INTRODUCTION

Vagus nerve stimulation (VNS) is a new promising therapeutic methodology that has been used to treat epilepsy and depression (1, 2). It has also been investigated for managing various anxiety disorders (2), Alzheimer’s disease, migraines (3), fibromyalgia (4) and tinnitus (5). Moreover, VNS influences cytokine production and improves survival in experimental models of sepsis, hemorrhagic shock and ischemia-reperfusion injury (6). Surprisingly, in some patients treated with VNS therapy, changes in food intake and weight reductions were observed. These findings have inspired many researchers to investigate the possible mechanisms underlying VNS-induced weight loss and to introduce VNS as a novel effective method for obesity treatment (7, 8).

Body weight, food intake and body fat content are regulated by multiple factors (9-12). Despite fluctuations in the amount of food consumed, body weight remains stable within a relatively narrow range. Food intake and body mass are controlled by both short- and long-term regulatory mechanisms. For short-term regulation, the glucostatic hypothesis of regulation of food intake was proposed by Mayer over 50 years ago (13). Hypoglycemia increases food intake and stimulates vagal activity (14). Campfield and Smith first demonstrated that a vagally mediated increase in plasma insulin concentrations resulted in a transient decrease in blood glucose that then induced spontaneous feeding in rats (15). In addition, food transported into the stomach and duodenum activates both stretch- and mechanoreceptors. These signals are also transmitted via the vagus nerve to the hind brain, where they are integrated and play a major role in short-term regulation by limiting the size of the meal consumed (9, 10). Randich and Cox (16, 17), using extracellular recordings from the vagus nerve, found that the vagus nerve transmits a “satiety signal” from the jejunum that is activated by fatty acid infusion. Although not significant, serum nesfatin-1 levels were also elevated. These results support the theory that VNS leads to reductions in food intake, body weight gain and adipose tissue by increasing brain satiety signals conducted through the vagal afferents. VNS also evoked a feed-related hormonal response, including elevated blood concentrations of nesfatin-1.

**Key words:** body weight, c-Fos, fat pad, food intake, ghrelin, high-fat diet, leptin, nesfatin-1, nucleus of the solitary tract, obesity, rat, vagus nerve stimulation
of central and peripheral signals (12). It is also hypothesized that the VNS decreases food intake and body weight gain by mimicking the “satiety” signals transmitted from the gut to the brain, leading to the activation of the hypothalamic neurons that initiate the state of satiety. As vagal afferents transmit information to the brain not only from activated gastrointestinal mechanoreceptors, but also from osmoreceptors, duodenal chemoreceptors and hepatic glucoreceptors (18), this hypothesis has considerable support evidence.

Previously, we showed that short-term vagus stimulation affects food intake and decreases body weight in rats (19-21). Bugajski et al. (22) reported a decrease in meal size, body weight and in epididymal fat pad weight in obese rats, and Ziomber et al. (23) showed similar results with left VNS in growing animals. Recently, a study by Val-Laillet et al. reported significant weight loss and reduced food consumption in mini-pigs implanted with vagus nerve constant current stimulators (24). In contrast, in humans subjected to VNS, no changes in body weight were observed (25, 26), or the data are controversial (7, 8, 27, 28). These discrepancies could result from completely different patterns of applied vagus nerve stimulation. Additionally, the appropriate frequency, impulse amplitude and method of stimulation still need to be established in experimental models. In our study, we decided to stimulate the left vagus nerve with a 10 Hz frequency; although this value is relatively high, it does not block vagus nerve transmission (8).

Because the left and right vagal trunks are connected to different parts of the gastrointestinal tract and the contributions of both are important, the decision of which trunk should be stimulated remains controversial. Although previous work from our laboratory (19, 20) demonstrated that bilateral VNS is more effective than unilateral VNS, other studies have shown that unilateral stimulation can also be effective. However, to limit possible side effects on the heart or the lungs, we decided to apply chronic VNS using a microstimulator placed on the left vagus nerve. Electrodes were placed close to the gastroesophageal junction to stimulate the small unmyelinated C fibers; this placement also avoids the stimulation of fibers that join the trunk from the heart and lungs, as discussed by Val-Laillet (24). We used constant voltage microstimulators that were implanted for at least 6 weeks.

The aim of the current work was to evaluate the effects of high frequency (10 Hz) chronic left VNS on long-term regulation of body weight and food intake in rats with high-fat diet-induced obesity. This animal model of obesity induced by a high-fat diet has been widely accepted (22, 23, 29). To determine whether VNS activated the food-related brain areas in our study, the c-Fos response of neurons in the nucleus of the solitary tract (NTS) was measured. Furthermore, the fat compartments of experimental animals were evaluated by measuring the epididymal fat pads of the rats, which reflect total body fat mass (30-32). As vagus nerve manipulations are thought to influence the circuits controlling food intake, we also investigated the circulating levels of several appetite regulating hormones: leptin, ghrelin and nesfatin-1. Leptin levels are related to body mass and fat content. While ghrelin is a potent orexigenic factor, nesfatin-1 is a newly described peptide of anorexigenic character.

MATERIALS AND METHODS

Twenty-four adult male Wistar rats were studied. The animals were housed in individual cages, and all were fed the same obesity-inducing high-fat diet (Bento Kronen Products, Belgium) throughout the entire experimental period. The caloric distribution of the diet was as follows: protein, 29.5%; fat, 45.6%; and carbohydrates, 24.9%. The metabolizable energy was 4.34 kcal/g. All animals were housed in the same optimal conditions, and food and water were provided ad libitum. The temperature was maintained at 23±2°C, and animals were placed on a 12:12 h dark/light cycle. The Jagiellonian University Bioethical Committee approved the care and use of the animals (ethical approval number - 36/2008).

After approximately 2 weeks of adaptation to the new environmental conditions and high-fat diet, rats were starved for 12 hours and operated on under general anesthesia induced with sodium pentobarbital given intraperitoneally at a dose of 0.25 mg/kg (Vetbutal, Biowet, Pulawy, Poland).

The rats were randomly divided into the following three groups: 1) rats with an active microstimulator (MS) connected to the left vagus nerve by electrodes (MS group, n=8), 2) animals with an inactive MS without electrodes on the vagus nerve (sham group, n=8), and 3) intact rats without an MS or electrodes (control group, n=8). To eliminate the possible effects of surgical procedures and microstimulator implantation on the parameters examined, a control group of intact rats was included in the study. The sham group served as an important reference group for the assessment of food intake, body weight and epididymal fat pad weight manipulations.

The MS for chronic vagus stimulation were designed by the Institute of Electron Technology, Cracow, Poland. In the MS group animals, an MS was surgically placed into a subcutaneous pocket, and the ends of the MS silver electrodes were wrapped around the subdiaphragmatic left vagus nerve; the cathode and anode were positioned at a distance of 0.5 cm. In the second group, laparotomies were performed, and an inactive MS was implanted (sham group). In the control group, no surgeries or manipulations were performed.

Food was restored on the day following the operation. After a one week recovery period, rats with a mean body weight of 475±25 g were considered ready for experimentation. Recovered animals from the MS and sham groups were then placed into individual plastic cages with electromagnetic field exposure, as previously described (23). The cages were connected to a neurostimulator NSE 002 sinusoidal wave generator with an amplifier (Institute of Electron Technology, Cracow, Poland) that generated a 30-kHz pulsating magnetic field. The magnetic field served as an external source of current in the MS wires connected to the left vagus nerve. The parameters of the magnetic field were set experimentally to match the amplitude, duration and frequency of the impulses used in our experiment. The rats in the control group were placed into individual cages outside of the magnetic field.

The parameters of the unipolar rectangular impulses generated by the MS were based on our previous studies (19, 20, 23, 29) and set as follows: duration, 10 ms; amplitude, 200 mV; and frequency, 10 Hz. As food intake is predominantly nocturnal in rats, the amount of food consumed during the light phase generally does not exceed 20% (33). Therefore, animal stimulation commenced each day at 6 p.m. and lasted 12 h until 6 a.m. the next morning (dark phase stimulation). Daily observations of the rats were performed to check for any visible behavioral symptoms of discomfort.

During the study, daily food intake and body weight were measured each morning. For each rat, the amount of daily food intake was determined by subtracting the amount of food remaining from that given 24 h before. At the end of the experiment (day 42), all of the rats were weighed and euthanized by decapitation. Both epididymal fat pads, located between the cauda epididymis and the distal extremity of the testis, were dissected from each rat and weighed. The epididymal fat pad/body weight ratio was calculated by dividing the fat pad weight by the total body weight.
Blood samples collected at the end of the experiment were taken into tubes containing aprotinin (0.6 TIU per 1 ml of blood) (Sigma-Aldrich, USA) and incubated for 30 minutes at 4°C for clotting formation. After centrifugation at 1500 g for 20 min at 4°C (Megafuge 1.0R, Heraeus Instruments), serum samples were collected and frozen at −80°C until further analysis. Three serum aliquots were prepared for each sample, and ghrelin, leptin, and neesfatin-1 levels were measured using radioimmunoassays (RIAs); all assays were performed according to the manufacturer’s directions (Phoenix Pharmaceuticals Inc., USA). All measurements were performed in duplicate.

Following decapitation, the whole brain was removed from the skull and placed in a solution of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 24 h. Neurons of the nucleus of the solitary tract (NTS) were visualized according to the methodology described by Osharina (34); NTS localization was determined using brain maps from Swanson (35). According to Osharina et al. (34), c-Fos positive neurons in NTS are the most prominent following VNS in the brain section where the area postrema is localized close the NTS. Subsequently, the same brain hemi-section was selected for the evaluation of c-Fos positive neurons in our experiment (bregma - 14.0 mm). Each whole brain was positioned in special rat brain blocker (PA001, KOPF), and the required slice was incised. To identify the right or contralateral side of the brainstem, each specimen was marked with a small puncture prior to further tissue processing. The brain fragments were then routinely processed, embedded in paraffin blocks, and cut using an ultra-microtome; slices that were 1–2 μm thick were then mounted on silanized glass. Additionally, sections from stimulated, sham and control rats were processed for routine hematoxylin-eosin staining and for c-Fos immunohistochemistry. First, antigen retrieval was performed using the heat-induced epitope retrieval (HIER) method by incubating slides in a target retrieval solution at 95°C for 20 min. Next, peroxidase block (DAKO) was added to remove the endogenous peroxidase activity in the tissue. The sections were then incubated with protein block (DAKO) with 0.05% Triton X-100 (DAKO) for 30 min; primary polyclonal rabbit antibodies against c-Fos (Santa-Cruz, sc-52, diluted 1:200) were then added. The sections were incubated for 48 h at 4°C, washed and then incubated with the UNIVISION anti-rabbit visualization system (Labvision; antibodies were conjugated to horseradish peroxidase (HRP), and diaminobenzidine (DAB) was used as a chromogen. The specimens were counterstained with Mayer hematoxylin and closed with Ultracruz Mounting Medium (Santa Cruz). The brown-stained cells stained were considered positive. Positive cells in the NTS in the ipsilateral and contralateral brain hemi-sections were visualized using an AXIOPHOT light microscope (Zeiss); cells were counted using the MULTISCAN semi-automated image analysis system (CSS, Warszawa, Poland).

Data are expressed as the mean and standard deviation (S.D.). The results were analyzed using a one-way analysis of variance (ANOVA) followed by a post hoc LSD test; all statistical tests were performed using STATISTICA 8.0 software package (StatSoft, Tulsa). Statistical significance was set at P<0.05.

RESULTS

Food intake and body weight

Electrical stimulation of the left vagus nerve reduced the daily and total food intake in the MS group compared with the sham and control groups. The differences were statistically significant (P=0.042, MS vs. sham) and (P=0.037, MS vs. control). No differences between the sham and control groups were observed (P=0.088). VNS-induced changes in food intake, body weight and epididymal fat pad changes are shown in Table 1.

As a consequence of the reduced food intake, the final body weight was significantly lower in the MS group compared with the sham group (P=0.043) and the control group (P=0.05). No differences between the sham and control groups were observed (P=0.037) (Table 1). VNS also significantly reduced body weight gain. The mean body weight gain in the MS group was 21.1% of initial body weight, while it was 30.5% in the sham group and 29.8% in the control group. Body weight differences between the MS, sham and control groups became visible and significant after 3 weeks of stimulation; these differences persisted until the end of experiment (Fig. 1).

Epididymal fat pad weight

Fat pad weight, which reflects total body fat content, was significantly higher in the sham group compared with the MS group (P=0.011). The control group fat pad weight was also significantly higher than that in the MS group (P=0.034). The mean epididymal fat pad weight relative to body weight (fat pad/body weight ratio) was significantly lower in the MS group rats compared with the sham group rats (24.8%) or control group rats (22.1%) (Table 1). No differences between the sham and control groups were observed.

Table 1. Mean food intake, body weight and epididymal fat pad weight in MS left vagus nerve stimulated (10 Hz)–, sham operated- and control-treated rats (n=8 for each group). Asterisks (*) indicate significant differences between the MS-10 Hz group and the sham and control groups.

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>MS - 10Hz</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake during experiment (g)</td>
<td>980.5±54.9</td>
<td>890.7±92.5*</td>
<td>986.8±78.3</td>
</tr>
<tr>
<td>Initial body weight (g)</td>
<td>473.7±25.8</td>
<td>469.7±22.5</td>
<td>477.7±26.5</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>618.4±43.36</td>
<td>568.9±50.0*</td>
<td>620.3±49.9</td>
</tr>
<tr>
<td>Body weight gain (g)</td>
<td>144.6±27.3</td>
<td>99.1±33.1*</td>
<td>142.7±35.1</td>
</tr>
<tr>
<td>Body weight gain increment over initial body weight (%)</td>
<td>30.5</td>
<td>21.1*</td>
<td>29.8</td>
</tr>
<tr>
<td>Epididymal fat pad weight (EFP) (g)</td>
<td>11.1±2.9</td>
<td>8.4±1.3*</td>
<td>10.8±2.6</td>
</tr>
<tr>
<td>EFP/body weight ratio (%)</td>
<td>18.1±3.1</td>
<td>14.5±1.8*</td>
<td>17.7±2.7</td>
</tr>
<tr>
<td>EFP/body weight ratio increase over MS rats (%)</td>
<td>24.8*</td>
<td>--</td>
<td>22.1*</td>
</tr>
</tbody>
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Ghrelin

VNS significantly increased serum ghrelin levels compared with the sham (P=0.04) and control (P=0.018) groups; the levels for the MS, sham and control groups were 520.6±96.7, 418.4±97.2, and 391±65.1 pg/mL, respectively (Fig. 1). No differences between the sham and control groups were observed (P=0.97).

Leptin

Serum leptin levels were significantly decreased in the MS group compared with the sham (P=0.044) and control (P=0.04) groups; levels for the MS, sham and control groups were 5.4±2.8, 7.9±2.9, and 7.8±3.4 ng/L respectively (Fig. 2). No differences between the sham and control groups were found (P=0.97).

Nesfatin-1

Although not significantly, vagus nerve stimulation increased serum nesfatin-1 levels compared with the sham (P=0.19) and control (P=0.27) groups; nesfatin-1 levels were 913.1±139.5, 824.6±127.7, and 831.6±76.9 pg/mL in the MS, sham, and control groups, respectively (Fig. 3). No differences between the sham and control groups were observed (P=0.92).
Activated neurons in the nucleus of the solitary tract

C-Fos–positive neurons in the NTS were found in all of the rat brain hemissections examined. The amounts of c-Fos positive cells were similar in the left and right NTS in the control and sham groups. No differences were observed between the left and right NTS inside the control and sham groups (P>0.05). However, the number of c-Fos-positive cells increased above basal levels following left vagus nerve stimulation in both the left (P<0.0001) and the right (P<0.001) NTS (Fig. 5). Although the VNS stimulation predominately affected c-Fos expression on the ipsilateral (left) side, it also significantly affected c-Fos expression on the contralateral side (right NTS). The increase in c-Fos positive neurons on the ipsilateral side was highly significant (P<0.0001 ipsilateral vs. contralateral side).

**DISCUSSION**

This animal study was performed using rats fed a high-fat diet because high-fat diet-induced obesity mimics human obesity and is widely considered to be an appropriate model for studying dietary obesity (36, 37). We previously showed that low frequency vagal stimulation affects short-term volume regulation of food intake and decreases body weight in rats (19, 20, 21); however, these studies were conducted in rats fed with...
hypothesized that peripheral electrical vagus nerve stimulation now widely accepted as a marker of neuronal activation. We regulation of neuronal metabolism (34, 44), c-fos expression is forms of stimulation. As the protein c-Fos participates in the expressed rapidly in the central nervous system after various levels insufficient for stimulation (43).

Voltage drop that could decrease the current at the nerve level to of constant voltage stimulation to avoid a possible additional should be noted that Val-Laillet used current stimulation instead of voltage drop (humoral vs. electrical) or the anatomical placement confirming that VNS appears to act predominately through factors, such as appetite regulating hormones, on activations of NTS neurons cannot be omitted.

Peripheral vagus nerve stimulation is sufficiently effective to evoke a response in central nervous system neurons due to afferents stimulation. However, the efferent effects of VNS must also be considered. A study by Poci (48) showed decreased hepatic glucose production involving parasympathetic circuits (vagal efferents). Our data, however, did not reveal any glucose level changes related to VNS (data not shown). This discrepancy could be caused by the different experimental models of stimulation (humoral vs. electrical) or the anatomical placement of the electrodes (above the division of common hepatic branch from the left vagus nerve). These facts support our findings, confirming that VNS appears to act predominately through afferent signaling.

Vagus nerve stimulation influences the brain centers controlling appetite, body weight and adipose tissue accumulation. In rodents, fat tissue is localized in different accumulation. In rodents, fat tissue is localized in different
deposits, and visceral fat accumulates in the epididymal fat pad. This visceral fat is well delimited and easy to excise. Although the epididymal fat pad represents only a small portion of the total body weight, previous studies have shown that the epididymal fat pad weight calculated as a proportion of total body weight is highly correlated with total body fat in mice and rats (30-32). Moreover, the caudal epididymal fat pad is under strong hormonal and nervous regulation (49-51). In the present study, body composition was altered by vagus nerve stimulation; epididymal fat pad mass relative to body weight was significantly lower in rats that received VNS compared with control and sham-treated animals. Significant reduction of fat pad weight in rats following long-term VNS has been previously reported (22, 29).

In our study, the decrease in fat content could be related to the significant reduction in serum leptin levels. Most published reports agree that leptin expression and its release correlate with the amount of body fat and number of adipocytes. As fat cells produce leptin, serum leptin concentrations depend mainly upon an organism’s adipose tissue content (52-54). It is well documented that leptin is involved in the regulation of feeding behavior, body temperature, energy expenditure and blood pressure (53, 55, 56). In physiological conditions, leptin decreases food intake. However, obesity in humans is associated with leptin resistance, which is progressively established when a hypercaloric diet is maintained. Leptin resistance is accompanied by a progressive increase of serum leptin levels (52), and leptin levels in obese humans are high. Recently, a study by Morton et al. (45) found that this effect could be circumvented by the action of oxytocin. It should also be noted here that vagus nerve circuits play a role in leptin secretion. In 2003, Cigaina reported that decreased leptin levels correlated significantly with weight loss in patients with gastric pacing (57). Wang and Liu (58) did not find any changes in leptin levels after either vagus nerve dissection or gastric bypass in rats. However, leptin concentrations were significantly diminished and correlated with weight loss in both studies. In a previous study (23), we reported that the rats with VNS had decreased serum leptin concentrations, which correlated with decreased food intake and body weight gain. If VNS reduces food intake through leptin release in our current VNS model, then we would expect to observe an increase in serum leptin levels. However, our results showed the opposite. Therefore, we hypothesize that the decrease in leptin concentrations observed following VNS is connected with diminished body weight and loss of the adipose tissue rather than with vagus nerve manipulation.

Because VNS decreased food intake in our study, we expected the release of ghrelin, an orexigenic factor, to be suppressed. However, we obtained different results. The main sources of ghrelin are the stomach and duodenum, although it is also secreted by pituitary cells, the hypothalamus, liver, kidneys, and placenta (59). Ghrelin evokes an orexigenic effect, increasing food intake both in animal models and humans (60), and its mechanism of action involves the vagus nerve and activation of neurons in the hypothalamus (61, 62). Ghrelin level increase before a meal and fall sharply after the meal starts (63). Ghrelin levels are negatively correlated with body weight (64). Obese individuals have decreased ghrelin levels (65), which can normalize after diet-induced weight loss (66). We found a significant increase in serum ghrelin levels following electrical VNS. Although the data are potentially controversial, several reports must be mentioned. Cigaina (67) performed gastric pacing in morbidly obese patients and observed increased serum ghrelin levels throughout the entire (6 months) stimulation period. Although gastric pacing does not directly stimulate the vagus nerve, the authors postulated the involvement of the vagus nerve in ghrelin release after gastric pacing. These observations are partially supported by data published by Gallas et al. obtained in rats after short (1 h) electrical gastric stimulation (68). They found that increased ghrelin mRNA levels in stomach cells were responsible for the increased ghrelin secretion and significantly elevated serum ghrelin levels. In a murine brain trauma injury model, Bansal et al. (69) reported that short term (2-6 h) VNS significantly increased serum ghrelin levels; however, the underlying mechanism remains unclear. One possibility that should be considered is based on report by Broux et al. (70). He reported that ghrelin does not stimulate food intake in patients who underwent vagotomy, suggesting a peripheral mechanism of ghrelin action on food intake that involves the vagus nerve. These observations partially confirm data from Murakami et al. (71), who postulated that two separate (peripheral and central) mechanisms of action for ghrelin are involved in the regulation of body weight and food intake. Our results are most supported by data from Cummings et al. (66), who demonstrated that ghrelin significantly increased with weight loss and that gastric bypass is associated with markedly suppressed ghrelin levels. On the other hand, Kamiji et al. (72) reported that ghrelin levels are decreased in vagotomized and gastrectomized patients. Consistent with this, Wang and Liu (58) reported that ghrelin levels decreased in parallel to body weight reduction after gastric bypass and vagus dissection in an animal model of obesity treatment.

In light of such numerous but controversial reports, it remains difficult to present a clear rationale for our data. Moreover, our data became even more complicated to understand following the recent release of work by Huang et al. (73), who postulated that stimulation of the dorsal or ventral trunk of the vagus nerve evokes opposite effects on ghrelin release and that ghrelin release depends on the frequency of the VNS. We conclude that the increase in serum ghrelin levels observed in our study is associated with the organism’s response secondary to VNS-induced decreases in food intake and weight loss. However, it is also possible that VNS somehow influences peripheral circuits to induce increased ghrelin release.

Oh et al. recently identified nesfatin-1, an 82 amino acid anorexigenic peptide derived from NEFA/nucleobindin2 (NUCB2) (74). When administered into the third cerebral ventricle, nesfatin-1 significantly reduces food intake in rats. Twenty-four hour fasting effectively reduces NUCB2 expression in the paraventricular nucleus (PVN) (75). Additionally, nesfatin-1 immunopositive neurons in the PVN are activated by refeeding (76), indicating that nesfatin-1 plays an important role in regulating food intake. Nesfatin/NUCB2 immunopositive neurons are also located in the arcuate nucleus (ARC), where they co-localized with proopiomelanocortin (POMC) and cocaine-and-amphetamine responsive transcript (CART) neurons (74, 75). Additionally, nesfatin-1 crosses the blood-brain barrier in both directions (77, 78).

There are only few reports that examine nesfatin-1 in the context of VNS and obesity. Shimizu et al. (79) reported that intraperitoneal injection of nesfatin-1 diminished food intake. A study by Stengel et al. (80) found that nesfatin-1 immunopositive cells are present in the rat gastric mucosa and that the majority of nesfatin-1 immunopositive cells co-expressed ghrelin. The authors also observed a down-regulation of NUCB2 mRNA in gastric endocrine cells after 24 hours of fasting and concluded that nesfatin/NUCB2 gene expression might be regulated by nutritional status. To elucidate the mechanism underlying nesfatin-1 regulation, Li et al. (81) examined fluctuations in nesfatin-1 levels in diabetes mellitus patients after oral glucose ingestion. He observed a reduction in fasting nesfatin-1 concentration. However, glucose ingestion did not affect plasma nesfatin-1 levels, suggesting that gastric chemosensation is not sufficient for a nesfatin-1 response. To date, the influence of other nutrients, such as the high-fat diet
used in our study, on serum nesfatin-1 levels has not been investigated. However, in a study of obese patients, Tan et al. (82) found that increased nesfatin-1 levels correlated positively with body mass index (BMI), body fat mass and obesity; additionally, plasma nesfatin-1 levels were significantly negatively correlated with nesfatin-1 levels in cerebrospinal fluid, suggesting nesfatin-1 resistance in obese subjects.

In our experiment, vagus nerve stimulation elevated serum nesfatin-1 levels. Because there are not sufficient data from other studies to determine whether the VNS or the high-fat diet, either by peripheral or central mechanisms, reduced food intake, body weight and fat content, we report an increase of serum nesfatin-1 levels concomitant with VNS, which caused the anorexigenic effects observed in our study. However, the specific mechanisms need to be further elucidated. The data from Shimizu et al. (83, 84) partially support our findings, as intraperitoneally administrated M30, which is an active mid-segment of nesfatin-1, induced anorexia via the vagus nerve in mice; importantly, this effect was abolished in animals pretreated with capsaicin.

The present study demonstrates that vagus nerve stimulation exerts anorexigenic effects on food intake and body weight gain in rats with high-fat diet-induced obesity. Electrical signals generated by a microstimulator were conducted by the vagal afferents as satiety signals and modified the central neural circuits regulating body weight, food intake and body fat content. Additionally, VNS increased serum nesfatin-1 levels, which also may contribute to the observed reductions in body weight and fat content. Thus, vagus nerve stimulation may represent a promising, effective method for obesity treatment.

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Author’s address: Dr. Krzysztof Gil; Department of Pathophysiology, Jagiellonian University Medical College, 18 Czysta Street, 31-121 Cracow, Poland; Phone: (+48)12 6333947; Fax: (+48)12 6329056; E-mail: mpgil@cyf-kr.edu.pl