The Gut Microbiota Reduces Leptin Sensitivity and the Expression of the Obesity-Suppressing Neuropeptides Proglucagon (Gcg) and Brain-Derived Neurotrophic Factor (Bdnf) in the Central Nervous System

Erik Schéle, Louise Grahnemo, Fredrik Anesten, Anna Hallén, Fredrik Bäckhed, and John-Olov Jansson

Institute of Neuroscience and Physiology/Endocrinology (E.S., L.G., F.A., J-O.J.), Sahlgrenska Center for Cardiovascular and Metabolic Research (E.S., A.H., F.B., J-O.J.), The Wallenberg Laboratory (A.H., F.B.), Department of Molecular and Clinical Medicine, The Sahlgrenska Academy at the University of Gothenburg, S-413 45 Gothenburg, Sweden; and Novo Nordisk Foundation Center for Basic Metabolic Research (F.B.), Section for Metabolic Receptology and Enteroendocrinology, Faculty of Health Sciences, University of Copenhagen, Copenhagen, DK-2200, Denmark

The gut microbiota contributes to fat mass and the susceptibility to obesity. However, the underlying mechanisms are not completely understood. To investigate whether the gut microbiota affects hypothalamic and brainstem body fat-regulating circuits, we compared gene expression of food intake-regulating neuropeptides between germ-free and conventionally raised (CONV-R) mice. We found that CONV-R mice had decreased expression of the antiobesity neuropeptide glucagon-like peptide-1 (GLP-1) precursor proglucagon (Gcg) in the brainstem. Moreover, in both the hypothalamus and the brainstem, CONV-R mice had decreased expression of the antiobesity neuropeptide brain-derived neurotrophic factor (Bdnf). CONV-R mice had reduced expression of the pro-obesity peptides neuropeptide-Y (Npy) and agouti-related protein (Agrp), and increased expression of the antiobesity peptides proopiomelanocortin (Pomc) and cocaine- and amphetamine-regulated transcript (Cart) in the hypothalamus. The latter changes in neuropeptide expression could be secondary to elevated fat mass in CONV-R mice. Leptin treatment caused less weight reduction and less suppression of orexigenic Npy and Agrp expression in CONV-R mice compared with germ-free mice. The hypothalamic expression of leptin resistance-associated suppressor of cytokine signaling 3 (Socs-3) was increased in CONV-R mice. In conclusion, the gut microbiota reduces the expression of 2 genes coding for body fat-suppressing neuropeptides, Gcg and Bdnf, an alteration that may contribute to fat mass induction by the gut microbiota. Moreover, the presence of body fat-inducing gut microbiota is associated with hypothalamic signs of Socs-3-mediated leptin resistance, which may be linked to failed compensatory body fat reduction. (Endocrinology 154: 3643–3651, 2013)

The gut microbiota has been suggested as an environmental factor that contributes to the development of obesity. Obese humans and mice display an altered gut microbiota with reduced diversity and increased capacity to absorb energy (1, 2). Germ free mice (GF) have reduced adiposity and are resistant to diet-induced obesity compared with conventionally raised (CONV-R) or conventional GF counterparts (3–5). Furthermore, treatment

Abbreviations: AgRP, agouti-related protein; ANCOVA, analysis of covariance; ARC, arcuate nucleus; BDNF, brain-derived neurotrophic factor; CART, cocaine- and amphetamine-regulated transcript; CNS, central nervous system; CONV-R, conventionally raised; ER, endoplasmic reticulum; fwd, forward; GF, germ free; GLP-1, glucagon-like peptide-1; NPY, neuropeptide-Y; POMC, proopiomelanocortin; PTP1B, protein-tyrosine phosphatase-1B; qRT-PCR, quantitative real-time PCR; rev, reverse; SOCS-3, suppressor of cytokine signaling-3; UTR, untranslated region; XBP1, X-box-binding protein 1.
of obese mice with antibiotics similarly reduces body weight and improves glucose metabolism (6, 7). The underlying mechanisms for how the gut microbiota modulates host metabolism and contributes to obesity are at present largely unknown but may involve numerous pathways including regulation of lipogenesis (3), fatty acid oxidation (4), inflammation (8), and the gut endocannabinoid system (9). Interestingly, the reduced adiposity in GF mice occurs despite increased food intake (3) and thus also suggests that the gut microbiota directly or indirectly may affect the central nervous system (CNS).

Leptin, which is produced by adipocytes in relation to adipose tissue mass, regulates food intake and energy expenditure by acting through hypothalamic neuronal circuits to suppress fat mass (10–12). In particular, leptin influences the arcuate nucleus (ARC) of the hypothalamus by binding to the leptin receptors on neurons that express neuropeptide-Y (NPY) and agouti-related protein (AgRP) (13), and neurons that express proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) predominantly located in the ARC (14). NPY and AgRP are considered obesity-promoting peptides because they stimulate food intake and decrease energy expenditure (15, 16), whereas the melanocortin-α-MSH, which is cleaved from the POMC precursor molecule, and CART exhibit anorexigenic effects and accordingly suppress food intake and favor weight loss (17, 18). Leptin activates POMC/CART neurons (18, 19) and inhibits NPY/AgRP neurons (20–23).

As discussed above, circulating leptin levels are increased in relation to body fat (10). However, chronic elevated leptin levels are associated with gradually increasing leptin resistance (24, 25). The reason for leptin resistance is unknown, although several mechanisms have been proposed, including dysfunction of the intracellular leptin receptor-signaling pathway. Suppressor of cytokine signaling-3 (SOCS-3) and protein-tyrosine phosphatase-IB (PTP1B) are believed to promote leptin resistance, because whole-body and brain-specific inactivation of the Soc3 gene or the Ptp1b gene result in enhanced leptin sensitivity (26–29). SOCS-3 may reduce leptin sensitivity by inhibition of the phosphorylation of signal transducer and activator of transcription-3, an intracellular leptin-signaling mediator (28). Endoplasmatic reticulum (ER) stress in the hypothalamus may also contribute to leptin resistance. ER stress is increased in the hypothalamus of obese mice and has been shown to impair leptin receptor signaling, causing leptin resistance that is reversed by improved ER protein-folding capacity (30, 31).

Central glucagon-like peptide-1 (GLP-1) is produced from, and positively correlated with, Gcg mRNA in the nucleus of the solitary tract of the brainstem (32). GLP-

1-producing neurons in the brainstem project to several parts of the brain including the hypothalamus. The expression of GLP-1 is reduced by food restriction and induced by leptin (32), thus suggesting that GLP-1 has a role in central regulation of feeding and body fat (33).

The brain-derived neurotrophic factor (BDNF), mostly known as a neuronal survival, neuronal growth, and neurogenesis control factor, is now also considered to be involved in energy balance regulation through hypothalamic and brainstem circuits (34, 35). Within the hypothalamus, BDNF suppresses appetite and obesity through the ventromedial hypothalamus, where it is also most abundant (34). In the brainstem, BDNF is present in high density in the dorsal vagal complex, another integrator of energy balance regulation (36). The expression of BDNF in the ventromedial hypothalamus and dorsal vagal complex is induced by circulating leptin (37, 38) and by activation of the melanocortin-4 receptor MC4R (39–41). The MC4R binds α-MSH and has a critical role in energy balance as an obesity-suppressing receptor and is indirectly activated by leptin (42, 43). BDNF is most likely an important downstream target of the MC4R-mediated signaling (39–41). There are also indications that BDNF regulates fat mass in humans, because mutations affecting the TrkB gene, coding for the BDNF receptor, or the BDNF gene are associated with obesity (44).

In this study, we investigate the expression of body fat-regulating genes in the CNS in GF and CONV-R mice, to elucidate possible mechanism for regulation of body fat mass by the gut microbiota.

**Materials and Methods**

**Animals**

GF 12- to 14-week-old C57Bl/6J male mice were maintained in flexible film isolators under a 12-hour light cycle and fed an autoclaved chow diet (Labdiet) ad libitum. The sterile environments in the isolators, on a regular basis, were controlled for by autoclaving and analyzing the presence of 16S rRNA using PCR. Mice were killed by cervical dislocation; the hypothalamus and brainstem were immediately dissected and snap frozen. Animal protocols were approved by the Research Animal Ethics Committee in Gothenburg at the University of Gothenburg.

**Leptin treatment and leptin measurement**

Recombinant murine leptin (PeproTech) was dissolved in 0.9% NaCl solution and sterile filtered through a 0.22-μm filter. Both GF and CONV-R mice were injected ip with 60 μg of leptin or vehicle twice daily for 3 days. Body weight was measured before and after the treatment. The mice were euthanized the day after the final injection.

Serum leptin was measured with a mouse leptin ELISA kit (catalog no. 90030; Crystal Chem).
RNA extraction and cDNA synthesis

The frozen hypothalami and brainstems were homogenized in QIAzol lysis reagent using a TissueLyser (Qiagen), and chloroform was subsequently added to the homogenates prior to centrifugation. RNA was isolated from the received aqueous phase using RNeasy micro kit (Qiagen). DNA was removed by DNase treatment (Qiagen). The RNA was reversed transcribed by using iScript cDNA synthesis kit (Bio-Rad Laboratories).

Quantitative real-time PCR (qRT-PCR) analysis

A 100-µl PCR was prepared by mixing 100 ng of cDNA with TaqMan Universal PCR Master Mix (Applied Biosystems) for each sample. Eight samples were loaded onto a 48-gene customized TaqMan low-density array card (Applied Biosystems) for qRT-PCR. Specific primers and probes for each of the 48 genes had been spotted onto the card by the manufacturer (see Supplemental Table 1 published on The Endocrine Society’s Journals Online web site at http://endo.endojournals.org). Single assay was used for Trkb (Mm 00435422_m1), Socs3 (Mm0054913_s1), and Ptp1b (Mm00448427_m1). For Gcg (forward [fwd], TGGCAGCAGGCCCTTC; reverse [rev], GGCGTTCTGTCTGGAGA) and long 3'-untranslated region (UTR) BDNF (fwd, CAGGAGGAATTCTGAGTGGCCA; rev, GCAGAAGGCCATAAGCAACTTGGACA), primers in combination with SYBR green fluorescence detection were performed with an ABI Prism 7900HT sequence detection system and analyzed with ABI Prism 7900HT SDS software (Applied Biosystems). The cycle threshold (Ct) was set automatically by the SDS software. The data were normalized to the reference gene Gusb, which was evaluated as the most appropriate of 8 reference genes (18S, Actb, Gapdh, Hprt1, Ppiα, Rplp0, and Rplp2). Relative mRNA expression levels were calculated by using the 2-ΔCt equation, where the ΔCt value was obtained by subtracting Ct value of the reference gene from the Ct value of the studied gene.

PCR analysis of Xbox-binding protein 1 (XBP1) mRNA splicing

XBP1 is a transcription factor that activates genes needed for ER-stress response. To measure ER stress in the hypothalamus, the presence of the 575-bp spliced isoform of the Xbp1 gene, designated Xbp1s, was analyzed using PCR. For each sample a PCR was prepared by mixing 200 ng cDNA from hypothalami of GF or CONV-R mice, HotStarTaq Master Mix (Qiagen), and 1 ng of each primer (fwd, AAACAGAGTAGCAGCGCAAGC; rev, GGATCTCTAGAGGCTTGGTG). The samples were amplified with PCR using the following thermal cycling protocol: 1) 1 cycle (95°C, 5 minutes); 2) 5 cycles (95°C, 30 seconds; 68°C, 30 seconds; 72°C, 45 seconds); 3) 5 cycles (95°C, 30 seconds; 64°C, 30 seconds; 72°C, 45 seconds); 4) 30 cycles (95°C, 30 seconds; 58°C, 30 seconds; 72°C, 45 seconds); 5) 1 cycle (72°C, 10 minutes); 6) cooling to 4°C. The PCR products were mounted on a 12-well E-Gel 2% agarose (GP) gel (Invitrogen). The gel was run for 40 minutes on an E-Gel iBase (Invitrogen) using the developer’s program number 1. After the run, the gel was photographed under UV light. As positive control, cDNA from thapsigargin (0.1 µM)-treated mouse embryonic fibroblasts was used, and as negative control cDNA from nontreated mouse embryonic fibroblasts was used.

Statistical analysis

The data were analyzed using either Student’s t test or analysis of covariance (ANCOVA). ANCOVA was used whenever data were presented as a result of more than one experiment but where the outcomes (ΔCt values) could not be pooled due to interexperiment variations (Figure 1 and see Figure 3, A–D, and Figure 5). P < .05 was considered statistically significant.

Results

The gut microbiota suppresses Gcg expression in the brainstem

To investigate whether the gut microbiota regulated expression of appetite-regulating genes in the hypothalamus and brainstem, we measured mRNA levels of a panel of neuropeptides known to be involved in energy balance using multiple qRT-PCR assays (see Supplemental Table 1). The brainstem mRNA levels of Gcg that code for the obesity-suppressing neuropeptide GLP-1 was decreased by 54% in the CONV-R mice compared with GF mice (Figure 1). The expression of the receptor Glp1r tended to be decreased (P = .08) in CONV-R compared with GF mice (Figure 1). Decreased levels of both Gcg and Glp1r mRNA levels in the brainstem were also observed in young CONV-R mice compared with GF counterparts with equal fat mass (see Supplemental Table 2, A and B).

The gut microbiota suppresses Bdnf expression in the hypothalamus and the brainstem

The hypothalamic mRNA levels of the obesity-suppressing neuropeptide Bdnf were decreased by 23% in the CONV-R mice compared with GF counterparts (Figure 2A). In addition, the mRNA levels of Bdnf were also de-

![Figure 1](https://academic.oup.com/endo/article-abstract/154/10/3643/2423894)
creased by 39% in the brainstem of CONV-R mice (Figure 2B). The levels of mRNA coding for the BDNF receptor (TrkB) were decreased in the brainstem (B), but not in the hypothalamus (A). Levels of mRNA were measured with qRT-PCR. Data are combined from 2 experiments and presented as group means ± SEM. The levels in CONV-R mice are relative to the mean level of GF mice, which is set to 100%. ANCOVA with experiment as covariate was used for statistical comparison. **, P < .01; ***, P < .001.

Figure 2. CONV-R mice have reduced Bdnf mRNA levels compared with GF mice in the hypothalamus (A; n = 13 in both groups) and the brainstem (B; n = 12 and 9, respectively). The levels of mRNA coding for the BDNF receptor (TrkB) were decreased in the brainstem (B), but not in the hypothalamus (A). Levels of mRNA were measured with qRT-PCR. Data are combined from 2 experiments and presented as group means ± SEM. The levels in CONV-R mice are relative to the mean level of GF mice, which is set to 100%. ANCOVA with experiment as covariate was used for statistical comparison. **, P < .01; ***, P < .001.

Increased hypothalamic expression of the obesity-suppressing neuropeptides Pomc and Cart were increased by 46% and 19%, respectively, in CONV-R mice compared with GF animals (Figure 3, C and D, left bars). Importantly, these regulations appeared to be specific because the expression levels of other fat-regulating hypothalamic neuropeptides were unchanged (see Supplemental Table 1). No differences in levels of Npy, Agrp, Pomc, or Cart mRNA were observed in the brainstem (Figure 3, A–D, right bars). As expected, the levels of circulating leptin were higher in CONV-R mice than in GF animals (Figure 3E). This is in line with the increased epidymal fat mass observed in CONV-R male mice, aged 12 weeks, compared with GF counterparts (1.6 ± 0.3% of total body weight in CONV-R mice compared with 0.8 ± 0.1% in GF mice; n = 5 and 4, respectively; P = .038), and in agreement with previous findings (3–5, 47).

No difference in Npy, Agrp, Pomc, or Cart mRNA levels were observed in CONV-R mice, aged 6 weeks, compared with GF counterparts with comparable fat mass (see Supplemental Table 2).

Decreased leptin responsiveness in CONV-R mice

Because colonized mice have increased serum levels of leptin and altered expression of leptin-regulated ARC peptides (Figure 3, A–D, left bars) we next investigated whether the response to exogenous leptin was altered in CONV-R mice compared with GF mice. Leptin treatment caused a nonsignificant tendency for weight loss in CONV-R mice (P = .08), whereas leptin induced a marked and significant body weight reduction in GF mice (Figure 4A). This indicates that leptin responsiveness was reduced in CONV-R mice.

Leptin treatment of GF mice decreased the Npy and Agrp mRNA to the same levels as in CONV-R mice (Figure 4, B and C). In contrast, leptin treatment had no effect on Npy or Agrp mRNA expressions in CONV-R mice (Figure 4, B and C). This supports the notion above that leptin responsiveness is decreased in CONV-R. The levels of Pomc, Cart, and Bdnf mRNA were not modulated by leptin in neither CONV-R nor GF mice (data not shown).

Increased hypothalamic expression of the leptin-signaling suppressor Socs3

We next investigated whether the reduced leptin responsiveness was associated with markers of leptin resistance in the hypothalamus. SOCS-3, PTP1B, and ER stress represent 3 pathways that reduce leptin sensitivity by suppressing leptin signaling in the hypothalamus. The hypothalamic levels of Socs3 mRNA were increased by 33% in CONV-R mice compared with GF mice, whereas the levels of Ptp1b mRNA were unchanged (Figure 5 A). The spliced

The gut microbiota affects expression of ARC neuropeptides

Expression of the obesity-promoting neuropeptides Npy and Agrp was reduced by 34% and 35%, respectively, in CONV-R mice compared with GF counterparts (Figure 3, A and B, left bars). In contrast, the mRNA levels of the obesity-suppressing neuropeptides Pomc and Cart were increased by 46% and 19%, respectively, in CONV-R mice compared with GF animals (Figure 3, C and D, left bars). Importantly, these regulations appeared to be specific because the expression levels of other fat-regulating hypothalamic neuropeptides were unchanged (see Supplemental Table 1). No differences in levels of Npy, Agrp, Pomc, or Cart mRNA were observed in the brainstem (Figure 3, A–D, right bars). As expected, the levels of circulating leptin were higher in CONV-R mice than in GF animals (Figure 3E). This is in line with the increased epidymal fat mass observed in CONV-R male mice, aged 12 weeks, compared with GF counterparts (1.6 ± 0.3% of total body weight in CONV-R mice compared with 0.8 ± 0.1% in GF mice; n = 5 and 4, respectively; P = .038), and in agreement with previous findings (3–5, 47).

No difference in Npy, Agrp, Pomc, or Cart mRNA levels were observed in CONV-R mice, aged 6 weeks, compared with GF counterparts with comparable fat mass (see Supplemental Table 2).

Decreased leptin responsiveness in CONV-R mice

Because colonized mice have increased serum levels of leptin and altered expression of leptin-regulated ARC peptides (Figure 3, A–D, left bars) we next investigated whether the response to exogenous leptin was altered in CONV-R mice compared with GF mice. Leptin treatment caused a nonsignificant tendency for weight loss in CONV-R mice (P = .08), whereas leptin induced a marked and significant body weight reduction in GF mice (Figure 4A). This indicates that leptin responsiveness was reduced in CONV-R mice.

Leptin treatment of GF mice decreased the Npy and Agrp mRNA to the same levels as in CONV-R mice (Figure 4, B and C). In contrast, leptin treatment had no effect on Npy or Agrp mRNA expressions in CONV-R mice (Figure 4, B and C). This supports the notion above that leptin responsiveness is decreased in CONV-R. The levels of Pomc, Cart, and Bdnf mRNA were not modulated by leptin in neither CONV-R nor GF mice (data not shown).

Increased hypothalamic expression of the leptin-signaling suppressor Socs3

We next investigated whether the reduced leptin responsiveness was associated with markers of leptin resistance in the hypothalamus. SOCS-3, PTP1B, and ER stress represent 3 pathways that reduce leptin sensitivity by suppressing leptin signaling in the hypothalamus. The hypothalamic levels of Socs3 mRNA were increased by 33% in CONV-R mice compared with GF mice, whereas the levels of Ptp1b mRNA were unchanged (Figure 5 A). The spliced
The short isoform of Xbp1 gene mRNA (Xbp1s; 575 bp), used as a marker for ER stress, was not found in the hypothalamus of any of the examined CONV-R or GF mice, indicating that ER stress was not present. As expected, the spliced isoform of mRNA (Xbp1s) was present in positive control mRNA from stressed cells (Figure 5B). Thus, the reduced leptin sensitivity in CONV-R mice was associated with increased Socs3 expression in the hypothalamus, a well-known mediator of decreased leptin sensitivity.

**Discussion**

In this study we demonstrate that the gut microbiota, previously shown to contribute to fat mass, reduces the expression of Gcg, which codes for the fat-suppressing peptide GLP-1 in the brainstem. The gut microbiota also decreased the expression of the body fat-suppressing neuropeptide Bdnf in the hypothalamus and in the brainstem, 2 parts of the brain that are important for regulation of energy balance. Moreover, we found that the gut microbiota affected the levels of energy balance regulating peptides in the ARC of the hypothalamus in a way that would reduce body fat. This regulation is most likely a compensatory effect that could be a consequence of elevated fat mass by the gut microbiota.

Moreover, we found evidence that gut microbiota accompanied by chronically increased leptin levels can induce leptin resistance via induction of Socs3 expression in the hypothalamus. The latter effect may explain the decreased response to the body weight-reducing effect of exogenous leptin seen in mice with gut microbiota in the present study. Finally, the increased Socs3 expression in the hypothalamus may explain the increased body weight and body fat despite increased serum leptin that we observed, in agreement with previous studies (3, 4).

In the present study we found the brainstem expression of the GLP-1 precursor Gcg, which has been demonstrated to correlate with GLP-1 protein levels (32), to be reduced by gut microbiota. Because central GLP-1 has been shown to reduce body weight in experimental animals (32, 33, 48), the decreased Gcg expression induced by the gut microbiota may contribute to the increased fat mass in the host. Furthermore, GLP-1-producing neurons are almost exclusively located in the nucleus of the solitary tract, and these neurons seem to receive input from the gut via vagal afferents (49), providing a possible route for information about gut microbiota to the GLP-1-producing neurons. It has been suggested that gut microbiota also modulate GLP-1 in the periphery, because GLP-1 secretion from enteroendocrine L-cells is stimulated by short-chain fatty acids, a product from microbiota-fermented polysaccharides (50).

The microbiota suppressed hypothalamic and brainstem Bdnf expression. BDNF has been reported to suppress obesity in experimental animals (35), and mutations of BDNF and the BDNF receptor TRKB are associated with
obesity in humans (44). Therefore, the low expression of Bdnf seen in the hypothalamus and the brainstem in the presence of gut microbiota may partly explain the increased host adiposity. BDNF is believed to be an important antiobesity factor (51) mediating the downstream signaling of leptin and POMC/MC4R in the hypothalamus and in the brainstem (37–41). Therefore, it is remarkable that Bdnf expression was reduced in mice with gut microbiota despite high levels of leptin and increased Pomc expression in this study. Bdnf expression was not affected by leptin treatment in the present study, neither in the presence or absence of microbiota (data not shown). These results suggest the gut microbiota exert an additional suppressing effect on hypothalamic and brainstem BDNF, and that this effect is strong enough to counteract the stimulation of hypothalamic BDNF that one would expect to be exerted by the leptin/POMC/MC4R pathway.

Previous studies have indicated that gut microbiota influences expression of Bdnf in the areas without obvious relation to energy balance regulation (52, 53). The effects by the gut microbiota on BDNF in various brain regions warrant further investigation.

The expression of the obesity-promoting neuropeptides Npy and Agrp were reduced, whereas the expression of feeding- and obesity-suppressing neuropeptides Pmc and Cart were elevated in the hypothalamus of CONV-R mice studied at 12–14 weeks of age. These hypothalamic changes could be a consequence of the elevated fat mass in CONV-R mice at this age. Because
CONV-R mice have higher body weight than GF mice, the decreased expression of Npy and Agrp, as well as the increased Pomp and Cart, may be regarded as a failed compensatory homeostatic mechanism to reduce body weight. In agreement with this assumption, we found here that the hypothalamic expression of Npy, Agrp, Pomp, and Cart in younger mice with equal fat mass did not differ between CONV-R and GF mice.

Administration of leptin in CONV-R mice did not further reduce the levels of Npy and Agrp. In contrast, leptin administration reduced Npy and Agrp expression in GF mice, suggesting that the gut microbiota may decrease leptin sensitivity. In agreement, exogenous leptin induced a marked body weight reduction in the absence of gut microbiota, whereas there was only a tendency for reduced body weight by leptin in the presence of gut microbiota. Decreased leptin sensitivity in CONV-R mice may explain their higher body weight despite elevated leptin levels and may account for the failed compensatory mechanism discussed above. It is well known that enhanced fat mass is associated with reduced leptin sensitivity (24, 25).

There are at least 3 mechanisms, SOCS-3, PTP1B, and ER stress, that have been proposed to be important hypothalamic factors in the development of leptin resistance (28, 54). We found the leptin-signaling inhibitor Socs3 to be up-regulated in the hypothalamus in the presence of gut microbiota, which may explain the decreased leptin sensitivity as a consequence of gut microbiota (28). SOCS-3 has been shown to inhibit intracellular leptin signaling in the hypothalamus by decreasing the phosphorylation of signal transducer and activator of transcription-3 (28, 29). Because Ptp1b and ER stress, which are also proposed to suppress intracellular leptin signaling (26, 27, 30, 31), were not regulated by gut microbiota, we speculate that SOCS-3 specifically could mediate leptin resistance induced by gut microbiota.

It is well established, including in publications from our laboratory, that the food intake is lower in CONV-R mice than in GF mice (3, 47). However, the presence of the gut microbiota increases host adiposity despite reduced food intake (3), possibly due to increased capacity to absorb energy (1, 2). In part, our findings support this, because we found the hypothalamic expression of Npy, Agrp, Pomp, and Cart to be modulated by the gut microbiota in a direction known to be anorectic (18–22). However, the gut microbiota seems to increase adiposity in mice despite this anorectic neuropeptide profile of the hypothalamic arcuate nucleus, possibly due to modulation of GLP-1 and BDNF systems, as discussed above. The possibility that reduced expression of Gcg and Bdnf contributed to increased fat mass in CONV-R mice is further supported by the fact that reduced Gcg and Bdnf expression was seen already in younger mice before differences in adiposity were observed. In contrast, expression of Npy, Agrp, Pomp, and Cart was unchanged in young mice as would be expected by factors regulated by fat mass rather than by the gut microbiota per se.

Gut microbiota has been reported to elevate serum insulin, and to either have no effect or to reduce serum glucagon (3, 4, 47, 55–57). It remains to be investigated whether the changes in CNS neuropeptide expression seen in the present study, in addition to regulating body fat, are of importance for glucose metabolism, including pancreatic hormone secretion (58, 59).

In conclusion, our results indicate that the gut microbiota, known to affect body fat mass, influence body fat-regulating genes in the hypothalamus and the brainstem. The decreased expression of the body fat-suppressing neuropeptide Bdnf in the hypothalamus and brainstem, 2 areas involved in body fat regulation, in CONV-R mice may be linked to their increase in body fat. Similarly, this may be the case for the decreased expression of the body fat-suppressing GLP-1 precursor Gcg in the brainstem. On the other hand, microbial effect on expression of several neuropeptides of the ARC of CONV-R mice is in line with a failed compensatory effect by increased body fat. Indeed, our results indicate that gut microbiota is associated with decreased leptin sensitivity in the ARC of the hypothalamus, an effect that could be due to enhanced expression of Socs3 in CONV-R mice. Thus, the body fat stimulation by the gut microbiota could be mediated by changes in the expression of body fat-regulating neuropeptides such as GLP-1 and BDNF. Moreover, a relative leptin resistance may accentuate the obesity in CONV-R mice.

**Acknowledgments**

We thank Genomics core facility for providing equipment and valuable support; Frida Larsson for excellent technical support; and Dr Martin Bergö for expert help with measurement of ER stress.

Address all correspondence and requests for reprints to: John-Olov Jansson, Sahlgrenska Academy at the University of Gothenburg, Institute of Neuroscience and Physiology/Endocrinology Medicinaregatan 11, Goteborg-41390, Sweden. E-mail: john-olov.jansson@medic.gu.se.

This work was supported by grants from Swedish Research Council (K2013-54X-09894–19-3 and 325–2008-7534), Johan och Jakob Söderbergs Foundation, Marcus Borgströms Foundation, Nilsson-Ehle Foundation, NovoNordisk Foundation, Inga-Britt och Arne Ljunbergs Foundation, Swedish Medical Society, Swedish Society for Medical Research, and Sahlgrenska Center for Cardiovascular and Metabolic Research.
References

5. Rabot S, Membrez M, Bruneau A, et al. Germ-free C57BL/6j mice are resistant to high-fat-diet-induced insulin resistance and have altered cholesterol metabolism. *FASEB J*. 2010;24:4948–4959
39. Nicholson JR, Peter JC, Lecourt AC, Barde YA, Hofbauer KG. Melanocortin-4 receptor activation stimulates hypothalamic brain-derived neurotrophic factor release to regulate food intake, body


