Hypoglycemic Effects and Mechanisms of Electroacupuncture on Insulin Resistance

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Short running title: Electroacupuncture on glycemic control

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The aim of this study was to investigate effects and mechanisms of electroacupuncture (EA) on blood glucose and insulin sensitivity in mice fed with a high fat diet. **Methods:** Both wild type (WT) and adipose Ecto-nucleotide pyrophosphate phosphodiesterase (ENPP1) transgenic (TG) mice were fed with high-fed diet for 12 weeks; each mouse was studied intra-peritoneal glucose tolerance test (IPGTT) and insulin tolerance test (ITT) with or without EA at abdomen or auricular areas. **Results:** 1). High fat diet induced insulin resistance in both WT and TG mice. 2). In the WT mice, EA at 3Hz and 15Hz but not at 1Hz or 100Hz via CV4+CV12 significantly reduced postprandial glucose levels; EA at 3Hz was most potent. The glucose level was reduced by 61.7% at 60 min and 74.5% at 120 min with EA at 3 Hz (all P<0.001 vs. control). 2) Similar hypoglycemic effect was noted in the TG mice; 3) on contrary, EA at auricular points increased postprandial glucose level (P<0.03). 4). EA at 3Hz via CV4+CV12 significantly enhanced the decrease of blood glucose after insulin injection, suggesting improvement of insulin sensitivity. 4). Plasma free fatty acid was significantly suppressed by 42.5% at 15 min and 50.8% at 30 min with EA (P < 0.01) in both WT and TG mice. **Conclusions:** EA improves glucose tolerance in both WT and TG mice fed with high fat diet, and the effect is associated with stimulation parameters and acupoints, and is probably attributed to the reduction of free fatty acid.

**Key words:** Electroacupuncture, insulin resistance, glucose, vagal activity, free fatty acid
INTRODUCTION

Diabetes affects about 8.3% of Americans with an estimated total cost of over $174 billion (1). In addition, about 79 million American adults have pre-diabetes (1) and about 34% of American adults have metabolic syndrome (14). Insulin resistance is one of the major contributing factors for diabetes, pre-diabetes and metabolic syndrome. Obesity is virtually always associated with insulin resistance (16). Insulin resistance increases with weight gain and decreases with weight loss (44), indicating that fat accumulation is not only associated with but in fact causes insulin resistance (5). In addition, insulin resistance is known to be attributed to elevated plasma free fatty acid (FFA) (6). Elevated plasma FFA and intracellular lipid inhibit insulin signaling, leading to a reduction in insulin-stimulated muscle glucose transport (13, 27). The resulting suppression of muscle glucose transport leads to reduced muscle glycogen synthesis and glycosis.

Obese patients with insulin resistance have been reported to have increased adipose tissue. Ectonucleotide pyrophosphate phosphodiesterase (ENPP1) (17). ENPP1 modulates insulin action by physical interaction with the α-subunit of the insulin receptor and inhibition of β-subunit activation (20, 24, 36). In transgenic model, previous studies elucidated that ENPP1 overexpression in insulin-sensitive tissues (liver, muscle and brain) plays a role in insulin resistance and hyperglycemia, suggesting an important animal model to investigate insulin resistance. Recently, one study has shown that in the presence of high-fat diet, transgenic model with ENPP1 over expression in adipocytes induces fatty liver, hyperlipidermia and dysglycemia, recapitulating key manifestation of the metabolic syndrome (41).

Acupuncture has been shown to be effective in treating patients with diabetes and seems more effective in treating type 2 diabetes than type 1 diabetes (23). Patients with diabetes treated with acupuncture showed improved clinical manifestation as well as reduction in fasting blood glucose and improvement in oral glucose tolerance. Other studies indicated that acupuncture improved both hyperglycemia and insulin resistance (11, 15). In animals, electroacupuncture (EA) has been shown to improve insulin resistance or sensitivity (10, 25, 26, 31, 37) and reduce plasma FFA (32). In the current study, we hypothesized that EA reduces elevated blood glucose level in a mouse model of insulin resistance and the mechanism may be via the suppression of FFA.
Our aims of this study were therefore to investigate the effects and mechanisms of EA on blood glucose and insulin sensitivity in regular mice fed with high fat diet and ENPP1 transgenic mice fed with high fat diet. The experiments were designed to study the effects of EA on glucose tolerance, insulin tolerance and plasma insulin and mechanisms involving FFA.

MATERIALS AND METHODS

Subjects

Thirty-two female wild type (WT, C57Bl/6J) mice and sixteen female transgenic (TG) mice were used in the study. The transgenic mice were produced by the Department of Endocrinology, University of Texas Medical Branch in Galveston, TX. The breeding method was established in Dr. Abate’s lab and has been previously published (41). The mice were housed in the microisolator cage equipped with filter hoods under controlled temperature (20°C) and with a 12:12-h light:dark cycle and free access to water and solid food. The experimental protocol was approved by the Institutional Animal Care and Use Committee, University of Texas Medical Branch at Galveston, Texas.

Diet

The “ad libitum” diet was started at 8 weeks of age in both WT and TG mice and the animals had free access to high-fat chow (60% fat by calories; Research Diets D12492, New Brunswick, NJ). For the paired-feeding WT group, each animal was given the same amount of high-fat chow consumed by the corresponding paired transgenic mouse the day before. The experiments were initiated after 12 weeks’ feeding.

Electroacupuncture

The study was conducted under anesthesia (inhalation of isoflurane 1.5-2%) after the mouse was fasting for 5 hours. Electroacupuncture was performed either in the abdominal acupoints CV4 and CV12 or bilateral auricular acupoints. CV4 (Guanyuan) is located at 2 units above the upper crest of pubis bone (total 14 units from pubis bone to the top of xiphoid process) while CV12 (Zhongwan) is located at 7 units above CV4 (9, 30). The ear acupoints are located around auricular concha bilaterally. In the abdominal EA, the positive charge was introduced at CV4 and the negative charge was connected to CV12. The needles (short and tiny needle used for...
auricular acupuncture in clinics. 0.22 x 1.8mm, AcuMaster, China) were inserted into the
acupoints with the depth of 0.5-1mm and connected an electrostimulator (PulseMaster A300,
World Precision Instrument, Sarasota FL) (Figure 1). Stimulation parameters was set at
continuous on, pulse width of 0.5ms, various pulse frequency of 1Hz, 3Hz, 15Hz or 100Hz and
amplitude of 4mA (abdominal EA) or 1mA (auricular EA).

Experimental Protocol

Experiment 1: Diet on glucose tolerance: Four groups of mice (n = 8 in each group) were used in
this experiment: 1). WT mice fed with regular chow “ad libitum”; 2). WT mice fed with high fat
diet “ad libitum”; 3). Paired feeding groups of WT and TG mice fed with high fat diet. The
pairing was decided by body weight and age. The intraperitoneal glucose tolerance test (IPGTT)
was performed after 5-hour fasting by intraperitoneal injection of 20% glucose (1g/kg body
weight). About 5 µl blood sample was then collected from the tail vein at each time point 0
(baseline) then 15, 30, 60, 90 and 120 minutes after the injection for the assessment of blood
glucose level. The blood glucose level was measured by a glucometer (Accu-Check Aviva,
Roche, Germany). No stimulation was performed in the experiment.

Experiment 2: EA on glucose tolerance with various parameters: Eight WT mice were used in
this experiment. Each mouse was studied in 5 sessions in a randomized order: control without
stimulation, EA-1Hz, EA-3Hz, EA-15Hz and EA-100Hz. The IPGTT was performed in each
session as described in Experiment 1. Abdominal EA at CV4+CV12 was performed during the
entire 120 minutes period. This is a pilot study and we didn’t know which time point the
hypoglycemic effect of EA would occur; therefore, we chose to perform EA through the entire
120min-OGTT study, this is similar to a study previously reported (25).

Experiment 3: EA on glucose tolerance with various stimulating locations: Both WT and TG
mice were used in the experiment (n = 8 in each group). Each mouse was studied in three
randomized sessions: control without stimulation, EA-3Hz at the abdominal location
CV4+CV12, and EA-3Hz at the auricular location. The 3Hz was chosen because it yielded best
hypoglycemic effect based on the preliminary results from the Experiment 1. After 5-hr fast,
IPGTT was performed as described in the Experiment 1. In addition to the tail blood sample
taken from each time point for glucose measurement, 30-40µl blood samples were collected
from the tail at 0, 15, 60 and 90 minutes after the glucose injection for the assessment of plasma insulin level. EA was performed during the entire experimental period.

Experiment 4: EA on insulin sensitivity: Six WT mice and 6 TG mice from the Experiment 1 and 2 were used in this experiment. Each mouse was studied in two sessions randomly: control without stimulation and EA-3Hz at CV4+CV12. Insulin tolerance test (ITT) was performed after the mice were fasted for 5 hours. Regular insulin (Humulin U-100, Lilly, IN) in a saline solution (0.5 U/kg) was injected to the intraperitoneal cavity. A drop of blood sample (less than 5 µl) was then collected from the tail vein at the following time points: 0 (baseline) and 15, 30, 60, 90 and 120 minute for the assessment of glucose level (Accu-Check Aviva, Roche, Germany). In addition, 30-40µl blood samples were collected during the test at 3 time points: baseline, 15 and 30 minutes for measuring plasma free fatty acid level. Abdominal EA was performed for 120 minutes.

Analysis of Peptides

Blood samples were collected in chilled EDTA tubes (Microvette CB300, Sarstedt, Germany) then centrifuged at 4°C at 3000rpm speed for 30 minutes. Plasma was obtained and stored at -80°C. Insulin was assayed using Ultra Sensitive Mouse Insulin ELISA kit (catalog # 90080; Crystal Chem). FFA was assayed using Non-Esterified Fatty Acids Detection ELISA kit (catalog # SFA-1, Zen-Bio, Inc, NC)

Statistical Analysis

Data are presented as means ± SE. One way ANOVA was used for multiple comparisons such as different diets, different parameters or locations. A two-tailed Student t-test was used for comparison between control and EA. In the insulin tolerance test, the percentage of glucose change compared to the baseline at each time point was used to represent the insulin sensitivity. Significance was defined as P < 0.05.
RESULTS

**High fat diet and glucose tolerance**

The regular chow had no effects on glucose tolerance. As shown in Figure 2, the peak blood glucose level in the WT mice with “ad libitum” regular chow feeding was at 60 minutes (356.0 ± 16.6mg/dl) and back to the baseline after 120 minutes (220.1 ± 17.2mg/dl).

However, the high fat diet induced glucose tolerance in all three groups of rats: the ad libitum group and the paired feeding WT and TG groups. In the ad libitum regular mice fed with high fat diet, the blood glucose level after glucose injection was 491.7 ± 21.9mg/dl at 60 minutes and sustained at 456.7 ± 28.1mg/dl at 120 minutes, these were significantly higher than those in the regular chow group (P < 0.001). Same findings were observed in the paired feeding WT and TG groups; the blood glucose levels in the paired feeding WT and TG group was also high and even higher than the WT “ad libitum” high fat diet group at certain points. As shown in Figure 1, at 120 minutes, the blood glucose level was 576.8 ± 23.3mg/dl in the WT paired group (P = 0.02 vs. WT “ad libitum” high fat diet group) and 558.5 ± 21.6mg/dl in the TG paired group (P = 0.01 vs. WT “ad libitum” high fat diet group).

**Parameter-dependent effects of EA on postprandial glycemic control**

EA at 3Hz and 15Hz significantly reduced postprandial blood glucose level from 30min to 120min, and 3Hz was more potent than 15 Hz. The postprandial blood glucose level was substantially reduced from 396.9 ± 9.7mg/dl in the control session to 281.9 ± 36.1mg/dl with EA-3Hz at 30 minutes (P < 0.01), from 436.8 ± 26.2mg/dl to 167.1 ± 28.1mg/dl at 60 minutes (P < 0.001) and from 300.0 ± 55.8 mg/dl to 76.5 ± 18.1mg/dl at 120 minutes (P < 0.01) (Figure 3).

However, neither EA at 1Hz nor EA at 100Hz reduced postprandial blood glucose during the entire 120 min experimental period. At 120 minutes, EA at both 1Hz and 100 Hz actually increased blood glucose level; it was 410.5 ± 55.8 mg/dl in the EA-1Hz session (P = 0.02 vs. control) and 476.2 ± 44.7mg/dl in the EA-100Hz session (P = 0.001 vs. control, Figure 3)

**Location-dependent effects of EA on postprandial glycemic control**

In the wild type mice, EA with 3Hz at abdominal location CV4 and CV12 significantly reduced the postprandial blood glucose level at 30, 60, 90 and 120 min; the blood glucose level
was reduced by 29.0% at 30 minutes, 61.8% at 60 minutes, 71.0% at 90 minutes and 74.5% at 120 minutes (All points, $P < 0.03$ vs. control). In contrast, EA with 3Hz at the ear location increased the postprandial blood glucose level from 60 to 120 minutes. The blood glucose level was increased by 17.5% at 60 minutes, by 37.4% at 90 minutes and by 72.5% at 120 minutes (All points, $P < 0.03$ vs. control. Figure 4A), suggesting the opposite effect of EA at different locations on the blood glucose level.

Similar findings were observed in the TG mice. EA at CV4 + CV12 significantly decreased the postprandial blood glucose level from 15 minutes to 120 minutes. As shown in Figure 4B, EA at CV4 + CV12 reduced glucose level from 364.1 ± 12.7mg/dl to 303.0 ± 23.3mg/dl at 15 minutes ($P < 0.01$), from 489.1 ± 42.1mg/dl to 276.1 ± 37.5mg/dl at 60 minutes ($P < 0.01$) and from 399.7 ± 56.6mg/dl to 193.6 ± 59.6mg/dl at 120 minutes ($P = 0.04$). EA at the ear location significantly increased glucose level to 512.2 ± 38.8mg/dl at 120 minutes ($P < 0.002$ vs. control, Figure 4B).

**EA on insulin sensitivity**

In the WT mice, abdominal EA significantly increased insulin sensitivity represented by an enhanced decrease of blood glucose in the insulin sensitivity test (Figure 5A). It has to be mentioned that the blood glucose level decreases after the injection of insulin; therefore the percentages of glucose change were shown as negative values starting from 15 minutes after insulin injection. The percentage of blood glucose from baseline (before insulin injection) was substantially decreased from 18.5 ± 5.4% in the control session to 49.7 ± 6.5% with EA at 30 minutes ($P < 0.001$); from 34.7 ± 6.6% to 69.2 ± 8.3% at 60 minutes ($P = 0.0069$ vs. control) and from 33.7 ± 10.4 to 80.1 ± 4.4% at 120 minutes ($P = 0.01$ vs. control).

Similar findings were observed in the TG mice. EA significantly decreased the percentage of blood glucose change from baseline from 30 minutes to 120 minutes after insulin injection (all $P < 0.01$ vs. control), suggesting an increase of insulin sensitivity with EA-3Hz at the abdominal location (Figure 5B).

**EA on Plasma insulin and free fatty acid**

EA-3Hz at the abdominal locations significantly reduced plasma insulin level in both WT mice and TG mice (Figure 6). In the WT mice, the plasma insulin level was reduced from 0.8 ±
0.2ng/ml in the control session to 0.5 ± 0.03ng/ml with EA at 15 minutes (P = 0.046, Figure 6A); from 1.3 ± 0.2ng/ml to 0.6 ± 0.2ng/ml at 90 minutes (P = 0.006 vs. control). In the TG mice, EA showed the similar results, however the significance was only observed at 90 minutes (P = 0.01 vs. control, Figure 6B).

EA substantially suppressed plasma FFA in both WT and TG mice (Figure 7). In the WT mice, the plasma FFA level was reduced from 91.2 ± 10.6 ng/ml in the control session to 52.4 ± 3.7 with EA at 15 minutes (P = 0.005) and from 90.7 ± 10.4ng/ml to 44.6 ± 4.2ng/dl at 30 minutes (P = 0.004, Figure 7A). Similar findings were observed in the TG mice (P = 0.009 vs. control at 15 minutes, P = 0.01 vs. control at 30 minutes, Figure 7B).
In the current study, we have found that EA at frequencies of 3 Hz and 15 Hz at acupoints CV4 and CV12 significantly reduced blood glucose, increased insulin sensitivity, reduced plasma insulin and suppressed plasma FFA in mice with high fat diet induced insulin resistance, suggesting the hypoglycemic effect of EA for insulin resistance.

Two murine models of insulin resistance were used in this study: regular mice with high fat-induced insulin resistance and TG mice with ENPP1 over expression. It was reported that adipose ENPP1-TG and WT littermates had similar body weights after being fed with 16-week regular chow diet; however, when exposed to high fat diet, WT mice consumed more food and gained more weight than the transgenic mice (41). Our data indicated that with either free feeding or paired feeding, high fat diet (60%) induced hyperglycemia and glucose intolerance in both WT and TG mice. The results of our study demonstrated similar hypoglycemic effects of EA on both TG mice and WT mice with high fat-induced insulin resistance.

Low frequency EA is applied more frequently for the treatment of insulin resistance with beneficial results (25, 29, 30). In the current study, we tested various frequencies and found EA with 3Hz and 15Hz reduced blood glucose; whereas neither 1Hz nor 100Hz had any hypoglycemic effect in the mice. In general, low frequency is defined as below 4Hz and high frequency is defined as above 100Hz. In a chart with a log scale, 15Hz is in the middle point (21). Previous studies have shown EA at CV4 and CV12 with 15 Hz reduced plasma glucose concentration in rats with or without hyperglycemia (9); using the same EA method, Ishizaki et al reported glucose tolerance was improved and insulin sensitivity was enhanced during EA in diabetic Goto-Kakizaki rats (25). With 3Hz EA at CV4 and ST36, Liang et al reported a reduction of fasting blood glucose after 8 weeks of treatment in obese diabetic mice, an effect mediated via improvement in insulin sensitivity (30). Our findings were consistent with what have been reported in the literature; however, we explored EA at more frequencies and different locations. We found that EA at both 15Hz and 3Hz improved glucose tolerance and 3Hz was more effective. In the current study, we also tried EA with 1Hz and 100Hz. Sixty minutes after EA at 1Hz, there was a trend of decrease in blood glucose but it was not significant; however, during the second 60 minutes, this minor hypoglycemic effect disappeared, a trend of increase of blood glucose was observed instead. Our data on 100Hz confirmed the ineffectiveness of EA...
with high frequency on blood glucose and insulin sensitivity. During 90-120min IPGTT test, we noted an increase of blood glucose. It is interesting that both 1Hz and 100Hz increased blood glucose during the 90-120 min in the current study, however, both changes were statistically not significant. Due to limitation in the total number of sessions we could perform in the same group of animals, we did not study the effect of EA at 2Hz; however, based on our data on 3Hz and other studies (21), we would speculate a hypoglycemic effect with reduced potency on EA at 2 Hz.

The underlying mechanisms involved in the hypoglycemic effect of EA are not clear. Previous studies have shown EA with different frequencies releases different kinds of neuropeptides. For example, EA of 2Hz accelerates the release of enkephalin, \( \beta \)-endorphin and endomorphin, while that of 100Hz selectively increases the release of dynorphin (21, 22). Increased \( \beta \)-endorphin was associated with decreased plasma glucose. In obese Zucker rats, an increase of plasma \( \beta \)-endorphin-like immunoreactivity was obtained in parallel with the reduction of plasma glucose (46). In an earlier study, Chang et al indicated that EA stimulation at CV12 could induce the secretion of \( \beta \)-endorphin to produce hypoglycemia in an insulin-dependent manner in rats (9). Another study demonstrated that serotonin may activate 5-HT\(_7\) receptor on rat adrenal gland to enhance \( \beta \)-endorphin secretion and then stimulates the opioid receptor to increase peripheral glucose utilization, resulting in decreased plasma glucose level in STZ-diabetic rats (12). In the present study, we believe low frequency but not high frequency EA accelerated the release of \( \beta \)-endorphin, which further decreased the glucose tolerance in insulin resistance mice.

Autonomic nerve system dysfunction in terms of either an over-activation of sympathetic activity or a blunted vagal/parasympathetic activity has been shown to be associated with insulin resistance or diabetes (8, 19, 33, 39, 43). In obese patients, increased visceral fat was reported to be associated with sympathetic overactivity and insulin resistance (29). Chronic sympathetic overactivity decreases insulin sensitivity. EA has been frequently and consistently shown to improve vagal activity and suppress sympathetic activity. EA reduced high sympathetic nerve activity in women with polycystic ovary syndrome (45). In animal models and humans with gastrointestinal motility disorders, EA was shown to increase vagal activity and reduce sympathovagal balance (34, 40, 48). Based on these previous findings, a possible autonomic
mechanism is hypothesized: EA improves vagal activity and suppresses sympathetic activity, leading to a reduction in free fatty acid and improvement in insulin sensitivity. Although no direct evidences were available to support such a hypothesis, our data from the insulin tolerance test did demonstrate increased insulin sensitivity with EA. There is a close relation between the peripheral insulin sensitivity and insulin secretion because subjects with low insulin sensitivity or insulin resistance are adapted to secrete more insulin (2, 4, 28). Evidence has shown that increased cholinergic activation is involved in this adaptation. It was reported that cholinergic activation by carbachol returned insulin secretion and glucose intolerance to normal in insulin resistant mice fed with high fat diet (3). Based on these previous studies, the current findings are understandable: 3Hz EA increased insulin sensitivity, yielding a reduced demand for amount of insulin, resulting in a reduced secretion in insulin that was originally elevated in insulin resistant mice fed with high fat diet, especially in the TG mice (41).

Impaired autonomic nervous system in insulin resistance contributes to the pathogenesis through increase in lipolysis from adipose tissue and increase in plasma FFA (38, 47). In type 2 diabetic patients, an increased postprandial plasma FFA concentration was found to be associated with increased sympathovagal balance (38). Sympathetic overactivity is believed to result in elevated plasma FFA; the β3-adrenergic receptor has been found to play a crucial role in catecholamine-induced rate of FFA mobilization from mental fat cells (35). Increased fatty acid flux from adipose to peripheral circulation is known to reduce glucose utilization in skeletal muscle and increase triglyceride deposition in the liver (6). Increasing vagal or reducing sympathetic tone would therefore have a positive effect on fatty acid metabolism. Reduced fatty acid flux from the adipose tissue would improve insulin-mediated peripheral glucose utilization, mainly occurring in skeletal muscle, and reduce fatty infiltration of the liver. In the current study, we found 3Hz abdominal EA reduced plasma FFA concentration in mice with insulin resistance and this reduction was believed to play a major role in the improvement of glucose tolerance. Since the close relation between FFA and sympathetic activity, we believe the suppression of FFA is considered to be related to the decrease of sympathetic activity and improve insulin sensitivity.

Surprising but interestingly, the hypoglycemic effect of EA was observed only at the abdominal location but not at the auricular location. The points we stimulated at the auricular area were considered to receive innervations from auricular branch of vagus nerve (18, 42).
Electrical activation of the vagus nerve was shown to stimulate insulin secretion both in vivo and
in vitro in several different species including humans (2, 7). In the literature, little was reported
on auricular acupuncture for treating diabetes, we hypothesized that similar to abdominal EA, by
stimulating the auricular branch of the vagus, vagal activity might be increased and sympathetic
activity might thereby be reduced so that insulin resistance could be improved. However, our
results on auricular acupuncture failed to confirm such a hypothesis. This might be attributed to:
1). in addition to the auricular branch of vagus nerve, other sensory nerves innervating the ear
may also be involved; 2). low frequency and low energy stimulation was applied in current
study, which might not enhance vagal activity. In the literature, it was reported that strong or
even painful stimulation was required to produce an autonomic response with auricular
acupuncture (18). Surprisingly, our data indicated a trend of increasing blood glucose during
auricular EA, implying that it may even cause hyperglycemia. Studies on auricular EA and
glucose metabolism are required to further investigation. . .

The weakness of the study is the lack of chronic data. However, a positive chronic and
sustained effect is speculated. Although not investigated in the present study, EA has been shown
to have sustained effects in other studies; for example, the liquid/solid gastric emptying was
continuously accelerated after the termination of EA (40, 49). Different from most of other
methods of electrical stimulation, such as cardiac stimulation and spinal cord stimulation with
which stimulation has to be performed continuously, EA is typically performed once a day or a
few times a few. This practice also implicitly suggests sustained effects of EA. Further studies
are being performed in our lab to study the hypoglycemic effect of chronic EA.

**Perspectives and Significance**

Diabetes, pre-diabetes and metabolic syndrome are common in general population. Therefore it is of great clinical significance to develop new therapies for these disorders. While
acupuncture or EA has been shown to improve insulin resistance and effective in treating
diabetes in some studies, its clinical application has not been popularized due to the limitation of
study design, inconsistency of acupuncture locations and parameters. In the current study, we
have investigated the effects of EA on hyperglycemia in mouse models of insulin resistance and
various parameters and locations have been tested. We found EA with low frequency
substantially improved glucose intolerance and increased insulin sensitivity by suppressing free
fatty acid, possibly attributed to the activation of vagal activity and/or the suppression of sympathetic activity. This pilot study provides important information in the development of an alternative therapy for pre-diabetes or diabetes. Future chronic study with low frequency EA at abdominal location should be performed to access the therapeutic potential of EA for insulin resistance and diabetes.

In conclusion, acute 3Hz EA at abdominal points CV4 and CV12 exerts a hypoglycemic effect in both wild-type mice and ENPP1 TG mice with insulin resistance. The inhibitory effect of EA is attributed to an increase in insulin sensitivity at least partially mediated via the inhibition in free fatty acid. EA with low frequency at CV4 and CV12 may have a therapeutic potential for diabetes; however, chronic studies are needed.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).
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Figure Legends

Figure 1: Experimental setup of abdominal electroacupuncture.

Figure 2: Diet on glucose tolerance in both wide type and transgenic mice. High fat diet induced less glucose tolerance in both wild type and transgenic mice.

Figure 3: EA at different frequencies on glucose tolerance in wild type mice. EA at 3Hz and 15 Hz significantly reduced blood glucose level, however 1Hz and 100Hz had no effects.

Figure 4: EA at different points on glucose tolerance in WT (A) and TG (B) mice. EA with frequency of 3Hz at abdominal points significantly reduced blood glucose level; while EA with same frequency at auricular points increased blood glucose level.

Figure 5: EA-3Hz on insulin sensitivity in both WT (A) and TG (B) mice. EA significantly increased insulin sensitivity in both WT and TG Mice.

Figure 6: EA-3Hz on plasma insulin level in both WT (A) and TG (B) mice. EA significantly reduced plasma insulin level after 15 and 90 minutes in WT mice; the reduction in TG was less significant.

Figure 7: EA-3Hz on plasma free fatty acid in both WT (A) and TG (B) mice. EA significantly suppressed plasma free fatty acid in both WT and TG mice at 15 and 30 minutes.
Figure 1

[Diagram showing a stimulator connected to CV12 and CV4]
Figure 2

* P < 0.04 vs. regular chow

Blood Glucose Concentration (mg/dl)

- WT-Reg Chow
- WT-HFD ad libitum
- WT-HFD Paired
- TG-HFD Paired

Minutes

0 15 30 45 60 75 90 105 120
Figure 3

* P < 0.03 Control vs. 3Hz

Blood Glucose Concentration (mg/dl)

Minutes

Control, 1Hz, 3Hz, 15Hz, 100Hz
Figure 4

A Wild Type Mice

B ENPP1-Transgenic Mice

Blood Glucose Concentration (mg/dl)

Minutes
Figure 5

A. Wild Type Mice

B. ENPP1-Transgenic Mice

* P < 0.01 vs. control

* P < 0.02 vs. control

% of Glucose Change

Minutes

Control

EA-3Hz

Control

EA-3Hz
Figure 6

A. Wild-Type Mice

- Control
- EA-3Hz

* P < 0.05 vs. Control

B. ENPP1 Transgenic Mice

- Control
- EA-3Hz

* P = 0.01 vs. Control

Plasma Insulin (ng/ml) vs. Time (0, 15min, 60min, 90min)
Figure 7

A  Wild Type Mice

B  ENPP1-Transgenic Mice

* P < 0.004 vs. Control