Effect of Chronic Vagus Nerve Stimulation on Learning and Memory in Obese-Insulin Resistant Rats

ผลของการกระตุ้นเส้นประสาทเวกัสแบบเรื้อรังต่อการเรียนรู้และความจำในหนูอ้วนที่มีภาวะดื้อต่อฮอร์โมนอินซูลิน

Titikorn Chunchai (ฐิติกร จันทร์ไชย)* Bencharunan Samniang (เบญจารุนันท์ สำเนียง)**
Dr.Jirapas Sripetchwandee (ดร.จิรภาส ศรีเพชรวรรณดี)*** Dr.Nipon Chattipakorn (ดร.นิพนธ์ ฉัตรทิพากร)****
Dr.Siriporn Chattipakorn (ดร.สิริพร ฉัตรทิพากร)*****

ABSTRACT

Long-term high-fat diet (HFD) is one of the major causes of obese-insulin resistant condition. Our previous studies found that HFD consumption caused not only peripheral insulin resistance, but also the cognitive decline. Recently, VNS therapy in patients with depression demonstrated the beneficial effects on the improvement of insulin sensitivity. However, the effect of chronic VNS therapy on cognitive function in obese-insulin resistant condition has never been investigated. The present study aimed to test the hypothesis that chronic VNS therapy provides the protective effects on metabolic parameters and cognition. After 12 weeks of HFD consumption, animals were randomly divided into 2 groups: sham and VNS group. Either sham or VNS was implanted and stimulated into animal of each group for 12 weeks. At the end of the experimental protocol, Morris Water Maze test (MWM) for cognitive function was determined. Blood sampling in each animal was collected for the investigations of plasma glucose, insulin, cholesterol levels and HOMA index. The results showed that VNS treatment in obese insulin-resistant rats improved insulin sensitivity and attenuated the cognitive impairment, compared with sham treatment ($p<0.05$). These findings suggest the beneficial effects of VNS on learning and memory of obese insulin-resistant rats.

บทคัดย่อ

การรับประทานอาหารที่มีไขมันสูงเป็นเวลานานเป็นสาเหตุหลักที่ชักนำไปสู่ภาวะอ้วนที่ดื้อต่อฮอร์โมนอินซูลิน โดยการศึกษาของเราก่อนหน้านี้พบว่าการรับประทานอาหารที่มีไขมันสูงเป็นเวลานานไม่เพียงแต่ชักนำไปสู่ภาวะดื้อต่อฮอร์โมนอินซูลินส่วนปลาย แต่ยังทำให้มีการเรียนรู้ที่แย่ลง ในปัจจุบันการรักษาด้วยการกระตุ้นเส้นประสาทเวกัสในผู้ป่วยที่มีภาวะซึมเศร้าแสดงให้เห็นถึงผลการปรับเปลี่ยนความไวต่อฮอร์โมนอินซูลินไปในทางที่ดีขึ้น อย่างไรก็ตามผลของการกระตุ้นเส้นประสาทเวกัสแบบเรื้อรังต่อการเรียนรู้และความจำในหนูอ้วนที่มีภาวะดื้อต่อฮอร์โมนอินซูลินยังไม่มีผู้ทำการศึกษาจึงเป็นที่มาของการศึกษาครั้งนี้ โดยมีวัตถุประสงค์เพื่อทดสอบสมมติฐานที่ว่าการกระตุ้นเส้นประสาทเวกัสแบบเรื้อรังจะให้ผลที่ดีต่อค่าเมตาบอลิก การเรียนรู้และความจำ โดยหลังจากหนูได้รับอาหารที่มี

* *Student, Master of Science Program in Physiology, Faculty of Medicine, Chiang Mai University*

** *Student, Master of Science in Physiology, Faculty of Medicine, Chiang Mai University*

*** *Lecturer, Department of Physiology, Faculty of Medicine, Chiang Mai University*

**** *Professor, Cardiac Electrophysiology Research and Training Center, Faculty of Medicine, Chiang Mai University*

***** *Associate Professor, Department of Oral Biology and Diagnostic Sciences, Faculty of Dentistry, Chiang Mai University*

ไขมันสูงเป็นเวลา 12 สัปดาห์ จะถูกสุ่มแบ่งออกเป็น 2 กลุ่มได้แก่ กลุ่มที่ไม่ได้รับ และกลุ่มที่ได้รับการกระตุ้นเส้นประสาทเวกัสเป็นเวลา 12 สัปดาห์ เมื่อสิ้นสุดการทดลองหนูจะถูกวัดการเรียนรู้และความจำด้วยการทดสอบมอริสวอเทอร์เมซ และถูกเก็บตัวอย่างเลือดเพื่อนำไปตรวจวัดระดับ กลูโคส อินซูลิน คลอเรสเตอรอล และค่าดัชนีโฮมา ผลการศึกษาพบว่าการกระตุ้นเส้นประสาทเวกัสแบบเรื้อรังปรับเปลี่ยนความไวต่อฮอร์โมนอินซูลินไปในทางที่ดีขึ้น ลดความรุนแรงของการเรียนรู้และความจำที่ลดลงในหนูอ้วนที่มีภาวะดื้อต่อฮอร์โมนอินซูลินเมื่อเปรียบเทียบกับกลุ่มที่ไม่ได้รับการรักษา ดังนั้นผลจากการศึกษานี้จึงแสดงถึงคุณประโยชน์ของการกระตุ้นเส้นประสาทเวกัสแบบเรื้อรังต่อการเรียนรู้และความจำของหนูอ้วนที่มีภาวะดื้อต่อฮอร์โมนอินซูลิน

Key Words: Chronic vagus nerve stimulation, Obese-insulin resistant, Cognition

คำสำคัญ: การกระตุ้นเส้นประสาทเวกัสแบบเรื้อรัง ภาวะอ้วนที่ดื้อต่อฮอร์โมนอินซูลิน การเรียนรู้และความจำ

Introduction

Obesity has reached the epidemic proportions in many countries around the world. Several studies showed that obesity leads to the development of insulin resistance (Pintana et al., 2012; Pipatpiboon et al., 2013; Pratchayasakul et al., 2011). Long-term high-fat diet (HFD) consumption has been shown to be one of the major causes of obese-insulin resistant condition (Fung et al., 2001; Riccardi et al., 2004). Our previous studies demonstrated that obesity induced by 12-week HFD consumption increased brain oxidative stress, as indicated by increased brain mitochondrial ROS production (Pintana et al., 2012; Pipatpiboon et al., 2013). Moreover, obese-insulin resistant condition has been shown to cause cognitive impairment (Pintana et al., 2012; Pipatpiboon et al., 2013).

Vagus nerve stimulation (VNS) is currently used to treat refractory epilepsy and is being investigated as a potential therapy for a range of pathological conditions, including heart failure, tinnitus, obesity and Alzheimer's disease. Several studies demonstrated that VNS therapy generates weight loss and reduced body fat in pigs (Val-Laillet et al., 2010), dogs (Xi et al., 1993) and rodents (Bugajski et al., 2007; Krolczyk et al., 2001; Krolczyk et al., 2005; Laskiewicz et al., 2003). In addition, weight loss is the secondary outcome in patients, when VNS is used to treat refractory epilepsy (Burneo et al., 2002) or depression (Bodenlos et al., 2007; Pardo et al., 2007). The underlying mechanisms of VNS induced weight loss has been described that the stimulation of vagal afferents resulted in satiating effects via gut-brain feedback mechanism, then leading to lower food consumption and weight loss (Bray, 2000). Previous studies showed that vagal nerve activity in dorsal vagal complex plays an important role for the secretion of insulin and glucose homeostasis (Sobocki et al., 2006). However, the effects of VNS on peripheral insulin sensitivity in obese-insulin resistant condition have not been investigated.

It has been shown that obese-insulin resistant condition causes the deleterious effects on cognition, via increased pro-inflammatory cytokines (Clark et al., 1998), increased oxidative stress and brain mitochondrial dysfunction (Asato et al., 2012; Bray, 2014; Pintana et al., 2012). Previous studies demonstrated that VNS can suppress inflammation and decreased oxidative stress (Parada et al., 2010;

Shi et al., 2009). Moreover, acute and chronic VNS have shown to enhance early long term potential (LTP) and to modulate the synaptic plasticity in hippocampus of rats model (Biggio et al., 2009; Zuo et al., 2007). It has been shown that vagal stimulation during memory consolidation modulated retention for memory task via an increase in the exciting synaptic transmission, decreased synchronized action potential discharges of granule cells and increased progenitor cell in dentate gyrus (Clark et al., 1995; Revesz et al., 2008; Ura et al., 2013). Those findings suggest that VNS could enhance cognitive function.

However, the effects of VNS on cognitive function in obese-insulin resistant condition have not been determined.

Objective of the study

The aim of the present study was to examine the effect of chronic VNS therapy on insulin sensitivity and cognitive function in obese-insulin resistant rats.

Methodology

Animal Preparation

All experiments were conducted in accordance with an approved protocol from the Faculty of Medicine, Chiang Mai University Institutional Animal Care and Use Committee, in compliance with NIH guidelines. Male Wistar rats (body weight 180-200 g, n=10) from the National animal center, Salaya campus, Mahidol University, Bangkok were use in the present study. All rats were fed with high-fat diet (HFD: 59.28% E fat) for 12 weeks. Blood sample was collected in each animal before further dividing into two groups.

After 12 weeks of HFD, animals were randomly divided into 2 groups: sham and VNS groups.

Experimental protocol (as shown in Figure 1)

After 12 weeks of either sham or VNS treatment, each animal was tested for the cognitive function by using Morris Water Maze test. Then, blood sample of each animal was collected for the metabolic analysis, including plasma insulin, glucose and cholesterol levels.

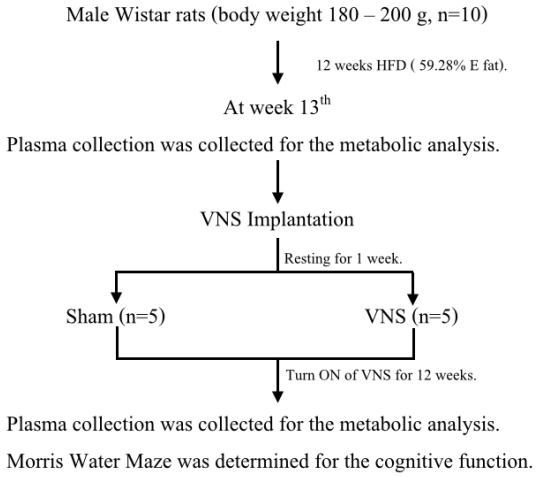

Figure 1 Diagram illustrates the experimental protocol of the present study.

High fat diet preparation

The high-fat diet (59.28% E fat) was prepared by mixing of the following ingredients; standard rat diet (365 g/kg food), casein (250 g/ kg food), lard (310 g/kg food), cholesterol (10 g/kg food), vitamins (60 g/kg food), DL-Methionine (3 g/kg food), yeast powder (1 g/kg food) and sodium chloride (1 g/kg food). The mixture was molded into a spherical shape and then refrigerated until utilization. (Pratchayasakul et al., 2011)

Vagus nerve stimulation

In either sham or VNS group, rats were anesthetized with xylazine (3 mg/kg) and zoletil (50 mg/kg). After hair shaving and skin cleaning, a bipolar cuff electrode was implanted around the left cervical vagus nerve and connected to a custom implantable pulse generator (Demipulse, Model 103, Cyberonics, Boston Scientific Corporation, St. Paul, MN, USA). After surgical implantation, a period of one week was allowed for recovery. VNS was continuously delivered at a frequency (20 Hz), pulse width (50 0 μs), current 0.5 - 0.75 mA, turn ON 14 s and turn OFF 48 s. Prior to data collection, VNS therapy was turned off for 5–10 min and remained off for the duration of Morris Water Maze (MWM) test. In the sham group, similar surgical procedure was done except that the programmed VNS was turn off.

Determination of plasma insulin level

Plasma insulin level was detected by sandwich ELISA kit (Millipore, MI, and USA). A microplate reader was used for measurement the intensity of enzyme activity at 450 nm. The intensity of absorbance is directly proportion to the amount of captured insulin in each sample. The insulin level was interpreted by calculation from the standard curve that was generated from the known-concentration standards (Pratchayasakul et al., 2011).

Determination of plasma glucose level

After 5-hour fasting, blood samples were collected from tail vein into NaF-coated tube. Blood was centrifuged at 6000 rpm for 10 minutes and plasma was collected. Plasma glucose was determined by colorimetric assay from a commercially available kit (Biotech, Bangkok, Thailand). The color intensity of each sample underwent enzyme reaction was measured, at 505 nm by spectrophotometry (BioTek, Winooski, VT, USA), using Trinder indicator reaction. A standard curve was generated and glucose level of each sample was interpreted by interpolation its absorbance to the standard curve (Pipatpiboon et al., 2012).

Determination of plasma cholesterol level

Plasma cholesterol level was determined by enzymatic colorimetric essay using a commercial kit (Biotech, Bangkok, Thailand). The color intensity of each sample was measured, at 505 nm by spectrophotometer (BioTek, Winooski, VT, USA). A standard curve of cholesterol was generated and concentration level of each sample was interpreted by interpolation its absorbance to the standard curve (Pipatpiboon et al., 2012).

Determination of Cognitive function via the Morris water maze (MWM) test

The MWM test was modified from the methods of Vorhees & Williams (2006) and used for the purpose of cognitive or learning and memory behaviors assessment. MWM set up with the round water pool was assigned with 4 cardinal points and separated into 4 quadrants. Animals were different 4 starting points per day. The round platform was located in the middle one of four quadrants. Each test included two different assessments: the acquisition test (existent platform) and the probe test (non-existent platform). The acquisition test was performed on five consecutive days of training with four trials per day. Animals were given 120 s to locate the hidden platform. After the platform was found, the animal was allowed to remain on the platform for 15 s

before the next test began by placing the animal at a starting point within the other three remaining quadrants. The acquisition time began at the moment the animal entered the water and ended at the moment the animal reached the submerged platform. In the probe test, animals were tested on the 6th day of training with only one starting point. The probe time was the amount of time the animals spent in the target quadrant during the 90-s testing period. The data analysis of MWM was done manually from video tape recording with the investigator blinded to the groups of rats (Pintana et al., 2012).

Statistical analysis

The data for each experiment were presented as mean ± SEM. For all comparisons, the significance of the difference between the means was calculated by one-way ANOVA followed by post-hoc LSD. MWM was performed using two-way ANOVA followed by post hoc LSD. $P<0.05$ was considered as significance.

Results

Vagus nerve stimulation (VNS) improved insulin sensitivity in obese- insulin resistant rats

At baseline before HFD feeding, the metabolic parameters were not different between animals for sham and VNS group. After 12 weeks o HFD consumption, all rats had increased the bod weight, plasma insulin and plasma total cholestero levels, indicating the obese-insulin resistant conditio occurring in those rats following 12-week HFI consumption. After 12-week VNS therapy in HFD fed rats, insulin sensitivity of animals in VNS group significantly increased compared with that in sham group, as indicated by decreased HOMA index (as shown in Table 1). These findings suggest that VNS therapy improved insulin sensitivity in obese-insulin resistant rats.

Table 1 Metabolic parameters of obese insulin-resistant rats treated with sham and VNS group (n=5 in each group).

Metabolic parameters	12 weeks	12 weeks post-treatment	
	High fat diet	Sham	VNS
Body weight (g)	540 ± 21.6	562 ± 41.5*	550 ± 4.1*
Plasma glucose (mg/dl)	120 ± 7.9	136 ± 7.1*	137 ± 7.7*
Plasma insulin (ng/ml)	5.33 ± 1.2	4.57 ± 1.6	3.57 ± 0.7
HOMA index	27.36 ± 4.4	27.14 ± 8.5	21.05 ± 3.4*
Plasma total cholesterol (mg/dl)	84.06 ± 4.9	79.97 ± 1.5	81.16 ± 2.5

* $P<0.05$ significantly different from 12 weeks HFD.

VNS improved cognitive function in obese-insulin resistant rats measured by the MWM test

To assess cognitive function, we measured time to reach the platform in acquisition test and time spent in the target quadrant in probe test.

After 12 weeks of VNS therapy, time to reach the platform during the acquisition test in VNS group was significantly less than that in sham group (Figure. 2A). In addition, time spent in the target quadrant of the probe test in VNS group was significantly greater than that in sham group (Figure. 2B). These findings suggest that VNS attenuated cognitive impairment in obese insulin-resistant rats.

Discussion and Conclusions

Major findings from the present study demonstrated that chronic VNS treatment 1) improved insulin sensitivity in obese-insulin resistant rats and 2) attenuated the cognitive impairment in obese-insulin resistant rats, The present study demonstrated that 12-week HFD consumption caused the development of obese-insulin resistance as indicated by increased plasma insulin and plasma total cholesterol levels. These findings are consistent with our previous studies (Pintana et al., 2012; Pipatpiboon et al., 2013; Pratchayasakul et al., 2011).

Our study has shown that 12 weeks of VNS treatment in obese-insulin resistant rats improved insulin sensitivity, by reducing HOMA index. Although several studies reported that VNS therapy generate weight reduction (Bugajski et al., 2007; Val-Laillet et al., 2010), previous study demonstrated no weight loss following VNS therapy (Bodenlos et al., 2014). In the present study, VNS therapy in obese-insulin resistant rats did not change in body weight parameter. The reason behind no weight loss in the present study could be that time course of VNS therapy might not be long enough. Although weight loss was not observed, the improvement of insulin sensitivity has been shown following VNS therapy in our study. It has been shown that VNS inhibited the synthesis of TNF-α and decreased inflammatory responses via nicotinic receptor activation leading to the inhibition of inflammatory cytokines release (Jiang et al., 2014; Parada et al., 2010; Shi et al., 2009). Decreased inflammation demonstrated increased insulin sensitivity in animal model and clinic (Gower and Goss, 2015; Hayashi et al., 2014; Jiang et al., 2014).

The underlying mechanism of cognitive impairment following long term HFD consumption may be that obesity after HFD leads to the development of insulin resistance, increased brain oxidative stress, impaired brain insulin signaling, as well as increased Aβ accumulation, resulting cognitive impairment as demonstrated in previous

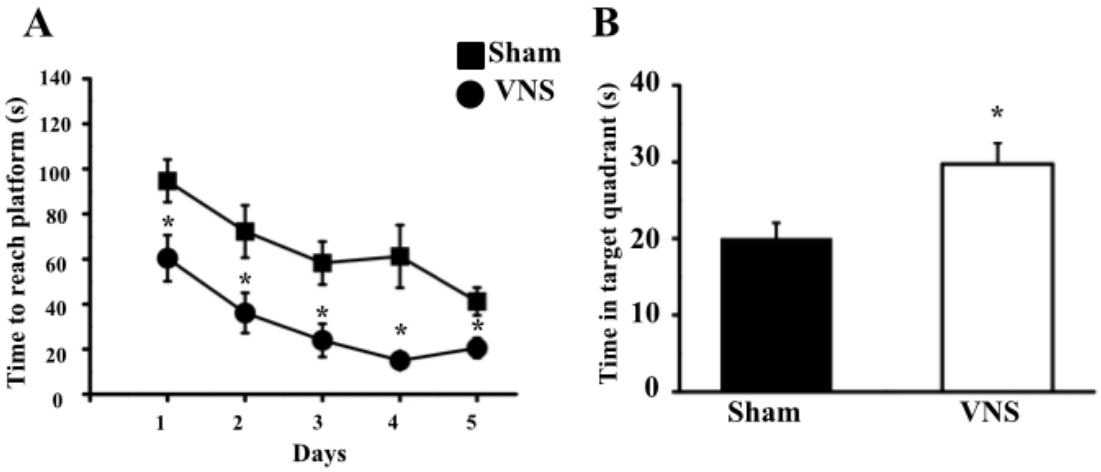

Figure 2 The effect of VNS on learning and memory in HFD-fed rats measured by Morris Water Maze test. (A) VNS improved learning and memory behaviors, indicated by decreased time required to reach the platform in the acquisition test, *p<0.05 in comparison with the sham group, and increased time spent in the target quadrant in the probe test (B). *p<0.05 in comparison with the sham group. (n=5 in each group)

studies (Pintana et al., 2012; Pipatpiboon et al., 2013; Pratchayasakul et al., 2011). The present study showed that VNS therapy in obese-insulin resistant rats improved cognitive impairment. The possible explanation is that VNS increased insulin sensitivity as well as possibly decreased brain oxidative stress. Previous studies demonstrated that VNS therapy decreased oxidative stress (Parada et al., 2010; Shi et al., 2009). In conclusion, the present study demonstrated that the effects of chronic VNS attenuated peripheral insulin resistance and preserved learning and memory behavior in obese insulin-resistant rats.

Acknowledgements

This work was supported by Thailand Research Fund grants: TRF-BRG 5780016 (SC), Chiang Mai University Excellent Center Award (NC), and a Research Chair Grant from the National Science and Technology Development Agency (NSTDA) (NC).

References

Asato, M., Ikeda, H., Kamei, J. Effects of diabetes and obesity on the higher brain functions in rodents. Nihon Shinkei Seishin Yakurigaku Zasshi 2012, 32(5-6): 251-5.

Biggio, F., et al. Chronic vagus nerve stimulation induces neuronal plasticity in the rat hippocampus. Int J Neuropsychopharmacol 2009, 12(9): 1209-21.

Bodenlos, J.S., et al. Vagus nerve stimulation and emotional responses to food among depressed patients. J Diabetes Sci Technol 2007, 1(5): 771-9.

Bodenlos, J.S., et al. Vagus Nerve Stimulation and Food Intake: Effect of Body Mass Index. J Diabetes Sci Technol 2014, 8(3): 590-595.

Bray, G.A. Afferent signals regulating food intake. Proc Nutr Soc 2000; 59(3): 373-84.

Bray, N. Neuroimmunology: Obesity inflames memory circuits. Nat Rev Neurosci 2014; 15(4): 204-5.

Bugajski, A.J., et al. Effect of long-term vagal stimulation on food intake and body weight during diet induced obesity in rats. J Physiol Pharmacol 2007; 58 Suppl 1: 5-12.

Burneo, J.G., et al. Weight loss associated with vagus nerve stimulation. Neurology 2002; 59(3): 463-4.

Clark, K.B., et al. Post-training unilateral vagal stimulation enhances retention performance in the rat. Neurobiol Learn Mem 1995; 63(3): 213-6.

Clark, K.B., et al. Posttraining electrical stimulation of vagal afferents with concomitant vagal efferent inactivation enhances memory storage processes in the rat. Neurobiol Learn Mem 1998; 70(3): 364-73.

Fung, T.T., et al. Association between dietary patterns and plasma biomarkers of obesity and cardiovascular disease risk. Am J Clin Nutr 2001; 73(1): 61-7.

Gower, B.A., Goss, A.M. A lower-carbohydrate, higher-fat diet reduces abdominal and intermuscular fat and increases insulin sensitivity in adults at risk of type 2 diabetes. J Nutr 2015; 145(1): 177S-83S.

Hayashi, M., et al. Angiotensin II Receptor Blocker Ameliorates Stress-Induced Adipose Tissue Inflammation and Insulin Resistance. PLoS One 2014; 9(12): e116163.

Jiang, Y., et al. Vagus nerve stimulation attenuates cerebral ischemia and reperfusion injury via endogenous cholinergic pathway in rat. PLoS One 2014; 9(7): e102342.

Krolczyk, G., et al. Effects of continuous microchip (MC) vagal neuromodulation on gastrointestinal function in rats. J Physiol Pharmacol 2001; 52(4 Pt 1): 705-15.

Krolczyk, G., et al. The effects of baclofen on the feeding behaviour and body weight of vagally stimulated rats. J Physiol Pharmacol 2005; 56(1): 121-31.

Laskiewicz, J., et al. Effects of vagal neuromodulation and vagotomy on control of food intake and body weight in rats. J Physiol Pharmacol 2003; 54(4): 603-10.

Parada, E., et al. Poststress treatment with PNU282987 can rescue SH-SY5Y cells undergoing apoptosis via alpha7 nicotinic receptors linked to a Jak2/Akt/HO-1 signaling pathway. Free Radic Biol Med 2010; 49(11): 1815-21.

Pardo, J.V., et al. Weight loss during chronic, cervical vagus nerve stimulation in depressed patients with obesity: an observation. Int J Obes (Lond) 2007; 31(11): 1756-9.

Pintana, H., et al. Effects of metformin on learning and memory behaviors and brain mitochondrial functions in high fat diet induced insulin resistant rats. Life Sci 2012; 91(11-12): 409-14.

Pipatpiboon, N., et al. PPARgamma agonist improves neuronal insulin receptor function in hippocampus and brain mitochondria function in rats with insulin resistance induced by long term high-fat diets. Endocrinology 2012; 153(1): 329-38.

Pipatpiboon, N., et al. DPP4-inhibitor improves neuronal insulin receptor function, brain mitochondrial function and cognitive function in rats with insulin resistance induced by high-fat diet consumption. Eur J Neurosci 2013; 57(5): 839-49.

Pratchayasakul, W., et al., 2011. Effects of high-fat diet on insulin receptor function in rat hippocampus and the level of neuronal corticosterone. Life Sci 2011; 88(13-14): 619-27.

Revesz, D., et al. Effects of vagus nerve stimulation on rat hippocampal progenitor proliferation. Exp Neurol 2008; 214(2): 259-65.

Riccardi, G., Giacco, R., Rivellese, A.A. Dietary fat, insulin sensitivity and the metabolic syndrome. Clin Nutr 2004; 23(4): 447-56.

Shi, F.D., et al., 2009. Nicotinic attenuation of central nervous system inflammation and autoimmunity. J Immunol 2009; 182(3): 1730-9.

Sobocki, J., et al., 2006. Does vagal nerve stimulation affect body composition and metabolism? Experimental study of a new potential technique in bariatric surgery. Surgery 2006; 139(2): 209-16.

Ura, H., et al., 2013. Vagus nerve stimulation induced long-lasting enhancement of synaptic transmission and decreased granule cell discharge in the hippocampal dentate gyrus of urethane-anesthetized rats. Brain Res 2013; 1492: 63-71.

Val-Laillet, D., et al., 2010. Chronic vagus nerve stimulation decreased weight gain, food consumption and sweet craving in adult obese minipigs. Appetite 2010; 55(2): 245-52.

Xi, L., et al., 1993. Effects of memory from vagal feedback on short-term potentiation of ventilation in conscious dogs. J Physiol 1993; 462: 547-61.

Zuo, Y., Smith, D.C., Jensen, R.A., 2007. Vagus nerve stimulation potentiates hippocampal LTP in freely-moving rats. Physiol Behav 2007; 90(4): 583-9.