The Effect of Transcutaneous Auricular Vagus Nerve Stimulation on Impaired Glucose Tolerance: A Pilot Randomized Study

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Abstract

Background
Impaired glucose tolerance (IGT) is a pre-diabetic state of hyperglycemia that is associated with insulin resistance and increased risk of type II diabetes and cardiovascular pathology. Recently, investigators hypothesized that decreased vagus nerve activity may be the underlying mechanism of metabolic syndrome including obesity, elevated glucose and blood pressure.

Objective
In this pilot randomized clinical trial, we compared the efficacy of transcutaneous auricular vagus nerve stimulation (tLVNS) and sham tLVNS on patients with IGT.

Methods
There were 72 participants with IGT were single-blinded, randomized allocated by computer generated envelope to tLVNS and sham tLVNS stimulation treatment groups. In addition, 30 IGT adults were also recruited without any treatment, to monitoring the natural fluctuation of glucose tolerance in IGT patients. There were no differences in weight, systolic blood pressure, diastolic blood pressure, fasting plasma glucose, 2-hour plasma glucose, and glycosylated hemoglobin and BMI between every two groups before the trial.

Results
100 participants were analysed. Mean change in each group was less, tLVNS (n=35) and sham tLVNS groups (n=35). Two female patients dropped out from the study due to stimulation-evoked dizziness. The symptoms were relieved after stopping the treatment. After 12 weeks intervention, significant difference in 2-hPG between tLVNS and sham tLVNS groups (F(2)=5.79, p=0.004). In addition, we also found that differed significantly in systolic blood pressure (F(1)=4.21, p=0.044), dropped from 123.69 ± 14.14 (mean ± SD) to 118.64 ±13.34. There were
significant differences in fasting plasma glucose ($F(2)=10.62, p<0.001$), 2-hour plasma glucose ($F(2)=25.18, p<0.001$) and glycosylated hemoglobin ($F(1)=12.79, p=0.001$) between TaVNS and no-treatment groups over the course of 12 weeks.

**Conclusions**

Our study suggests that as a simple, cost-effective therapeutic method with mild side-effects, taVNS may have the great potential in treatment of IGT / pre-diabetes.

**Trial Registration**

Chinese Cochrane Centre, International Clinical Epidemiology Network Resource and Training Center (ChiCTR-TRC-12002522).

**Funding**

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**Key words**

Impaired glucose tolerance, transcutaneous vagus nerve stimulation, pre-diabetes, metabolic syndrome, glycemic control.
Introduction

Pre-diabetes, defined as impaired glucose tolerance (IGT), or impaired fasting glucose (IFG) or both, represents an intermediate state between the normal glucose homeostasis and diabetes(1). Individuals with pre-diabetes often progress to overt diabetes within a few years(1). Studies suggest that the prevalence rate of pre-diabetes is about 35%, or approximately 79 million people, which is more than three times the number of diabetes patients (2; 3). 5–10% of individuals with pre-diabetes will progress to diabetes per year (1). It is also believed that individuals with pre-diabetes have the same vascular risk factors as patients with type 2 diabetes (1). Thus, pre-diabetes is also a risk factor for metabolic syndrome (4), stroke (2), and cardiovascular disorders(5).

Impaired glucose tolerance (IGT) is a pre-diabetic state of hyperglycemia that is associated with insulin resistance and increased risk of cardiovascular pathology. In patients with IGT, the main site of insulin resistance is muscle tissue; this is in contrast with another state of hyperglycemia, impaired fasting glucose (IFG), in which the site of insulin resistance is the liver (1). IGT affects about 11% of people aged 20-74 years in the United States (6) and 17% of those aged 40-65 years in Britain(7). The American Diabetes Prevention Program (DPP) showed that approximately 10% of individuals with IGT developed diabetes on an annual basis (8). In another study, investigators found that 30% of patients with IGT developed diabetes over a period of 8 years (9). In a 10 year follow up study, the results showed that 15% of people with IGT developed non-insulin dependent diabetes and 22% remained glucose intolerant(10). Although patients with transient IGT can revert to normal, they remain at increased long term risk of developing non-insulin dependent diabetes. By the time they develop diabetes, 50% will already have established
complications, 16% coronary artery disease, and 30% retinopathy(11).

Current opinion suggests that pre-diabetes should be treated either with lifestyle interventions, which target on obesity and physical inactivity, and/or with pharmacological intervention based on anti-diabetic drugs, in order to prevent progression to diabetes(1). For both interventions, compliance can be a serious problem, and the long term effects of pharmacological interventions remain to be tested (12).

In recent years, investigators have proposed the hypothesis that adequate vagus nerve activity can reduce the risk of metabolic syndromes such as obesity, elevated glucose level and blood pressures (13). Thus, increasing the activity and firing of the vagus nerve by direct stimulation may prevent metabolic syndromes such as obesities, elevated glucose level and blood pressure (13). However, the involvement of surgery, the perioperative risks and potentially significant side effects associated with surgery have limited the application of classic vagus nerve stimulation methods on the patient population.

More recently, investigators started using transcutaneous auricular vagus nerve stimulation (taVNS) to replace the classic VNS method to treat disorders such as epilepsy (14) and depression (15). The rationale for using taVNS is that anatomical studies have shown that the ear is the only place on the surface of the human body where there is afferent vagus nerve distribution(16). Thus, direct stimulation of the afferent vagus nerve fibers on the ear may produce an effect similar to classic VNS without the burden of surgical intervention (17).

In this study, we performed a pilot randomized clinical trial to test the efficacy of taVNS on IGT patients.

**Methods and Materials**
This study was registered at the Chinese Cochrane Centre, International Clinical Epidemiology Network Resource and Training Center (ChiCTR-TRC-12002522). This study was approved by the Institutional Ethics Committee of the China Academy of Chinese Medical Sciences prior to study initiation. All clinical investigation procedures were conducted according to the principles expressed in the Declaration of Helsinki. All patients signed a patient consent form prior to joining the study.

**Study population**

IGT outpatients were recruited from the China Academy of Chinese Medical Sciences acupuncture hospital for a randomized clinical trial to test the efficacy of taVNS. Subject recruitment and all study procedures took place between November 2009 and December 2010. Based on the minimum sample size of clinical study, 72 patients with IGT were randomized (Randomization sequence was created using SAS System 7.0) to taVNS and sham taVNS stimulation treatment groups, given an anticipated dropout rate of 20%. The taVNS and sham taVNS were described as 1 and 2 respectively in envelope and identical in appearance. They were prepacked in envelope and consecutively numbered for each participant according to the randomisation schedule. Each participant was kept blinded and received corresponding intervention as described in the envelope.

In order to monitor the natural fluctuation of glucose tolerance in IGT patients, an additional 30 IGT patients, with the same inclusion criteria, were recruited from free clinics in the community through advertisements in the local newspaper and posters during the same period of time as the no-treatment group, to explore the variation of glucose of the patient under the normal life condition for 12 weeks without treatment.

All patients met the World Health Organization (WHO) standard diagnosis of IGT (18), i.e. a fasting blood sugar level of less than 7.0 mmol/L and a 2-hour plasma glucose level (2hPG) between 7.8 and 11.1 mmol/L at the
time of study enrollment.

Intervention and comparison

This study was designed as a single blind randomized controlled trial (RCT). After screening, 72 IGT participants were randomly assigned to either the transcutaneous auricular vagus nerve stimulation group (taVNS) or sham taVNS group. After randomization, all patients received training, under supervision at the hospital on how to use the Transcutaneous Electrical Nerve Stimulator device to apply stimulation. All subsequent treatments were self-administered by the patients at home. Patients were also instructed to fill in a patient dairy booklet every day to describe the side effects after each treatment. The investigators checked all booklets at the 6 and 12 week assessments.

To further explore the natural fluctuation of glucose tolerance in IGT patients, an additional 30 patients were recruited as no-treatment controls. For these patients, no treatments were applied.

taVNS treatment:
Location: The points for taVNS are located in the auricular concha area where there is rich vagus nerve branch distribution (Figure 1).

Intervention procedure: taVNS were applied using a Hutuo ear vagus nerve stimulator (TENS-200) developed by Suzhou manufacture of Medical Device and Material (see Figure 4). Stimulation parameters for both the taVNS and sham taVNS groups were 1mA of electrical current at a frequency of 20Hz with pulse duration≤1ms, administered twice daily. The intensity was adjusted based on the individual tolerance of the patients.
Each postprandial treatment lasted 20 minutes and was applied 0.5 hour after eating. After the training session, all subsequent treatments were applied independently at the home of the patients.

**Sham taVNS treatment**

**Location:** The stimulation points for sham taVNS are located at the superior scapha (the midpoint of the outer ear margin), where there is no vagus nerve distribution (*Figure 1*).

**Intervention procedure:** All procedures performed in the sham taVNS treatment group are identical to the procedures for the taVNS group except the location of the stimulation.

**No-treatment control:**

All patients in the no-treatment control group were recruited from community free clinics that provide free physical exams, including blood glucose testing. No taVNS or sham taVNS treatments were applied.

**Outcome measurements**

The primary outcome measure of this study is the 2-hour plasma glucose level changes measured at baseline, 6-weeks, and 12-weeks. Secondary outcome includes fasting plasma glucose level (measured at baseline, 6-weeks, and 12-weeks), glycosylated hemoglobin, BMI (Body Mass Index) and blood pressure (assessed at baseline and 12-weeks). All measures of blood glucose were measured by the Glucose Oxidase method (BaiAnyi, Bayer HealthCare LLC.).

**Statistical Analysis**
Our analyses were based on the intention-to-treat principle. Statistical analysis was performed using SPSS 19.0 Software (SPSS Inc., Chicago, IL, USA). Repeated measurements were applied to compare primary and secondary outcomes. First, we compared the taVNS and sham taVNS groups; then, we separately compared real and sham taVNS with no-treatment controls, to further assess the treatment effects of taVNS and sham taVNS.

**Results:**
A total of 102 participants completed the study (72 females, 54.4±7.4 years (mean ± SD), range: 33-68). Two female patients (one in the taVNS group and one in the sham taVNS group) dropped out from the study due to stimulation-evoked dizziness. The symptoms were relieved after stopping the treatment.

The Flow diagram shows the detail information on the recruited and excluded participants. (Figure 2)

**Comparison between the taVNS and sham taVNS**
Comparison by Independent Samples t-test showed that the two groups did not differ in age (t(70)=1.51, p=0.14), weight (t(70)=-0.83, p=0.41) systolic blood pressure (t(70)=1.42, p=0.16), diastolic blood pressure (t(70)=0.22, p=0.16), or BMI (t(70)=64.07, p=0.61) at baseline. The gender distribution also did not differ significantly across groups (χ² (2, n=72) =3.29, p=0.07).

Measures of fasting plasma glucose (t(70)=0.3, p=0.77), 2-hour plasma glucose (t(70)=1.96, p=0.054) and glycosylated hemoglobin (t(70)=1.12, p=0.27) also did not differ between groups at baseline.
Comparison of the taVNS and sham taVNS groups using repeated measures indicated a significant difference in 2-hPG between the groups over the course of the experiment ($F(2)=5.79$, $p=0.004$) (Figure 3 and Table 2). The decrease in 2-hour plasma glucose was significantly greater in the taVNS group compared to the sham taVNS group (Table 3). After adjusting for age, gender, and BMI, there was still a significant difference between the two groups (Table 2). Measures of FPG ($F_{GG}(1.84)=2.48$, $p=0.093$, corrected for Greenhouse Geisser) and HbA1c ($F(1)=0.23$, $p=0.63$) did not differ significantly between the taVNS and sham taVNS groups over time in both crude analysis and after adjusting for age, gender, and BMI (Table 2). For FPG, Mauchly’s Test of Sphericity indicated that assumptions of sphericity were violated, thus Greenhouse Geisser corrected degrees of freedom were used.

Further analysis of the other secondary outcomes indicated that the taVNS and sham taVNS groups differed significantly in systolic blood pressure over time ($F(1)=4.21$, $p=0.044$). In the taVNS group, systolic blood pressure dropped from $123.69 \pm 14.14$ (mean $\pm$ SD) to $118.64 \pm 13.34$, while in the sham taVNS group, systolic blood pressure remained at $119 \pm 12$. No significant difference was observed for changes in diastolic blood pressure ($F(1)=0.75$, $p=0.39$), and BMI ($F(1)=0.069$, $p=0.79$).

Comparison between taVNS, sham taVNS and no-treatment control

In this study, we added a separate no-treatment control group recruited from a free community clinic physical exam program to better understand
the natural fluctuation of outcomes in patients with IGT and to isolate the pure treatment effects from other factors.

Analysis of variance indicated that the three experimental groups at baseline did not differ in age ($F(2)=1.95$, $p=0.15$), weight ($F(2)=0.85$, $p=0.43$), diastolic blood pressure ($F(2)=1.05$, $p=0.37$), or gender distribution ($\chi^2(2, n=102)=3.29$, $p=0.2$), BMI ($F(2)=2.96$, $p=0.057$). Measures of fasting plasma glucose ($F(2)=2.86$, $p=0.06$), 2-hour plasma glucose ($F(2)=2.03$, $p=0.14$) and glycosylated hemoglobin ($F(2)=1$, $p=0.37$) also did not differ between groups at baseline. There was, however, a significant difference in systolic blood pressure ($F(1)=1.02$, $p=0.01$).

Repeated measures between the taVNS and no-treatment control indicated significant differences in fasting plasma glucose ($F(2)=10.62$, $p<0.001$), 2-hour plasma glucose ($F(2)=25.18$, $p<0.001$) and glycosylated hemoglobin ($F(1)=12.79$, $p=0.001$) between groups over the course of 12 weeks. All effects remained significant after adjusting for age, gender, and BMI (Table 4).

Analysis of the other secondary outcomes between the taVNS and no-treatment control indicated that there was no significant difference between the two groups in systolic blood pressure over time ($F(1)=0.99$, $p=0.32$), diastolic blood pressure ($F(1)=1.27$, $p=0.27$), and BMI ($F(1)=0.003$, $p=0.96$).
Repeated measures between the sham taVNS and no-treatment control groups showed that the two groups differed significantly in their levels of 2hPG ($F_{GG}(1.72)=10.51$, $p<0.001$ corrected for Greenhouse Geisser) and HbA1c ($F(1)=5.94$, $p=.018$) over the course of the experiment. Measures of both 2hPG and HbA1c increased over the 12 weeks in the control group and decreased over the course of the 12 weeks in the sham taVNS treatment group. After controlling for age, gender and BMI, only the effect for change in 2hPG remained significant (Table 5).

Analysis of the other secondary outcomes between the sham taVNS and no-treatment control indicated that there was no significant difference between the two groups in systolic blood pressure over time ($F(1)=1.44$, $p=0.24$), diastolic blood pressure ($F(1)=0.047$, $p=0.83$), and BMI ($F(1)=0.024$, $p=0.88$).

**Safety**

According to the patients’ booklet reports, we found that 2 patients showed dizziness during or after the treatment. All participants recovered fully from the adverse events after stopping the treatment.

**Discussion**

As the epidemic of diabetes continues to rise, it becomes essential to develop and implement cost-effective preventative therapeutic strategies (12). In this study, we compared the efficacy of taVNS and sham taVNS on patients with IGT. Results showed that compared with sham taVNS, taVNS significantly reduced the two-hour glucose tolerance. In addition, we also
found that taVNS produced significant decreases in systolic blood pressure over time \( (F(1)=4.21, p=0.044) \) compared with control. Taken together, our results suggest that taVNS has the potential to be used as a preventive treatment for pre-diabetes / IGT.

The metabolic syndrome, which includes a constellation of risk factors such as obesity, elevated lipids, elevated glucose and blood pressure, has been increasingly drawing attention from investigators, as this syndrome is regarded as both a disease and a risk factor for other major diseases such as cardiovascular disease and Alzheimer’s disease (AD) (13). These diseases not only harm our well-being and longevity, but also have a tremendous economic impact (19). Prevention and treatment of these disorders require a considerable number of medical resources. A simple and cost-effective method to control these disorders may have a significant influence on the society.

Previous studies have suggested that the vagus nerve plays an important role in maintaining metabolic homeostasis and that efferent vagus nerve-mediated cholinergic signaling controls immune function and proinflammatory responses via the inflammatory reflex. Deregulation of metabolism and immune function are associated with chronic inflammation, which acts as a critical step in the pathogenesis of insulin resistance and type 2 diabetes mellitus(13; 20).

In a previous study, investigators (21) found that the metabolic syndrome had an independent inverse association with the vagal component of high-frequency heart rate variability. In another study, Licht and colleagues (22)
found that decreased parasympathetic and increased sympathetic activities are associated with increased likelihood of metabolic syndrome. Specifically, they found that lower respiratory sinus arrhythmia (RSA), a measurement of parasympathetic activity in which high RSA reflects high parasympathetic activity, was associated with glucose level and systolic blood pressure. Taken together, these studies suggest that low vagus nerve activity may underlie elevated glucose levels, which provides the biological mechanism of modulating IGT using taVNS.

Anatomical studies have shown that the ear is the only place on the surface of the human body where there is afferent vagus nerve distribution (16; 17). Thus, direct stimulation of the afferent nerve fibers on the ear can regulate the activity of vagus nerve, which can further regulate metabolic homeostasis. In this study, we found that taVNS reduced both IGT and systolic blood pressure. This result suggests that taVNS has the potential to be used as a therapeutic method for IGT and pre-diabetes, as well as other metabolic syndrome.

In this study, we found that both taVNS and sham taVNS significantly reduced the 2-hour glucose tolerance and glycosylated hemoglobin, indicating that sham taVNS could also produce a significant modulatory effect on glucose levels. We speculate that this may have been due to the residual vagus nerve distribution at the location where sham taVNS was applied. In addition, sham taVNS may produce these effects by influencing the lifestyle of the patients, for patients were asked to apply the postprandial treatment twice daily, which might have reminded the patients to pay more attention to food intake and daily activity.
There are several limitations in this study. Firstly, treatments in the study were self-administered by the patients, and thus the compliance of the patients may have influenced the result. To enhance the compliance, all patients were required to complete daily entries into a diary that was checked during the assessments. More importantly, however, this self-administration method provides a direct evidence for the feasibility of wide application of the method, which can significantly reduce the expenses related to the treatment. Secondly, the duration of the treatment was only 12 weeks, thus the results obtained only represent the short or mid-term effect and further study is needed to evaluate the long term effects of the treatment. Finally, the no-treatment control was not randomized with other two treatment groups and the participants were recruited from a different population. However, the purpose of this group was to add another layer of control to explore the effects of different treatments; thus, it should not influence the conclusion of this study.

In summary, this pilot study demonstrates that taVNS can significantly reduce two-hour glucose tolerance and systolic blood pressure. As a simple, cost-effective therapeutic method with mild side effects, it may have great potentials in treatment of pre-diabetes.

Acknowledgments

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invaluable contribution to the study. We would also like to thank the participants for volunteering their time to be involved in the study. We also thank Rupeng Liu for providing the consent for his ear on the publication.

Authors’ contributions

PjR designed the trial and was the principal clinical research investigator. PjR, FH, wrote the manuscript with JK. LX generated the random allocation sequence. FH, HM, ChW, XL, XZ were in charge of the recruitment and treatment of patients in each center and collected the data. FH prepared the figures. JK, Stephanie Camhi and Rosa Spaeth were responsible for data analysis and edited the manuscript with BZ.

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Competing interests

All authors claim no conflicts of interest.

Trials registration:

Clinical Trials. ChiCTR-TRC-12002522 http://www.chictr.org.cn/
Table 1. Baseline characteristics across three groups. All values are presented as mean (SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Age</th>
<th>Gender (M/F)</th>
<th>Height</th>
<th>Weight</th>
<th>BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>taVNS</td>
<td>55.3(7.1)</td>
<td>7/29</td>
<td>1.6(.06)</td>
<td>63.9(11.7)</td>
<td>24.5(3.5)</td>
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<tr>
<td>Sham taVNS</td>
<td>52.3(8.7)</td>
<td>14/22</td>
<td>1.7(.08)</td>
<td>66(10.1)</td>
<td>23.9(2.6)</td>
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<td>Control</td>
<td>55.7(5.6)</td>
<td>9/21</td>
<td>1.6(.07)</td>
<td>67.2(9)</td>
<td>25.7(3.4)</td>
</tr>
</tbody>
</table>

Table 2. Comparison of 2-hour plasma glucose (2hPG), fasting plasma glucose (FPG) and glycosylated hemoglobin (HbA1c) between taVNS and sham taVNS groups. Adjusted values reflect age, gender, and BMI as covariates.

<table>
<thead>
<tr>
<th></th>
<th>P-value</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
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<tbody>
<tr>
<td>2hPG</td>
<td>.004</td>
<td>8.02</td>
<td>8.522</td>
</tr>
<tr>
<td></td>
<td>.006</td>
<td>8.015</td>
<td>8.526</td>
</tr>
<tr>
<td>FPG</td>
<td>.093</td>
<td>5.946</td>
<td>6.203</td>
</tr>
<tr>
<td></td>
<td>.11</td>
<td>5.948</td>
<td>6.201</td>
</tr>
<tr>
<td>HbA1c</td>
<td>.63</td>
<td>6.057</td>
<td>6.282</td>
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<tr>
<td></td>
<td>.681</td>
<td>6.056</td>
<td>6.283</td>
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</tbody>
</table>

Table 3. 2-hour plasma glucose (2hPG), fasting plasma glucose (FPG) and glycosylated hemoglobin (HbA1c) before and after the intervention across different groups. All values are presented as mean (SD)

<table>
<thead>
<tr>
<th></th>
<th>Ta-VNS</th>
<th>Sham taVNS</th>
<th>No-treatment</th>
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<tbody>
<tr>
<td>2hPG Baseline</td>
<td>9.7(1.2)</td>
<td>9.1(1.2)</td>
<td>9.3(1.1)</td>
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<tr>
<td>2hPG 6-weeks</td>
<td>7.3(1.8)</td>
<td>8.0(1.6)</td>
<td>9.5(1.9)</td>
</tr>
<tr>
<td>2hPG 12-weeks</td>
<td>7.5(1.3)</td>
<td>8.0(1.4)</td>
<td>10.0(2.7)</td>
</tr>
<tr>
<td>FPG Baseline</td>
<td>6.2(0.6)</td>
<td>6.3(0.5)</td>
<td>6.5(0.3)</td>
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<td>FPG 6-weeks</td>
<td>5.9(0.8)</td>
<td>6.2(0.8)</td>
<td>6.6(0.8)</td>
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<tr>
<td>FPG 12-weeks</td>
<td>5.7(0.6)</td>
<td>6.2(0.8)</td>
<td>6.9(1.2)</td>
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<tr>
<td>HbA1c Baseline</td>
<td>6.3(0.5)</td>
<td>6.2(0.6)</td>
<td>6.2(0.4)</td>
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<tr>
<td>HBA1C 12-weeks</td>
<td>6.1(0.4)</td>
<td>6.0(0.4)</td>
<td>6.3(0.6)</td>
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<tr>
<td>BMI Baseline</td>
<td>24.5(3.5)</td>
<td>23.9(2.6)</td>
<td>25.7(3.4)</td>
</tr>
<tr>
<td>BMI 12-weeks</td>
<td>24.1(3.3)</td>
<td>23.5(2.6)</td>
<td>25.4(3.0)</td>
</tr>
</tbody>
</table>
Table 4. Comparison of 2-hour plasma glucose (2hPG), fasting plasma glucose (FPG) and glycosylated hemoglobin (HbA1c) between taVNS and no-treatment control groups. Adjusted values reflect age, gender, and BMI as covariates.

<table>
<thead>
<tr>
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<th>Upper 95% CI</th>
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<tbody>
<tr>
<td><strong>2hPG</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>&lt;.001</td>
<td>8.553</td>
<td>9.238</td>
</tr>
<tr>
<td>Adjusted</td>
<td>&lt;.001</td>
<td>8.546</td>
<td>9.241</td>
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<tr>
<td><strong>FPG</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>&lt;.001</td>
<td>6.164</td>
<td>6.473</td>
</tr>
<tr>
<td>Adjusted</td>
<td>&lt;.001</td>
<td>6.162</td>
<td>6.476</td>
</tr>
<tr>
<td><strong>HbA1c</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>.001</td>
<td>6.123</td>
<td>6.346</td>
</tr>
<tr>
<td>Adjusted</td>
<td>.002</td>
<td>6.124</td>
<td>6.342</td>
</tr>
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</table>

*Table 5. Comparison of 2-hour plasma glucose (2hPG), fasting plasma glucose (FPG) and glycosylated hemoglobin (HbA1c) between the sham taVNS and no-treatment control groups. Adjusted values reflect age, gender, and BMI as covariates.

<table>
<thead>
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<th>Upper 95% CI</th>
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<tbody>
<tr>
<td><strong>2hPG</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>&lt;.001</td>
<td>8.63</td>
<td>9.336</td>
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<tr>
<td>Adjusted</td>
<td>.003</td>
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References

13. De Couck M, Mravec B, Gidron Y: You may need the vagus nerve to understand pathophysiology and to treat diseases. *Clin Sci (Lond)* 2012;122:323-328
Legends

Figure 1 Location of taVNS and sham taVNS. Red dots indicate locations of taVNS, blue dots indicate the location of sham taVNS.

Figure 2 The Flow diagram shows the detail information on the recruited and excluded participants.
Figure 3 2-hour plasma glucose level changes among taVNS, sham taVNS and no-treatment control groups.

The 2hPG were $9.7 \pm 1.2$, $9.1 \pm 1.2$, $9.3 \pm 1.1$ of TaVNS, Sham taVNS and No-
treatment groups separately at baseline; 7.3±1.8, 8.0±1.6, 9.5±1.9 of these three groups at 6-weeks; 7.5±1.3, 8.0±1.4, 10.0±2.7 at 12-weeks.

Figure 4 Ear vagus nerve stimulator used for taVNS
30 additional IGT patients were recruited without treatment.

Assessed for eligibility (n=128):
- Excluded (n=26):
  - Not meeting inclusion criteria (n=21)
  - Declined to participate (n=3)
  - Other reasons (n=2)

Randomized (n=72)

Allocation:
- Allocated to taVNS (n=36):
  - Received taVNS intervention (n=36)
  - Did not receive allocated intervention (give reasons) (n=0)
- Allocated to sham taVNS (n=36):
  - Received sham taVNS intervention (n=36)
  - Did not receive allocated intervention (give reasons) (n=0)

Follow-up:
- Lost to follow-up (give reasons) (n=0)
- Discontinued intervention (give reasons) (n=1) stimulation-evoked dizziness

Analysis:
- Analysed (n=35)
  - Excluded from analysis (give reasons) (n=0)
Figure 3

2-hour Plasma Glucose

mmol/L

Mean

Baseline 6-weeks 12-weeks

TaVNS
Sham TaVNS
No-treatment
Figure 4

Ear Vagus Nerve Stimulator

1. TIME
2. MODE
3. FREQ
4. ADJUST INTENSITY
5. ADJUST INTENSITY
6. ADJUST INTENSITY
7. ADJUST INTENSITY
8. ADJUST INTENSITY
9. ADJUST INTENSITY
10. ADJUST INTENSITY
11. ADJUST INTENSITY
Additional files provided with this submission:

Additional file 1: References.docx, 15K
http://www.biomedcentral.com/imedia/9175989501109653/supp1.docx
Additional file 2: Ethica approval-IGT.jpeg, 725K
http://www.biomedcentral.com/imedia/9956351810976122/supp2.jpeg
Additional file 3: 2964_CONSORT+2010+checklist.doc, 219K
http://www.biomedcentral.com/imedia/1155200611123643/supp3.doc