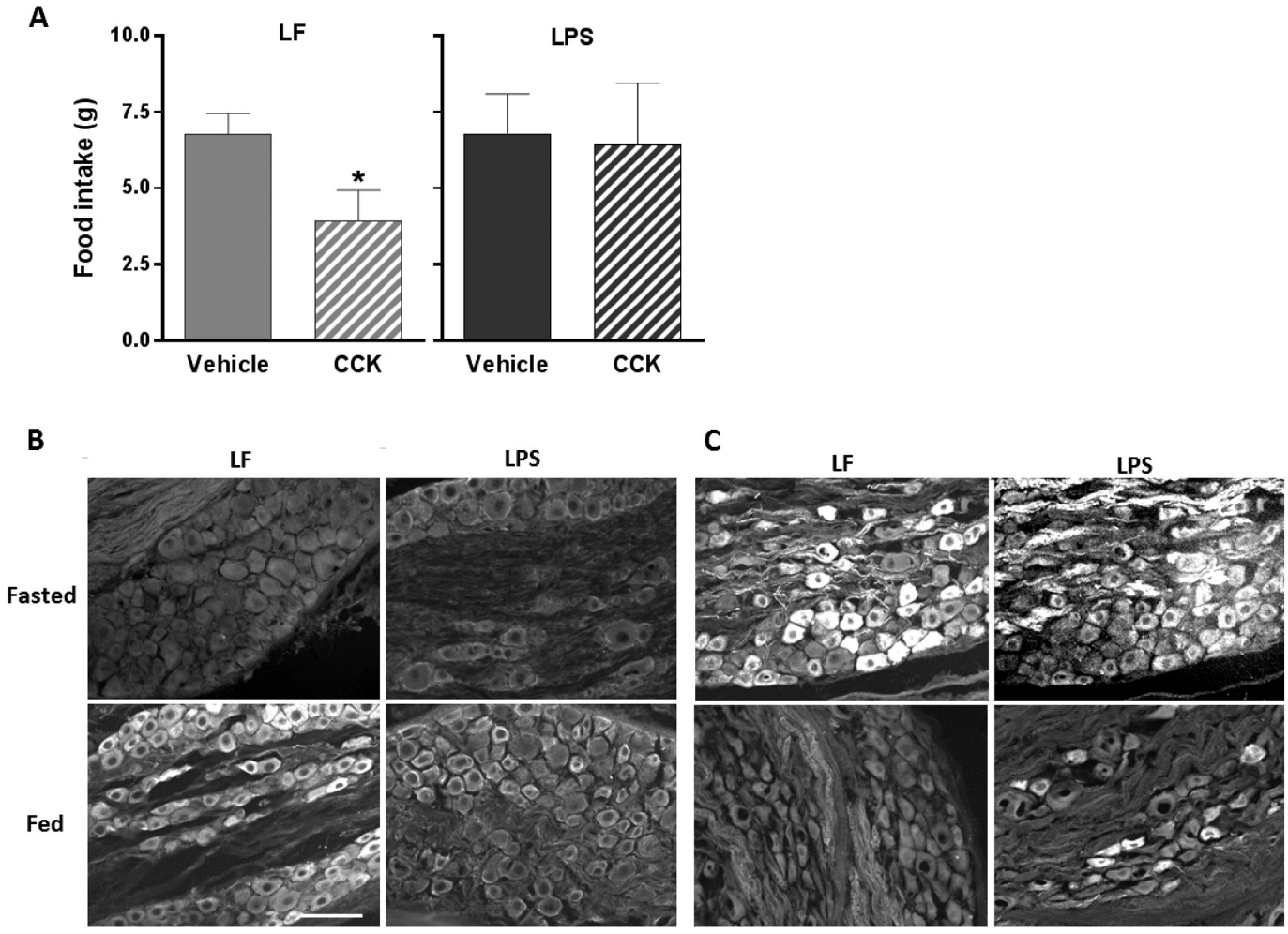

Fig. 4. Chronic low-dose LPS treatment markedly inhibits CCK-induced signaling and satiation. (A) Rats treated for 5 weeks were fasted 12 h and injected with CCK8S (0.22 nmol/kg; IP) or vehicle (400ul). CCK significantly inhibited food intake compared to vehicle in LF rats ($p < 0.05$), but not in LPS-treated rats. Representative immunohistochemistry of nodose ganglia of LF and LPS-treated rats after 6 weeks of treatment (upper panel, fasted; lower panel, fed ad libitum. Scale bars, 100um). (B) CART abundance is high in vagal afferent neurons of fed controls and reduced with fasting, but is constitutively low in LPS-treated rats. (C) MCH abundance is high in vagal afferent neurons of fasted controls and reduced with feeding, but is constitutively expressed in LPS-treated rats.

mesenteric fat, and although there was a trend towards an increase in circulating TNF-α in LPS-treated rats, the difference was not significant and did not provide evidence for systemic inflammation.

Acute administration of LPS (50-1000 μg/kg) induces hypophagia in rodents [28–31]. We observed LPS-induced hypophagia in the immediate post-surgical period in this study. Release of pro-inflammatory cytokines (interleukin-1, TNFα and IL-6) has been identified as mediating acute LPS-induced hypophagia [30,32,33]. There is also evidence that leptin is required for LPS-induced hypophagia; acute LPS treatment results in hyperleptinemia and enhanced leptin signaling in the arcuate nucleus [34] and the absence of functional leptin receptor in db/db mice and fa/fa rats reduces LPS-induced hypophagia [35,36]. We found no evidence of elevated serum leptin or TNF-α following chronic low-dose LPS treatment, indicating that the subsequent LPS-induced hyperphagia, which manifests after more than four weeks of treatment, likely occurs via different mechanisms.

We conclude that chronic low-dose LPS is sufficient to induce leptin resistance in vagal afferent neurons and this is associated with an increased in energy intake. High fat fed animals have also been observed to develop vagal leptin resistance [13,20] and metabolic endotoxemia [6,10,37]. A number of studies have reported that a HF diet alters CCK signaling in vagal afferent neurons characterized by an increase in orexigenic pathways and a failure to upregulate anorexigenic pathways by feeding [20,38,39]. Here we demonstrate that metabolic endotoxemia is sufficient to abolish CCK-induced signaling in vagal afferent neurons and CCK-induced satiation. Mimicking metabolic endotoxemia alters signaling in vagal afferent neurons in the same manner as that observed during HF diet treatment offering a potential pathway by which chronic ingestion of a HF diet disrupts transmission of anorexigenic signals from the gut. Moreover, the data provides a mechanism linking changes in gut microbiota induced by ingestion of HF diets to dysregulation of food intake and body weight.

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References

[1] Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. Proc Natl Acad Sci U S A 2005;102:11070–5.
[2] Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 2006;444:1027–31.
[3] Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. Nature 2006;444:1022–3.
[4] Jumpertz R, Le DS, Turnbaugh PJ, Trinidad C, Bogardus C, Gordon JI, et al. Energy-balance studies reveal associations between gut microbes, caloric load, and nutrient absorption in humans. Am J Clin Nutr 2011;94:58–65.

[5] Turnbaugh PJ, Backhed F, Fulton L, Gordon JI. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. Cell Host Microbe 2008;3:213–23.
[6] Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 2007;56:1761–72.
[7] Cani PD, Delzenne NM. Gut microflora as a target for energy and metabolic homeostasis. Curr Opin Clin Nutr Metab Care 2007;10:729–34.
[8] Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, et al. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut 2009;58:1044–55.
[9] Pendyala S, Walker JM, Holt PR. A high-fat diet is associated with endotoxemia that originates from the gut. Gastroenterology 2012;142:1100–1.
[10] de La Serre CB, Ellis CL, Lee J, Hartman AL, Rutledge JC, Raybould HE. Propensity to high-fat diet-induced obesity in rats is associated with changes in the gut microbiota and gut inflammation. Am J Physiol Gastrointest Liver Physiol 2010;299:G440–8.
[11] Tsukumo DML, Carvalho-Filho MA, Carvalheira JBC, Prada PO, Hirabara SM, Schenka AA, et al. Loss-of-function utation in Toll-like receptor 4 prevents diet-induced obesity and insulin resistance. Diabetes 2007;56:1986–98.
[12] Gakis G, Mueller MH, Hahn J, Glatzle J, Grundy D, Kreis ME. Neuronal activation in the nucleus of the solitary tract following jejunal lipopolysaccharide in the rat. Auton Neurosci 2009;148:63–8.
[13] de Lartigue G, Barbier de la Serre C, Espero E, Lee J, Raybould HE. Leptin Resistance in Vagal Afferent Neurons Inhibits Cholecystokinin Signaling and Satiation in Diet Induced Obese Rats. PLoS One 2012;7:595–607.
[14] de Lartigue G, Ronveaux CC, Raybould HE. Deletion of leptin signaling in vagal afferent neurons results in hyperphagia and obesity. Mol Metab 2014;3:595-60.
[15] Miller MJ, Sadowska-Krowicka H, Jeng AY, Chotinaruemol S, Wong M, Clark DA, et al. Substance P levels in experimental ileitis in guinea pigs: effects of misoprostol. Am J Physiol Gastrointest Liver Physiol 1993;265:G321–30.
[16] de Lartigue G, Dimaline R, Varro A, Dockray GJ. Cocaine- and amphetamine-regulated transcript: Stimulation of expression in rat vagal afferent neurons by cholecystokinin and suppression by ghrelin. J Neurosci 2007;27:2876–82.
[17] Burdyga G, Varro A, Dimaline R, Thompson DG, Dockray GJ. Feeding-dependent depression of melanin-concentrating hormone and melanin-concentrating hormone receptor-1 expression in vagal afferent neurones. Neuroscience 2006;137:1405–15.
[18] Dockray GJ. The versatility of the vagus. Physiol Behav 2009;97:531–6.
[19] Hosoi T, Okuma Y, Matsuda T, Nomura Y. Novel pathway for LPS-induced afferent vagus nerve activation: possible role of nodose ganglion. Auton Neurosci 2005; 120:104–7.
[20] de Lartigue G, Barbier de la Serre C, Espero E, Lee J, Raybould HE. Diet-induced obesity leads to the development of leptin resistance in vagal afferent neurons. Am J Physiol Endocrinol Metab 2011;301:E187–95.
[21] Cani PD, Delzenne NM. Interplay between obesity and associated metabolic disorders: new insights into the gut microbiota. Curr Opin Pharmacol 2009;9:737–43.
[22] Laugerette F, Vors C, Geloen A, Chauvin MA, Soulage C, Lambert-Porcheron S, et al. Emulsified lipids increase endotoxemia: possible role in early postprandial low-grade inflammation. J Nutr Biochem 2011;22:53–9.
[23] Burdyga G, de Lartigue G, Raybould HE, Morris R, Dimaline R, Varro A, et al. Cholecystokinin regulates expression of Y2 receptors in vagal afferent neurons serving the stomach. J Neurosci 2008;28:11583–92.
[24] Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science 2013;341: 1241214.
[25] Amar J, Burcelin R, Ruidavets JB, Cani PD, Fauvel J, Alessi MC, et al. Energy intake is associated with endotoxemia in apparently healthy men. Am J Clin Nutr 2008;87: 1219–23.
[26] Paulino G, Barbier de La Serre C, Knotts T, Oort PJ, Newman J, Adams S, et al. Increased expression of receptors for orexigenic factors in nodose ganglion of diet-induced obese rats. Am J Physiol Endocrinol Metab 2009;296: E898–903.
[27] Fontana L, Eagon JC, Trujillo ME, Scherer PE, Klein S. Visceral fat adipokine secretion is associated with systemic inflammation in obese humans. Diabetes 2007;56: 1010–3.
[28] Porter MH, Hrupka BJ, Langhans W, Schwartz GJ. Vagal and splanchnic afferents are not necessary for the anorexia produced by peripheral IL-1beta, LPS, and MDP. Am J Physiol 1998;275:R384–9.
[29] Schwartz GJ, Plata-Salaman CR, Langhans W. Subdiaphragmatic vagal deafferentation fails to block feeding-suppressive effects of LPS and IL-1 beta in rats. Am J Physiol 1997;273:R1193–8.
[30] Porter MH, Arnold M, Langhans W. TNF-alpha tolerance blocks LPS-induced hypophagia but LPS tolerance fails to prevent TNF-alpha-induced hypophagia. Am J Physiol 1998;274:R741–5.
[31] Hickey MJ. MPO and neutrophils: a magnetic attraction. Blood 2011;117:1103–4.
[32] Porter MH, Hrupka BJ, Altreuther G, Arnold M, Langhans W. Inhibition of TNF-alpha production contributes to the attenuation of LPS-induced hypophagia by pentoxifylline. Am J Physiol Regul Integr Comp Physiol 2000;279:R2113–20.
[33] Swiergiel AH, Dunn AJ. The roles of IL-1, IL-6, and TNFalpha in the feeding responses to endotoxin and influenza virus infection in mice. Brain Behav Immun 1999;13: 252–65.
[34] Borges BC, Rorato R, Avraham Y, da Silva LE, Castro M, Vorobiav L, et al. Leptin resistance and desensitization of hypophagia during prolonged inflammatory challenge. Am J Physiol Endocrinol Metab 2011;300:E858–69.
[35] Faggioni R, Fuller J, Moser A, Feingold KR, Grunfeld C. LPS-induced anorexia in leptin-deficient (ob/ob) and leptin receptor-deficient (db/db) mice. Am J Physiol 1997;273:R181–6.
[36] Lugarini F, Hrupka BJ, Schwartz GJ, Plata-Salaman CR, Langhans W. Acute and chronic administration of immunomodulators induces anorexia in Zucker rats. Physiol Behav 2005;84:165–73.
[37] Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, et al. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes 2008;57:1470–81.
[38] Nefti W, Chaumontet C, Fromentin G, Tome D, Darcel N. A high-fat diet attenuates the central response to within-meal satiation signals and modifies the receptor expression of vagal afferents in mice. Am J Physiol Regul Integr Comp Physiol 2009; 296:R1681–6.
[39] Savastano DM, Covasa M. Adaptation to a high-fat diet leads to hyperphagia and diminished sensitivity to cholecystokinin in rats. J Nutr 2005;135:1953–9.