A high-fat diet attenuates the central response to within-meal satiation signals and modifies the receptor expression of vagal afferents in mice

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Nefti W, Chaumontet C, Fromentin G, Tomé 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* 296: R1681–R1686, 2009. First published March 28, 2009; doi:10.1152/ajpregu.90733.2008.—During digestion, macronutrients are sensed within the small intestine. This sensory process is dependent upon the action of gut mediators, such as cholecystokinin (CCK) or serotonin (5-HT), on vagal afferents that, in turn, convey peripheral information to the brain to influence the control of food intake. Recent studies have suggested that dietary intervention effect on food intake could be linked to changes in vagal afferent neuron receptor expression. The results indicated that compared with an NF diet, and while increasing food intake and body weight gain, an HF diet altered the short-term response to CCK-8 and intragastric macronutrient loads, while decreasing vagal activation by CCK-8 and modifying the receptor expression of vagal neurons. These findings, therefore, suggest that dietary intervention effect on food intake could be linked to changes in vagal afferent receptor profiles.

**VAGAL AFFERENTS THAT EMERGE from the intestine and terminate in the nucleus of the solitary tract (NTS) play an important role in conveying satiation signals to the brain (1, 26, 30). Indeed, during digestion, a broad range of satiation signals generated within the gut wall, mostly consisting in the release of gut neuropeptides, such as cholecystokinin (CCK) or serotonin (5-HT), activate vagal afferents, which, in turn, activate the NTS (19, 20, 29). This phenomenon is related to increased satiation (early meal termination) (15, 27). In addition, the NTS is a major relay through which visceral information from the gastrointestinal tract travels to the brain to participate in complex eating behaviors and energy balance homeostasis (8). NTS neurons project extensively to other key regions involved in controlling food intake, including the hypothalamus, the amygdala, and the nucleus accumbens (13, 14, 25, 28).

Several pieces of evidence have also suggested that the efficiency of the NTS and its related peripheral inputs, principally the vagus nerve, are subject to neuroplasticity alterations. For instance, in rats, the vagal response to CCK has been shown to be downregulated after long-term exposure to a high-fat (HF) diet, this effect being consistent with the results of food intake experiments that showed that diminished satiation was induced by an HF diet (17, 24). Several mechanisms have recently been proposed for vagal pathway plasticity, including changes to dynamics of vagal afferent neuron receptors (7), peripheral synergistic interplays between gut mediators, such as CCK, 5-HT, leptin, orexins, or endocannabinoids (6) and adult-neurogenesis within the NTS (3, 16). These phenomena may be of importance because they could cause early body weight drifts and obesity, but the precise molecular and cellular bases for these adaptation processes remain unclear.

The present study was designed to investigate the involvement of vagal afferent neuroplasticity in behavioral changes consecutive to nutritional interventions, as well as the molecular substrates for this adaptive mechanism. For the purpose, the impact of exposing mice for 15 days to either a normal fat (NF) or HF diet was evaluated with respect to: 1) short-term food intake and sensitivity to CCK-8 and 2-methylserotonin maleate salt (2-methyl-5-HT); 2) gut mediator receptor profiles in vagal afferents characterized by real-time PCR for CCK1-R (CCK), 5-HT3-R (5-HT), CB1-R (endocannabinoids), Ob-LR (leptin), and OX1-R and OX2-R (orexin); and 3) vagal sensitivity to macronutrient intragastric loads and CCK-8 using c-fos expression as a marker of neuronal activation in the NTS (23).

**MATERIALS AND METHODS**

**Products and chemicals.** Neuromediators: 2-methyl-5-HT (a specific 5-HT3 receptor agonist) and CCK-8 (CCK fragment 26–33 amide, sulfated) were purchased from Sigma (St. Louis, MO). Real-time PCR products: Trizol reagent was purchased from Invitrogen, high-capacity complementary DNA (cDNA) reverse transcription kit and 2× SYBR Green master mix were obtained from Applied Biosystems. Antibodies: primary rabbit anti c-fos antibody (Ab-5) was purchased from Calbiochem (San Diego, CA), biotinylated anti-rabbit secondary antibody, and avidine FITC were obtained from Vector Laboratories (Burlingame, CA).

**Animals, diets, and feeding procedures.** Male (for gut mediator receptor profiles in vagal afferents characterized by real-time PCR only) and female (for all experiments) BalBc mice (4-wk-old and weighing 16.8 g on arrival; Harlan) were housed and kept under a 12:12-h light-dark cycle (lights on at 09:00), at a room temperature of 21 ± 1°C. The animals were given free access to food (standard pelleted chow) and water for 1 wk to recover from transport. All the experimental procedures described below complied with the guidelines of the French National Animal Care Committee and were approved by the Regional (Ile de France Sud) Animal Care and Ethical Committee. During the experiments, mice were subjected for 15 days to either a high-fat (HF) diet or a normal fat (NF) diet. Both the NF and HF diet formulas were based on American Institute of Nutrition recommendations [AIN-93 modified diet (21)]. In the HF diet, 30% of total energy was made up of lipids, 14% of protein, and 46% of carbohydrates. In the NF diet, 16% of total energy was made...
up of lipids, 14% of protein, and 70% of carbohydrates (see diet compositions in Table 1). Throughout the course of the experiment, all mice had free access to food and water. Unless specifically required by the experiment, the diets were supplied in a powdered form (the food cups being refilled every 2 days). The body weight of mice was 18.1 g at the beginning of the diet submission period.

Short-term food intake and CCK-8 and 2-methyl-5-HT efficiency in suppressing food intake. Two groups of mice (n = 6) were subjected for 15 days to the NF or HF diets. The mice were housed in individual cages and were habituated to consuming moistened diets (powder: water = 1:1) to minimize spillage and to allow precise food intake measurements. The animals were fasted overnight prior to experimental days. Their body weight and energy intake were measured 1 h prior to the onset of the dark cycle each day (starting at 09:00). On experimental days, the food intake of their habitual diet was measured after an acute intraperitoneal injection of CCK-8 (100 pmol/injection, injected volume = 100 µl), 2-methyl-5-HT (0.5 nmol/injection, injected volume = 100 µl) or saline (PBS), the volume thus being identical for all tests (100 µl) and injected 1 min prior to food cup presentation (i.e., 08:59). Food intake was then determined from 09:00 to 10:00 by measuring the difference in food cup weight before and after presentation to each mouse. The experimental plan was designed to ensure that each mouse had been tested for CCK-8, 2-methyl-5-HT, and saline responses by the end of the experiment. A minimum of 48 h was allowed between each trial in the same mouse.

For NTS response to an intragastric load of the NF or HF diet or to the intraperitoneal injection of CCK-8. To assess the effects of dietary conditions on the neuronal NTS response to an intraperitoneal injection of CCK-8, two additional groups of mice (n = 6 per group) were subjected for 15 days to the NF or HF diet. These mice received an intraperitoneal injection of PBS or CCK-8 (100 pmol/injection, injected volume = 100 µl, n = 6 mice) dissolved in PBS; 90 min after gavage or the intraperitoneal CCK-8 injection, the mice were euthanized using a lethal injection of pentobarbital (5.5 ml/kg ip); the brains were removed rapidly and fixed in a 4% paraformaldehyde PBS solution (0.1 M, pH = 7.4) for 6 h (at room temperature) and then 12 h in a 25% sucrose solution (+4°C). Then 40 µm-thick transverse brain stem sections were processed using a cryostat (Leica). Sixteen sections from each mouse, corresponding to the intermediate NTS (bregma: −7.76; −7.20), were collected in PBS (0.1M, pH = 7.4). For immunocytochemistry: NTS sections were rinsed for 10 min in PBS and incubated for 1 h in 2% BSA 0.5% Triton X-100 PBS. The sections were then incubated overnight at room temperature with rabbit anti-Fos antibody (1:10,000) and washed for 3 × 10 min in 0.01 M PBS containing 0.1% powdered skimmed milk. The sections were subsequently incubated in biotinylated anti-rabbit secondary antibody (1:200) for 3 h at room temperature, then washed for 2 × 10 min with PBS and 10 min with bicarbonate saline solution (BSS) (8.4g NaHPO4, 0.9% NaCl, pH = 8.2), and incubated in avidine-FITC diluted in BSS (1:500) for 30 min at room temperature. Finally, the sections were rinsed with BSS buffer for 10 min and PBS for 2 × 10 min, and then mounted using Vectashield hard-set mounting medium (Vector Laboratories, Biovalley, Marne la Vallée, France).

Quantification of the mRNA expression of gut mediators receptor transcripts in mouse nodose neurons using real-time PCR amplification. Two groups of female mice (n = 12) and two groups of male mice (n = 12) received the NF or HF diet for 15 days. One day prior to nodose removal, the mice were fasted overnight (with free access to water). On the day of the procedure, the animals were decapitated, and nodose ganglia were rapidly collected, stored into Trizol reagent, and snap-frozen in liquid nitrogen. To ensure a sufficient sample size in each group, nodose ganglia from four mice were pooled (one group thus comprised 3 pools). Total RNA were extracted from each pool with 200 µl Trizol reagent and homogenized using a Tissuelyser (Qiagen, Courtaboeuf, France). Total RNA concentrations were determined by absorbance measurements at 260 nm with a spectrophotometer (Nanodrop). The synthesis of complementary DNA strands was performed on 400 ng of total RNA using a high-capacity cDNA reverse transcription kit. Primers were designed using Oligo Explorer 1.1.0 software, and the 18S protein was used as a housekeeping gene to normalize the mRNA abundance of each gene. BLASTN searches were performed against GenBank to check the total gene specificity of the nucleotide sequences chosen for the primers (listed in Table 2). The synthesis of strand cDNA was performed using a high-capacity cDNA reverse transcription kit. Quantitative values for cDNA amplification were obtained from the threshold cycle number (Ct) at which a signal increase was associated with exponential growth of the PCR products that were starting to be detected. For real-time PCR amplification, PCR reactions were performed using a 7300 real-time PCR system (Applied Biosystems). Each cDNA was amplified in a 20-µl volume containing 15 µl of 2× SYBR Green master mix and 500 nM
concentrations of gene-specific primer. Thermal cycling conditions comprised an initial denaturation step at 95°C for 10 min and 40 amplification cycles at 95°C for 15 s and 60°C for 1 min. Quantitative values for cDNA amplification were obtained from the Ct. The Ct for each sample was determined at a constant fluorescence threshold line. For each run, a melt curve was subsequently performed to analyze the products generated for any contamination resulting from residual genomic DNA amplification (using a control without reverse transcriptase) and/or from the formation of primer dimers (controls containing no DNA template and no reverse transcriptase). The overall efficiency levels (E) of PCR were calculated from the slopes of the standard curves according to the equation: \( E = [10^{-1/slope}] - 1 \). Ribosomal 18 S RNA was used to normalize variability due to inequalities in the initial quantities of cDNA sampled. Gene expression was determined using the \( 2^{-\Delta Ct} \) formula where \( \Delta Ct = (Ct \text{ target gene: Ct 18S}) \).

**Statistics.** Data were analyzed separately and presented as means ± SE. Significant differences between response variables were analyzed using SAS (version 8), and a Generalized Linear Model was applied. Differences were considered to be significant for \( P \leq 0.05 \).

**RESULTS**

**Daily energy intake and body weight gain.** As expected, it was seen that mice receiving the HF diet consumed significantly more food than those receiving NF (\( P < 0.001 \), data presented in Table 3). In line with this, the body weight gain of mice given the HF diet was significantly higher than the body weight gain those receiving the NF diet (\( P < 0.001 \)).

**NTS response to intragastric gavage with an HF or NF load or to an intraperitoneal injection of CCK-8 in mice receiving the NF or HF diet for 15 days.** As shown in Fig. 1A, HF-treated mice given either an HF or NF load via intragastric gavage exhibited less Fos activation than NF-treated mice (\( P < 0.001 \), \( F = 25.72 \)). No difference was observed following the HF and NF loads in HF-treated mice, whereas in NF-treated mice, the HF load induced higher Fos activation than the NF load (\( P < 0.001 \)). This decreased in Fos induction in the NTS was due to the diet (\( P < 0.001 \)), not to the specific content of the load. Taken together, these data suggest that the changes to Fos induction were the only result of the 15-day diet (\( P < 0.001 \)). These findings were consistent with the observation of a significant difference in NTS activation in response to an intraperitoneal injection of 100 pmol CCK-8 when comparing HF-treated and NF-treated mice (Fig. 1, B and C) (17.8 ± 2.2 vs. 28.4 ± 2.3 Fos-positive neurons per NTS section; \( P < 0.05 \)). It is noteworthy that an intraperitoneal saline injection induced no difference in c-fos expression in either HF or NF-fed mice.

**Table 3.** Daily food intake and body weight changes in mice fed the normal fat or high-fat diet

<table>
<thead>
<tr>
<th></th>
<th>Normal Protein Diet, control</th>
<th>High-Fat Diet</th>
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<tbody>
<tr>
<td>Daily food intake, g</td>
<td>5.9 ± 0.3</td>
<td>6.2 ± 0.5</td>
</tr>
<tr>
<td>Daily energy intake, kJ</td>
<td>89.7 ± 5.0</td>
<td>109.3 ± 8.4*</td>
</tr>
<tr>
<td>Initial body weight, g</td>
<td>18.1 ± 0.3</td>
<td>18.1 ± 0.2</td>
</tr>
<tr>
<td>Body weight gain, g</td>
<td>0.5 ± 0.3</td>
<td>+1.9 ± 0.3*</td>
</tr>
<tr>
<td>Body weight gain, %</td>
<td>+3%</td>
<td>+11%</td>
</tr>
</tbody>
</table>

Results are presented mean ± SE *Significant effect of diet \( P \leq 0.05 \).

**Short-term food intake and the efficiency of intraperitoneal CCK-8 or 2-methyl-5-HT injections in suppressing short-term food intake in mice fed an NF or HF diet for 15 days.** Short-term food intake did not differ between NF- and HF-treated mice under control conditions. The efficiency of an intraperitoneal 100 pmol CCK-8 injection in suppressing 1 h food intake was significantly weaker (\( P < 0.001 \)) in HF-treated mice than in NF-treated mice (14.9 kJ ± 0.9 vs. 5.3 ± 1.7 in HF- and NF-treated mice, respectively). By contrast, there was no significant difference regarding the reduction in food intake induced by an intraperitoneal injection of 0.5 nmol 2-methyl-5-HT between HF- and NF-treated mice (14.3 kJ ± 1.9 vs. 14.6 kJ ± 0.9, in HF- and NF-treated mice, respectively) (Fig. 2).

**Quantification of gut mediator receptor transcripts in nodose neurons from mice fed the NF or HF diet for 15 days.** Nodose mRNA levels of CB1-R, CCK1-R, Ob-L, OX1-R, OX2-R, and 5-HT3-R transcripts were determined in NF and HF-treated mice after fasting for 12 h. As shown in Fig. 3, nodose neuron mRNA levels were significantly lower regard-
ing CB1-R \( (P < 0.001) \), CCK1-R \( (P < 0.05) \), Ob-L \( (P < 0.01) \), and OX1-R \( (P < 0.05) \) transcripts and were significantly higher for OX2-R transcripts \( (P < 0.05) \) in HF-treated mice compared with NF-treated mice. By contrast, no significant difference was found between groups of mice adapted to NF or HF diets with respect to 5-HT3-R mRNA levels. No difference was observed between male and female mRNA profiles.

**DISCUSSION**

The present study shows that feeding for 15 days with HF rather than NF affected daily food intake, the short-term control of ingestion, body weight gain, and sensitivity to both CCK-8 and macronutrient mixture loads, while modifying vagal afferent receptor transcript levels, especially those of the peripheral CCK-8 receptor, CCK1-R. This study was performed in female mice for practical reasons, but verifications (mRNA profiles) conducted in male mice fed identical diets yielded identical results.

First of all, these findings suggest that, in mice, 15 days of an HF diet reduced the propensity of satiation signals to elicit meal termination and thus inhibit short-term food intake. This phenomenon thus resulted in elevated food intake and increased body weight gain. Indeed, in mice maintained for 15 days under HF, the propensity of CCK-8 to inhibit food intake was found to be significantly reduced compared with mice maintained for 15 days under an NF diet. The doses used here can be considered as being physiologically effective (24). These findings corroborate previous reports that rats fed an HF diet were less sensitive to satiation signals and consumed more food than those receiving a low-fat diet (24). Interestingly, 1 h of food intake tended to be lower under the HF diet than under the NF diet, and this observation was not inconsistent with daily food intake. Indeed, it has previously been shown that animals fed an HF diet eat shorter, but more frequent, meals (17). Other studies have reported a reduced sensitivity to the anorexigenic effect of CCK-8, contributing to excessive eating and weight gain in rats maintained under an HF diet (10, 9, 11). In terms of our results and previously published works, adaptation to an HF diet seemed, at least in part, to be mediated by changes in sensitivity to the anorexigenic effects of CCK-8. Although dietary conditions induced decreased sensitivity to CCK-8, it is noteworthy that sensitivity to 2-methyl-5-HT remained unchanged under the same conditions. Consistent with this observation, nodose ganglia 5-HT3-R mRNA levels were unaffected by both the HF and NF diets \( (P = 0.71) \). These results suggest that vagal sensitivity to 5-HT is not involved in HF-induced desensitization to satiation signals.

In line with these results on food intake, it was also found that intragastric loads of macronutrient mixtures (designed to mimic the effects of a single meal) induced less c-fos transcription in the NTS neurons of mice receiving the HF diet than those under NF, regardless of load composition. Although it may be problematic to compare loads that differ significantly in terms of their composition, the outcomes of this study suggest that after habituation to the LF and HF diets, the composition of intragastric loads impacted c-fos much less than the composition of the habitual diet. Again, these data showed that 15 days of an HF diet led to decreased sensitivity to within-meal satiation signals, thus suggesting involvement of the vagal pathway; c-fos expression is a reliable indicator of neuronal activity, and NTS activation can be used to reflect vagal activity (5, 12, 22). In addition, HF-induced diminished sensitivity to CCK-8 was confirmed by measurements of Fos induction in the NTS in

![Fig. 2. Food consumption 1 h following food cup presentation after injection of a vehicle (100 \( \mu \)l saline injection) or of 100 pmol ip CCK-8 injection or 0.5 nmol ip 2-methyl-5-HT, in mice fed for 15 days with an NF or HF diet. Results are presented as means \( \pm \) SE. *Significant effect of diet \( P \leq 0.05 \).](http://ajpregu.physiology.org/)}

![Fig. 3. Anorexigenic and orexigenic receptor transcript levels in nodose ganglia in male and female mice fed for 15 days with an NF or HF diet \( (n = 3; 4 \) mice pooled in each observation) and after fasting for 12 h. Results are presented as means \( \pm \) SE. *Significant effect of diet \( P \leq 0.05 \).](http://ajpregu.physiology.org/)
response to intraperitoneal CCK-8, with HF-fed mice exhibiting a lower level of activation than NF-fed littermates, while this difference was not observed in the absence of stimulation. Mechanisms for macronutrient-driven NTS activation are now well documented (18), and several studies have reported that during digestion, macronutrients elicit the release of anorectic mediators (such as CCK and 5-HT) by enteroendocrine cells located within the gut wall. In turn, CCK and 5-HT activate vagal endings located in the intestinal wall. Vagal afferents convey this information to the NTS where satiation will be generated (27). If any desensitization occurs, it is likely to be at this level, so it is legitimate to investigate modifications to the vagal pathways.

The mechanism underlying HF-induced reduced sensitivity to CCK-8 is poorly understood. It has however, been shown that neurons can downregulate their activity when subjected to repeated stimulations. This phenomenon has been described extensively for neurotransmitters specific to G-protein coupled receptors (4), such as CCK1-R receptors (31). Chronic stimulation can lead to neuronal desensitization via either receptor internalization or changes to de novo receptor expression by vagal afferent neurons. This phenomenon has already been shown to occur under specific dietary conditions (7). Accord-ingly, the present results showed that the HF diet decreased mRNA levels of CCK1-R transcripts in nodose ganglia when compared with the NF diet. An HF diet-induced decrease in CCK1-R transcript expression may lead to diminished CCK1-R expression and would ultimately reduce the numbers of receptors at the plasma membrane of vagal afferents. These mechanisms may be responsible for reduced sensitivity to CCK-8. Furthermore, when comparing the profiles of vagal sensitivity receptors to anorexigenic and orexigenic gut mediators under the HF and NF diets, we observed that the gut-hormone receptor profile was more markedly modified by the HF diet than by the NF diet. Interestingly, in fasting animals, CB1 and Ob-LR levels were decreased under HF conditions, while OX-R1/2 levels increased. Synergistic interactions between CCK and leptin (2), CCK and orexins (6), or CCK and endocannabinoids (7) can lead to modulations of vagal afferent discharge. Ob-LR interact with CCK signaling and potentiate the CCK response, so that decreased levels of this receptor under HF may decrease CCK-mediated signals. By contrast, orexin action on OB-R1/2 has been shown to attenuate CCK action. As we report here on increased levels of OX-R1/2 under HF conditions, this result is consistent with a greater attenuation of CCK signaling. Surprisingly, CB1 levels were decreased under HF, so this effect was not consistent with the orexigenic properties of the endocannabinoid system, although because CB1 is a highly versatile receptor, it is likely that this receptor exhibits very rapid changes around a meal. Further studies are therefore necessary to clarify the involvement of CB1 in this phenomenon.

Taken together, these data show that when compared with 15 days of an NF diet, feeding with an HF diet for 15 days reduced vagal sensitivity to macronutrients by lowering sensitivity to CCK, a key peripheral mediator in satiation. This effect probably involved decreased levels of CCK1-R receptors and increased levels of OX-R1/2 receptors affecting vagal afferents.

Perspectives and Significance

These data support the hypothesis that the macronutrient composition of diets can significantly alter the control of ingestive behavior, and especially satiation, by influencing satiety pathways and particularly vagal sensitivity. These findings suggest a new role for the vague nerve as an integrative component of the gut-brain axis underlying diet-induced adaptive processes.

REFERENCES


