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MAGNETICALLY INDUCED VAGUS NERVE STIMULATION AND FEEDING BEHAVIOR IN RATS

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Vagus nerve (VN) contribute to the bidirectional communication between the gastrointestinal tract and the central nervous system. Stimulation of the VN by a magnetically-driven solenoid with parameters similar to those during food-induced stomach distension has been thought to mimic short-term signaling of satiety and suppress food intake. In this study, the determination of optimal parameters of vagal neuro-modulation to achieve decreased food intake with a resulting reduction in body mass of rats is explored as therapy to treat obesity. The experimental design consisted of three groups of obese adult male Wistar rats: Group 1: VEMF - with solenoid's electrodes placed on the left VN in the magnetic field exposure (MFE); Group 2: EMF - without solenoid's electrodes on the VN in MFE; Group 3: CON - without solenoid's electrodes on the VN outside the MFE. This study suggests that the rats with solenoid's electrodes placed on the left VN significantly decreased their food intake, weight gain and serum leptin concentrations when compared to that of the CON group. PP levels were found to be higher in the VEMF group when compared to the controls groups. It was found that the most effective parameters of vagal stimulation on eating behavior were 3631, 7861, 14523 A^2 x h/m^2. The magnetic field by unknown mechanisms also influences feeding behavior. This study suggests that vago-vagal reflexes are involved in the feeding homeostasis and that neuromodulation might be an effective method for managing obesity. Further studies are required to confirm these effects in humans.

Key words: *vagus nerve stimulation, feeding behavior, body weight, electromagnetic fields*

INTRODUCTION

The management of obesity is directed primarily to reduce energy intake and increase energy expenditure. Unfortunately, it remains to be a big challenge because biological mechanisms have evolved to prevent weight loss. The discrepancy between evolutionally preset weight loss prevention and readily availability of food with sedentary lifestyles contribute to over-eating thus increasing the prevalence of obesity (1).

Food consumption is regulated through a number of complex biological mechanisms designed to ensure that body weight remains relatively constant. The two neuronal populations of the arcuate nucleus in the hypothalamus is thought to play a pivotal role in the integration of appetite and satiety signals (2). The CNS communicates with enteric nervous system *via* brain-gut axis, where the vagal nerves (VNs) provide the major linkage between gastrointestinal sites stimulated during food intake and CNS sites that control feeding behavior. The presence of food in the stomach stimulates mechanical and chemical receptors to transmit signals to the brainstem (NTS) *via* vagal afferent fibres. Stomach distension is more important than nutrient content in the neural suppression of food intake. Load-sensitive vagal afferents are dose-dependent and are activated by increasing gastric volume and thus suppress feeding, independent of nutrient content (3).

Neuromodulation is a well-known method used in different areas of medicine. VN stimulation is of greatly effective in the management of: pharmaco-resistant epilepsy, chronic pain due to cancer, inflammatory pain, headaches and neuropathies (4). Stimulation of VN by magnetically-driven solenoid *via* parameters similar to those during stomach distension by food may mimic short-term signaling of satiety and suppress food intake. Repetitive VN stimulation may prevent compensatory mechanisms of energy homeostasis and may lead to weight loss.

The aims of our study is 1. Examine the effect of long-term VN stimulation *via* magnetically-derived solenoid on energy homeostasis in rats 2. Determine optimal effective parameters of vagal neuromodulation in rats on energy balance to achieve a decrease in food intake and subsequently body mass.

METHODS

Animals

Seventy-eight male Wistar rats with an initial body weight of 363 g were used in this study. Throughout the duration of the experiment, the animals were fed a diet with a higher percentage of fat (Perform, Bento Kronen Products, Belgium) than the standard diet (Labofeed B, Kcynia, Poland) to promote obesity. 100 g of DIO, contained: 21% of fats, 49% of carbohydrates and 30% of proteins, while the standard diet consisted of: 9% of fats, 65% of carbohydrates and 24% of proteins. All animals were

housed in the same lifestyle conditions (four rats per cage) with free access to food and water at a constant temperature of 23 +/- 2°C on a 12:12-hour dark/light cycle.

Jagiellonian University Bioethical Committee approved care and use of the animals.

Surgical procedures

Four weeks into the experiment, rats with an average body weight of 488 g were randomly divided into three groups and underwent surgical implantation of the solenoid (Institute of Electron Technology, Krakow, Poland) in the abdominal region. The solenoid used in the experiment comprised of a 1cm-diameter silicon-coated silver-wire (RTV 3140, Dow Corning). From the wire, 2 silver electrodes were connected to the VN. After 12 hours of food deprivation and under general anesthesia with pentobarbital intraperitoneally (Vetbutal 25 mg/ kg of body mass, Biowet, Pulawy, Poland), a longitudinal skin incision was made in the midline of the abdomen. In the subcutaneous pocket, formed by skin and underlining fascia, the solenoid was placed. Next, the left VN was localized in the subdiaphragmatic part of the esophagus and the solenoid's electrodes were brought into the abdominal cavity to connect with it. Un-isolated wires of the electrodes were wrapped around the left VN in VEMF group - cathode and anode were positioned 0.5 cm from each other. In the control groups (EMF and CON), laparotomy with subcutaneous implantation of the solenoid without electrodes was performed. After closing the wounds, the rats were placed into the cages with free access to food and water for a recovery period of five days. The operation was performed in sterile conditions with administration of prophylactic antibiotics (Erythromycinum pro suspensione, Polfa Tarchomin, Poland, 0.025g m/L) (5).

Group tested

The following groups were achieved after operation:

- Group 1: VEMF (n=34) - electrodes of solenoid on the left VN placed after recovery in the magnetic field exposure (MFE),
- Group 2: EMF (n=22) - inactive solenoid without electrodes on the left VN placed after recovery in the MFE,
- Group 3: CON (n=22) - inactive solenoid without electrodes on the left VN placed after recovery outside the MFE.

Magnetic field exposure

After the recovery period, the rats from VEMF and EMF groups were placed into plastic cages under MFE. The third cage with the CON group was placed outside the magnetic field. The cages with rats from VEMF and EMF groups, were connected to the generator of sinusoidal waves with amplifier (Neurostimulator equipment NSE 002, Electron Technology Institute, Krakow, Poland), where 10 kHz pulsating magnetic field was generated. The magnetic field served as an external source of current in the solenoids' wires because it was positioned vertically to the solenoid and thus generated electric current which was transmitted to the left VN. The parameters of the magnetic field were matched experimentally by regulating the amplitude and time of fulfilling impulse. This is in accordance to previous observations, that vagal impulses have a fulfilling cofactor beneath 10% and a repetition frequency of beneath 30 Hz. Following parameters of VN stimulation were used: of amplitude: 50, 100, 150 and 200 mV and frequency: 0.1 Hz; 0.2 Hz, 0.5 Hz and 1.0 Hz. The combination of applied parameters changed every three days, and are shown as magnetic field parameters: 227, 908, 3631, 7861, 14523 A2 x h/ m2. The stimulation period was of a 15 day duration. Food intake and body weight were measured every three days (6).

Biochemical examinations

At the end of the experiment, the animals were terminated and blood samples were taken for biochemical analysis. Leptin and insulin levels were assayed by conventional EIA and radioimmunoassay kits. Plasma PP radioimmunoassay was performed using guinea pig antirat PP serum, ^{125}I-Rat PP, and 2nd Antibody Precipitating System (Linco Research Inc, St. Louis, MO).

Data analysis

Five variables were analyzed: the average daily body weight gain, the average daily food intake, pancreatic polypeptide, insulin and leptin concentrations in blood. F-Snedecor test was performed to assess the differences among the average values of the analyzing parameters. Depending on similar or statistically different variances Student's T-test or Cochran-Cox's test were used. Results are expressed as mean ± SE. $P < 0.05$ was considered statistically significant. STATISTICA software for statistical analysis was used (Version 5.0).

RESULTS

Food intake

1. Pre-stimulation period

Food intake differed significantly between VEMF and CON groups in pre-stimulation period. There was no difference in food intake between VEMF and EMF or between EMF and CON groups (*Fig. 1*).

2. Post-stimulation period

After operation and throughout the stimulation period, the CON group started to eat significantly more food than VEMF and this tendency was also observed in the CON and EMF groups. From the first day of the stimulation period, the rats with the solenoid's electrodes on the left VN decreased their daily food intake thus achieving a significant difference to that of both control groups: inside or outside of the magnetic field. The group without VN stimulation but under MFE ate more food than rats with vagal stimulation, but less than in the control group outside magnetic field (*Fig. 1*).

3. Pre- and post-stimulation period in the same group

When comparing food intake during pre- and post-stimulation period in the same group, it was observed that the rats from VEMF had a lower appetite during the stimulation time than with the pre-operation period. This result infers that the presence of solenoid's electrodes on the VN changed their eating behavior. The rats from the CON group, increased their food intake during the second part of the experiment when compared to the pre-operating period, while the amount of food eaten by rats from the EMF group did not differ in the pre- and post-stimulation periods and also did not increase to the level of the CON group in the second part of the study (*Fig. 1*).

Body weight

1. Pre-stimulation period

There were no differences in body weight gain between the rats from VEMF and CON groups or between the EMF and CON groups. In the pre-operating period it was observed that the body weight gain in VEMF and EMF groups differed significantly and that the EMF group rats gained weight at a much faster rate (*Fig. 2*).

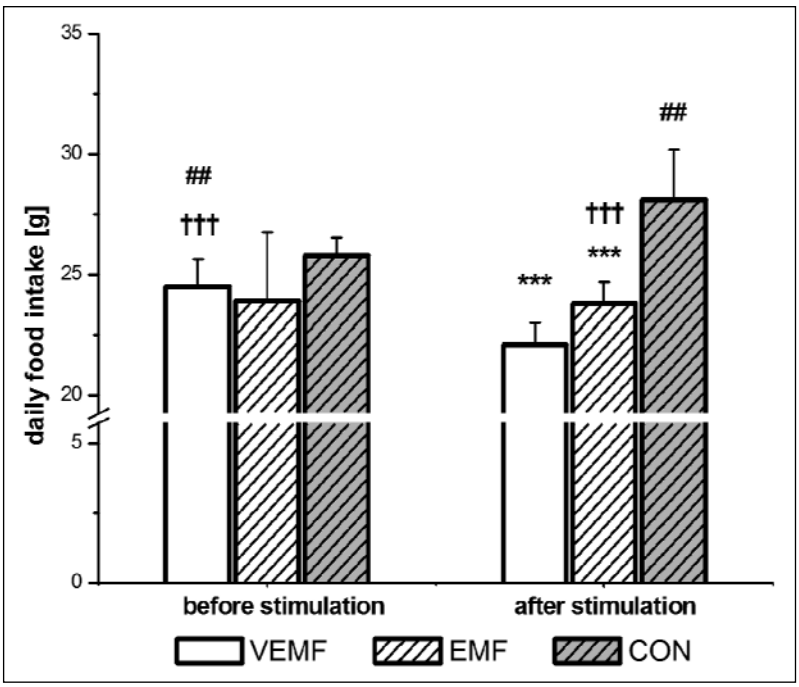

Fig. 1. Daily food intake [g] before and after left vagus stimulation period.
VEMF - group with vagus stimulation and magnetic field exposure; EMF - group with magnetic field exposure; CON - control group without vagus stimulation or magnetic field influence. ***P<0.001 *vs* CON after stimulation; †††P<0.001 *vs* VEMF after stimulation; ##P<0.01 *vs* CON before stimulation.

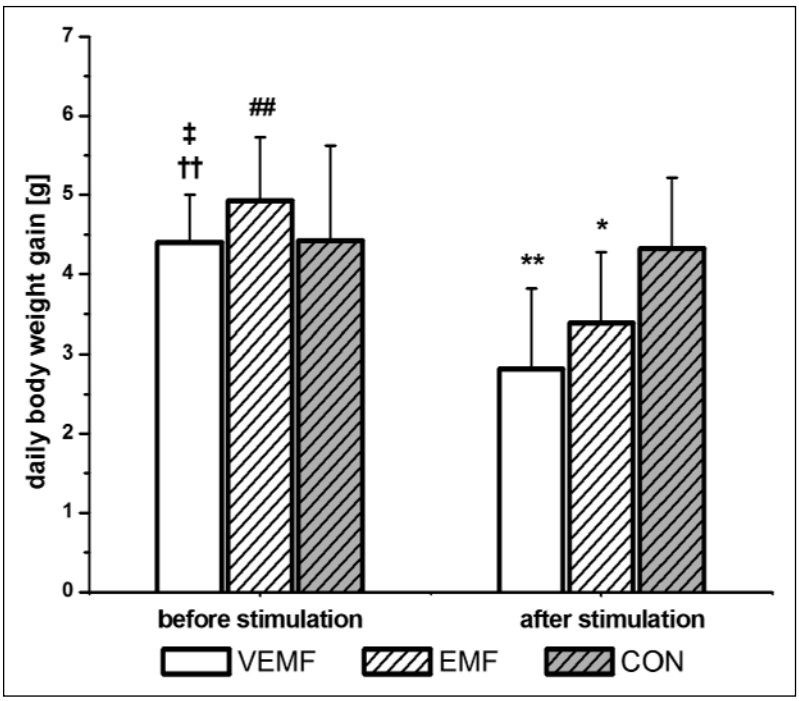

Fig. 2. Daily body weight gain [g] before and after left vagus stimulation period.
VEMF - group with vagus stimulation and magnetic field exposure; EMF - group with magnetic field exposure; CON - control group without vagus stimulation or magnetic field influence. *P<0.05; **P<0.01 *vs* CON after stimulation; ††P<0.01 *vs* VEMF after stimulation; ##P<0.01 *vs* EMF after stimulation; ‡P=0.046 *vs* EMF before stimulation.

2. Post-stimulation period

During the stimulation period, the rats from VEMF and EMF groups gained their body weight at a slower rate than the control group outside magnetic field (*Fig. 2*). No differences between the VEMF and EMF groups were observed. However, from the beginning of the vagal stimulation and/or magnetic field period, the differences in the body weights between VEMF and EMF groups were higher and achieved almost significant level at the end of the experiment (*Table 1*).

3. Pre-and post-stimulation period in the same group

When comparing body weight gain during pre- and post-operating time in the same group, it can be observed that the rates of weight gain in the rats from VEMF and EMF, were slower during the stimulation period of the experiment. This shows that vagal stimulation and the magnetic field influences energy homeostasis.

To assess the most potent parameters of VN stimulation on food intake and body weight gain, the rats used in the study, for statistical purposes, were divided into two groups: Group VNS (+): rats with vagal stimulation (the previous VEMF) and Group VNS (-): rats without vagal stimulation (the previous EMF and CON). This division allowed for the actions of vagal stimulation on food intake and body weight gain to compare and achieve statistically significant levels (VNS (+) *vs* VNS (-): 22.1±0.9 vs 25.95±1.5 g of food per day after stimulation period; and 2.82±1.0 *vs* 3.85±0.9 g of body weight gain per day after stimulation period) (*Fig. 3*).

The most effective parameters of vagal stimulation on the rate of weight gain are: 3631, 7861 and 14523 A^2 x h/ m^2. The

Table 1. Body weights (g) during stimulation period depending on given parameters of the left vagus stimulation (VNS) in A^2 x h/m^2.

VNS (A^2xh/m^2)	VEMF (± SD)	EMF (± SD)	Significance level (P)
207	489.2 (±22.7)	502.7 (±25.6)	0.16
908	493.1 (±23.1)	510.1 (±31.2)	0.12
3631	502.3 (±25.9)	523.2 (±32.2)	0.06
7261	512.1 (±24.6)	533.1 (±35.8)	0.07
14523	522.1 (±25.6)	546.2 (±39.2)	0.05

VEMF - group with vagus stimulation and magnetic field exposure; EMF - group with magnetic field exposure.

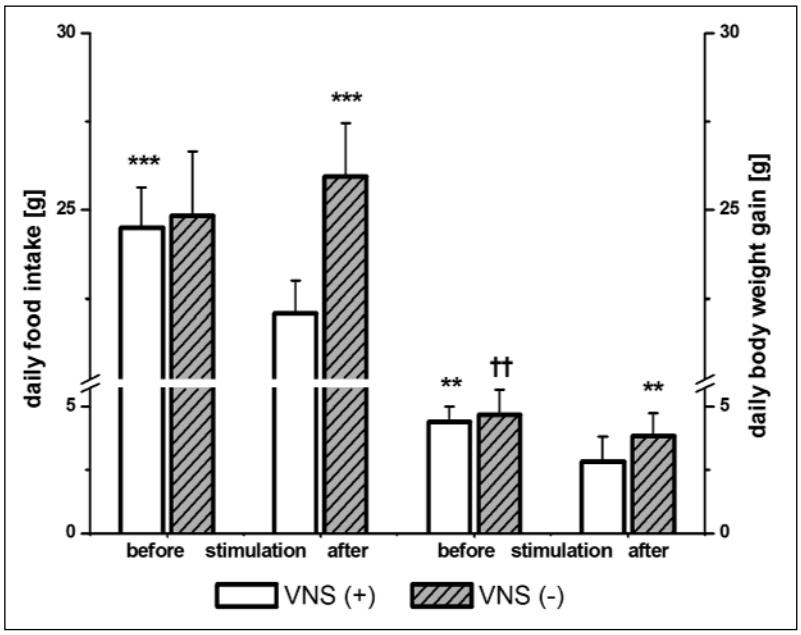

Fig. 3. Daily food intake (g) and daily body weight gain (g) before and after left vagus stimulation period. VNS (+) - group with VN stimulation (previous VEMF); VNS (-) - group without VN stimulation (previous EMF and CON). **P<0.01; ***P<0.001 *vs* VNS (+) after stimulation; ††P<0.01 *vs* VNS (-) after stimulation.

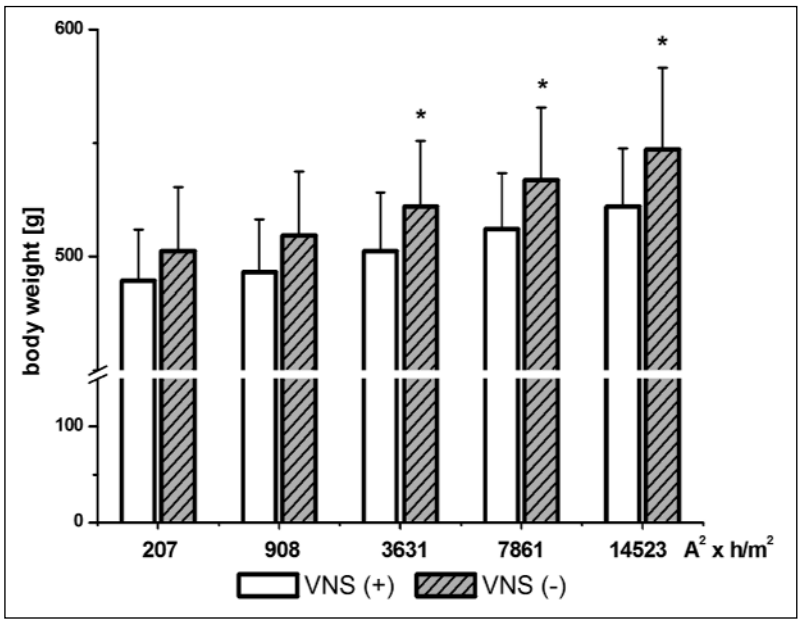

Fig. 4. Body weights (g) during stimulation period depending on given parameters of the left vagus stimulation in A^2 xh/m^2. VNS (+) - group with VN stimulation (previous VEMF); VNS (-) - group without VN stimulation (previous EMF and CON). *P<0.05 *vs* VNS (+).

frequency of those parameters ranged from 0.1 - 1.0 Hz, but the amplitude remained the same (200 mV - the highest one used in our experiment) (*Fig. 4*).

Biochemical analysis

1.Pancreatic polypeptide (PP)

The group with VN stimulation had markedly increased pancreatic polypeptide concentrations in blood than the rats from the control groups inside or outside the magnetic field. The

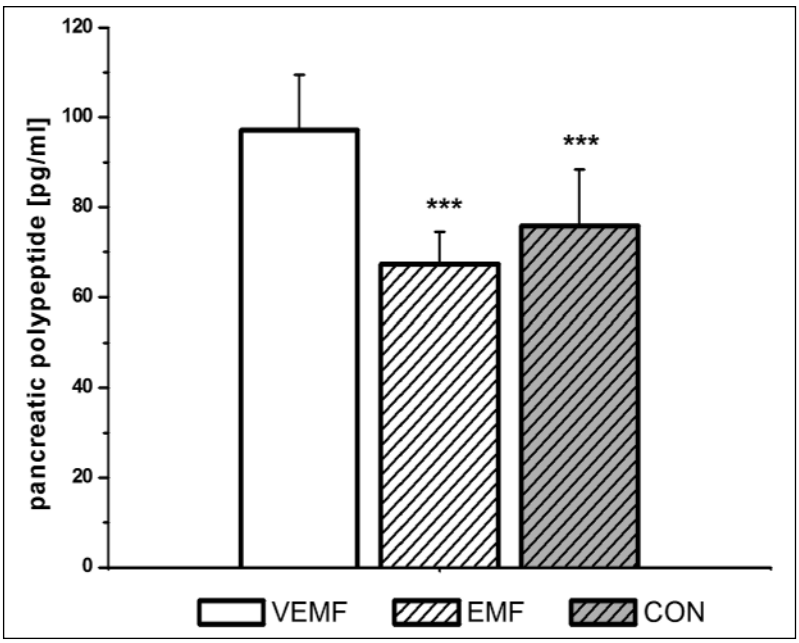

Fig. 5. Serum pancreatic polypeptide [pg/ml]. VEMF - group with vagus stimulation and magnetic field exposure; EMF - group with magnetic field exposure; CON - group without vagus stimulation or magnetic field influence. ***P<0.001 *vs* VEMF.

Table 2. Serum leptin concentrations [pg/ml].

	VEMF	EMF	CON
Leptin	2708,25*** (±1720,5)	3788** (±1175,1)	8324 (±541,6)

VEMF - group with vagus stimulation and magnetic field exposure; EMF - group with magnetic field exposure; CON - group without vagus stimulation or magnetic field influence. Data are given in ± SD. **P<0.01; ***P<0.001 *vs* CON.

serum PP levels between the EMF and CON groups did not differ (*Fig. 5*).

2. Leptin and insulin

Leptin serum concentrations were significantly lower in the VEMF and EMF groups when compared to the CON group and did not differ in the VEMF and EMF groups (*Table 2*). No differences in serum insulin levels were detected among the groups.

DISCUSSION

Attempts of using VN stimulation in feeding behavior disorders, like obesity, are based on the evidence that prove vagus influence in appetite control. The VN consists of mainly afferent fibres (80%) and transmits signals from the gastrointestinal tract to the brain. Amongst all the signals in the brain-gut axis, stomach distension is the most commanding indication of satiety. The mechanism of eating cessation is by negative feedback: activation of the gastric mechanoreceptors by stomach distension is transmitting *via* afferent fibres of vagus and lower ganglion to NTS, where the afferent neurons of the VN are located. NTS has connections to different places in the brain and of special interest are to those regulating feeding behavior. The brain response to stomach distension is then peripherally transmitted *via* vagal efferent fibres, which function in evoking eating cessation (7). Clinically, a similar effect is achieved after a bariatric operation in which a decrease in stomach size results in a decrease in food intake and an increase in vagal activity (8). Animals with total vagotomy or during sham-feeding, ate larger amounts of food which was suggested to be due to the lack of signals for meal termination associated with stomach distension and increased vagal nerve activity (9).

Traditional battery-driven microprocessors can provide only one particular parameter of stimulation during a single experiment, therefore a pulsating electromagnetic field (PEMF), as an external source of current in the solenoid, was connected to the left vagus. By changing the PEMF parameters, different parameters of vagus stimulation could be examined during the same study. Parameters of vagal stimulation used in this study were matched experimentally basing on this lab's previous experiments (5, 9, 10).

Decreased food intake and weight gain in the animals with vagal nerve stimulation is thought to be the result of stimulation on brain centres, peripheral action of vagal stimulation *via* short cholinergic reflexes, and the combination of central and peripheral signals. It was assumed that the VN stimulation decreased food intake and weight gain in the rats with the solenoid's electrodes on the vagus nerve by mimicking the signal transmitted from a distended stomach to the brain during physiological conditions. Stomach distension therefore, activates mechanoreceptors of the gastric wall and increases VN activity which leads to the stimulation of the hypothalamic neurons and thus the achievement of satiety. Vagal afferents transmit information to the brain not only from activated mechanoreceptors of the gastrointestinal tract but from duodenal chemoreceptors, hepatic glucoreceptors and from osmoreceptors (11). Therefore, in contrast to bariatric operations associated with stomach size reduction or gastric electrical stimulation that are aimed to evoke artificial anti-peristaltic waves and gastric mechanoreceptors activation, VN stimulation modifies signals from different sources that lead to satiety.

The reduced food intake and subsequent decrease in body weight during left VN stimulation observed in this study, may be partially due to the result of activation of short local cholinergic reflexes or by hormonal responses that are evoked by VN stimulation. The VN is the main communication between gastrointestinal tract and the brain. Receptors for most "satiety hormones" released from the gastrointestinal tract, especially CCK,

leptin and insulin, are present on vagal afferent nerve endings (12) therefore, VN stimulation influences their plasma concentrations, resulting in a decrease in food intake and body weight.

Comparable with the observations of Krizowa (13) and Boon (14), this study demonstrates marked influences of left vagal stimulation due to food intake and to a smaller extent on weight gain. The lack of paralleled changes in the rate of eaten food and weight gain, suggests that the VN is responsible mainly for short-term appetite control and other hormonal compensatory mechanisms are involved in the prevention of weight loss.

The rats with vagal stimulation in this experiment showed significant higher serum PP concentrations when compared to the control groups. Due to the fact that peripheral PP concentration is proportional to vagal nerve tension, it can be assumed that the stimulation of the vagal nerve in VEMF group was effective and its observed changes in feeding behavior were not associated with its unintentional damage during the current application. PP is an indicator of long-term energetic storage where low PP levels can be observed in obese people and high PP concentrations are present in persons with anorexia (15). Higher PP concentrations in this experiment were noticed in rats that consumed the lowest amount of food and had the slowest rate of weight gain. These changes in energy homeostasis may be the result of the effective stimulation of vagal afferents transmitting satiety signals and/or direct PP action released in higher amounts during VN stimulation. Anorectic effects of PP can be the consequence of the activation of central sites in the brain and/or delayed gastric emptying. Therefore these similar processes cannot be excluded in this study's rats with VN stimulation. The central satiating action of PP is thought to be associated with the activation of the central receptor Y4 or the inhibition action of PP on the hypothalamic NPY and orexin expressions, which result in higher oxygen consumption and increased metabolic rate (16). The satiety effects of PP in this study may be also evoked by its delaying action on gastric emptying. Previous data has shown that gastric emptying in obese individuals is accelerated (17) and the administration of PP to obese rats, delays gastric emptying and achieves a faster sensation of satiety. Hepatic branch vagotomy abolished the anorectic action of PP in mice underlining the role of vagus nerve in mediation of PP effect on gastric motility (16). In some studies, however, the anorectic action of PP was not so evident. The reduction of food intake was only moderate or not observed after high doses of PP (18).

The rats with vagal stimulation had decreased serum leptin concentrations, decreased food intake and decreased body weight gain when compared to the CON group. Leptin is involved in the regulation of: feeding behavior, body temperature, energy expenditure and blood pressure. Normally leptin decreases food intake; however it is generally observed that obesity is associated with leptin resistance which is progressively established during hypercaloric diet and accompanied by a progressive increase of serum leptin levels. In that case leptin expression and release correlate with the amount of body fat and number of adipocytes (19). This relationship of leptin correlates to this study's VEMF group that had decreased body weight gain and subsequent decreased leptin levels. The mechanisms underlying leptin resistance may include a failure of circulating leptin to reach its target cells in the brain, a blockage of leptin signaling or recently postulated an altered development of neuronal circuitry involved in food intake regulation programmed during foetal and neonatal life (20).

Leptin interplays with many peptides to control feeding behavior in humans and animals. Leptin stimulates GLP-1 release from L cells and GLP-1 receptor was found in vagal afferent terminals in the gut. Intact vagal transmission is necessary for the effect of both GLP-1 and leptin on food intake thus suggesting that the interaction between these two hormones may take place at the level of vagal sensory inputs. Theses effects cannot be excluded also in our experiment and electrical vagus stimulation could additionally enhance the transmission of satiety signals . It was shown that leptin and GLP-1 injected intraperitoneally act additively to reduce food consumption in the rat and the blockade of GLP-1 receptor prevented leptin effects on appetite (21). However, synergistic effects of exendin-4 (an analog of GLP-1) and leptin to suppress food intake was observed only with low dose but not high dose of leptin (22), explaining, at least in part, mechanisms responsible for leptin resistance in subjects with hyperleptinaemia.

Some studies have shown that modulation of vagal nerve activity stimulates insulin release. VN stimulation, however, did not change serum insulin concentration when compared to the control groups in this study. This discrepancy can be explained by the different parameters of vagal stimulation used in this experiment and the fact that insulin release can differ during chronic VN stimulation applied in our studyand during acute vagal modulation (23-25). Additionally, the rats in this experiment were obese and it was shown (26) that neural release of insulin is already impaired in the early life in obese Wistar rats.

Insulin is one of hormonal factors involved in the modulation of plasma leptin concentrations. Starvation decreases both plasma insulin and leptin concentration whereas obesity is strongly associated with hyperinsulinaemia and hyperleptinaemia. The effects of insulin on leptin secretion are contradictory: some groups reported that insulin increases plasma leptin levels, whereas others found that insulin does not appear to acutely regulate leptin expression or secretion (27). No differences in serum insulin levels among the groups and no correlation between serum insulin and leptin concentrations were found in our study indicating lack of insulin action on leptin secretion.

The magnetic field is useful for treatment of several disorders such as: headache, chronic fatigue syndrome, blood pressure abnormalities, sleep disorder, cardio-vascular disorders, stroke, Parkinson disease *etc*. (28, 29). Importance of magnetic field in feeding regulation is still unknown. This study shows that it can influence food intake, weight gain and subsequently serum leptin levels. Despite the fact that food intake in the EMF group was the same in the pre and post-stimulation periods, the lack of paralleled increased food intake in EMF and CON groups during PEMF exposure, demonstrates the appetite decreasing effect of the magnetic field. Additionally, the decreased daily body weight gain in the EMF group when compared to the CON group could be noticed during the MFE period, but not as significant as in the VEMF group. The lack of data focusing on influence of magnetic field on energy homeostasis makes it difficult to compare the ending results. Marino *et al*. (30) and Hilton *et al*. (31) have shown that the magnetic field effects body weight depended on the time of MFE. The outcomes of this experiment relate partially to these results; however, the parameters applied in this experiment were dissimilar from those used in other studies. Therefore it can be suggested that the parameters of the magnetic field stimulation used in this experiment had an inhibiting effect on feeding behavior and weight gain.

CONCLUSION

Although there are several surgical methods in the treatment of morbid obesity, operative management is not an attractive alternative for patients. VN stimulation, based on the findings in this study's results, seems to be a secure and better-tolerated method than surgical procedures as well as a less invasive "gate" to the hypothalamus. Gastric stimulation is not as precise as VN stimulation and therefore needs higher parameters of stimulation

resulting in greater amounts of side effects. Additionally, stimulation of the whole stomach may change its motility leading to adverse reactions such as: nausea, vomiting, intestinal malabsorption or "dumping syndrome." Obesity results from energetic imbalance, hence, our efforts to finding appropriate treatment should be concentrated on the modulation of central nervous system action.

Conflict of interests: None declared.

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