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Changes in Levels of Serum Insulin, C-Peptide and Glucose after Electroacupuncture and Diet Therapy in Obese Women

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Abstract: Our purpose was to investigate the effects of electroacupuncture (EA) therapy on body weight and on levels of serum insulin, c-peptide and glucose in obese women. 52 healthy women were included in this study and were allocated into three groups: 1) Placebo EA group (n = 15; mean age = 41.8 ± 4.6 and mean body mass index {BMI} = 33.2 ± 3.5); 2) EA group (n = 20; mean age = 42.1 ± 4.4 and BMI = 35.9 ± 3.6) and 3) Diet restriction group (n = 20; mean age = 42.9 ± 4.3 and BMI = 34.7 ± 2.7). EA was applied to the ear points Hunger and Shen Men on alternating days and to the body points LI 4, LI 11, St 36 and St 44 once a day for 30 minutes over 20 days. Diet restriction that entailed a 1450 kilocalorie (kcal) diet program was applied to the three groups for 20 days. An increase in weight loss was observed when weight loss in the EA group (p < 0.000) was compared to that in the diet restricted and placebo EA groups using the Tukey HSD test. There were increases in the serum insulin (p < 0.001) and c-peptide levels (p < 0.000) in the women treated with EA compared to those in the women treated with the placebo EA and diet restriction groups. A decrease was observed in the glucose levels (p < 0.01) in both the EA and diet restriction groups compared to those in the placebo EA group. Our results suggest that EA therapy is an effective method in treating obesity. EA therapy also helps serum glucose levels to decrease through the increase of serum insulin and c-peptide levels.

Keywords: Electroacupuncture; Obesity; Weight loss; Insulin; C-peptid.

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Introduction

Obesity is one of the major health problems in industrialized countries. It is often associated with chronic diseases like hyperlipidemia, hypertension, insulin resistance, type II diabetes, and coronary heart diseases (Lyznicki *et al.*, 2001). Obesity results from an imbalance in energy intake and consumption, defined as greater calorie intake than the body needs (Palou *et al.*, 2000). It is well known that metabolic changes associated with being overweight cause crucial health problems (Lyznicki *et al.*, 2001).

Acupuncture is one of the well-known traditional Chinese medical methods and is commonly (Ernst, 1997) used as an effective (Soong, 1975) method in the treatment of obesity. In recent years, intensive studies have been carried out to explain the underlying mechanisms of the efficacy of acupuncture. An increase in the release of endogen opioid peptides is generally accepted to be a key pathway that affects the metabolism after electroacupuncture (EA) application. It has been shown that EA application on specific points causes weight loss in obese people (Zhan, 1993; Sun and Xu, 1993). It has already been reported that EA reduces hunger sensation (Richards and Marley, 1998) and increases the beta endorphin (BE) in the plasma and central nervous system by affecting lipid and carbohydrate metabolism through the increase of insulin secretion from pancreas beta cells (Vettor *et al.*, 1993; Fu, 2000; Takeshige *et al.*, 1992; Takeshige *et al.*, 1993; Petti *et al.*, 1998; Pintov *et al.*, 1997).

In this study, we aimed to investigate the effects of EA therapy on body weight and on levels of serum insulin, c-peptide and glucose in obese women.

Materials and Methods

EA was performed in a private acupuncture clinic. Serum samples taken from the subjects were processed in the laboratories of the Meram Medical Faculty of Selcuk University.

Subjects

The protocols were approved by local institutional ethics committees. Informed consent was given by volunteers for EA application. Women aged between 35 and 50 and with a body mass index between 30 and 40 were included into this study. Fifty-two healthy women included in this study were allocated into three groups: 1) Placebo EA group (n = 15); 2) EA group (n = 20); and 3) Diet restriction group (n = 20).

Determination of Acupuncture Points

Acupuncture points were determined using an electronic detector. "Personal cun", used in Traditional Chinese medicine, was used as a measurement unit.
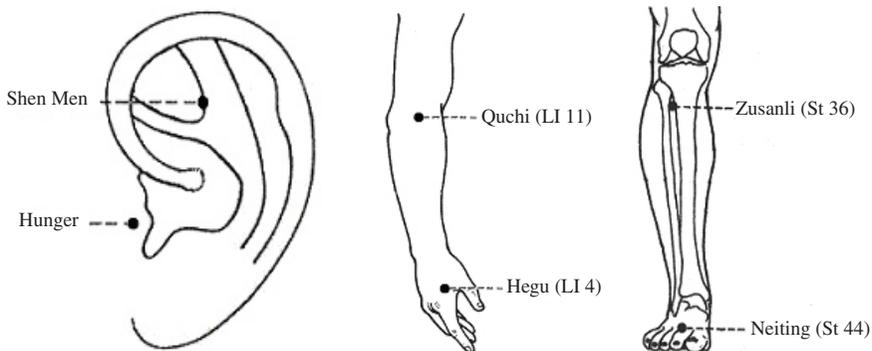
Figure 1. Schematic diagrams of the acupuncture points stimulated.

Selected Ear and Body Acupuncture Points

The Hungry and Shen Men were selected as ear points whereas the Hegu (LI 4), Quchi (LI 11), Zusanli (St 36), and Neiting (St 44) were used as body points (Fig. 1).

The Hungry ear point is placed at the junction of the lines drawn horizontally from the apex tragus and vertically from the intertragic notch and the Shen Men point is located at the one third point of the lateral side of the upper edge of the triangular fossa.

The stimulation of Hungry point creates a feeling of an increase of fullness and a suppression of hunger (Asomoto and Takeshige, 1992). Stimulation of the Shen Men point regulates cerebral cortex function and it has a sedative effect (Wang and Kain, 2001).

The LI 4 point on the body is found on the dorsal face of the hand between the first and second metacarpal bones and in the middle of the radial side of the second metacarpal bone. The LI 11 point is found between the Chize (Lu 5) point and lateral epicondilus of humerus when the elbow is in reflexion position at the end of transvers cubital line. This point is the most lateral point of the elbow transversal curve when the arm is in the maximum flexion position. The St 36 point is three cun below the patella's bottom edge and between the tibialis anterior muscle and flexor digitorium communis muscle. The St 44 point is between the second and third phalanges on the foot, and the lateral and distal side of the second metatarsodigital joint.

Stimulation of the LI 4 and LI 11 points has a regulatory effect on intestinal motility (Maciocia, 1989). Stimulation of St 36 and St 44 increase excitability of the satiety center in the ventral medial nucleus of the hypothalamus (Zhao *et al.*, 2000). EA to the St 36 point modulates the gastrointestinal motility causing an increase in bowel movement in people who have hypoactive gastrointestinal motility and a decrease in people who have increased bowel motility.

EA Application to Ear and Body Points

Ear and body EA application was performed daily for 30 minutes over 20 days, everyday at the same time from 8:00 am to 8:30 am. Body EA application was performed daily for

20 days, and ear EA was applied to each ear on alternating days. After EA application, permanent ear needles were placed on the Hungry points. The 0.22 mm diameter acupuncture needles used were 5 cm long for body EA and 3.5 cm long for ear EA. Electrical stimulation was given for 0.05 ms at a 2 Hz frequency at 3 V in a square wave form which had positive and negative alternances. EA application was performed with a "Biotron" instrument. The electrodes were connected to the Hungry and Shen Men points on both ears and on LI 4 and LI 11 with St 36 and St 44 on the body symmetrically in pairs.

   The twelve women in the control group were subjected to placebo EA applications as described below. In these women, the acupuncture needles were inserted into 2 points on the ear that were unrelated to weight loss and inserted superficially into selected body points that were not acupuncture points, but these points were near the acupuncture points used for the EA group.

Diet Program

A 1450 kcal diet was prepared for women on the diet restriction, placebo EA and EA groups. This amount was chosen in order to give a diet over their basal metabolism. Subjects continued their daily routine activities as before. Diet program was explained to the patients prior to the study and their full consent was taken and then calorie intake continuously checked everyday for placebo EA and EA groups during the study procedure.

Weight and Height of the Subjects

The weight of the subjects was measured with standard scales (sensitivity, ± 0.5 kg) before breakfast. The height of the subjects was measured with a steel rule (sensitivity, ± 0.5 cm). The body mass indexes (BMI) of the subjects were calculated by dividing the weights (kg) by the square of the corresponding heights (m^2).

Preparation of Samples

Blood samples (4 ml) were collected from each subject in the placebo EA, EA and diet restriction groups before and after therapy in the morning between 8.00–9.00 am just before breakfast. Blood samples were centrifuged at 1000 rpm for 10 minutes. The supernatants were collected and kept in −20°C until the analysis in the laboratory.

Determination of Serum Insulin Level

Serum insulin levels were determined by the immunometric method that detects human insulin, using the immulite kit (Diagnostic Products Corporation, Los Angeles, USA). The mean value of the test was 11.9 μIU/ml (CI 95%, between 6 and 27 μIU/ml).

Determination of Serum C-Peptide Level

Serum C-peptide levels were determined by the immunoassay method using the immulite kit. The mean value of the test was 1.6 ng/ml (CI 95%, between 0.9 and 4.0 ng/ml).

Determination of Serum Glucose Level

Serum glucose levels were determined by the hexokinase method using the Olympus System Reagent Kit (Lismeehan, Ireland) with a range from 75 to 115 mg/dl.

Statistical Analysis

The statistical analyses were performed using the SPSS for Windows program. The differences between groups, and values in each group before and after the treatment were calculated as mean ± standard deviation. One-way variation analysis and the Tukey HSD test were used in the statistical analyses; $p < 0.05$ was considered to be statistically significant.

Results

The mean age, the mean body weight and the mean BMI of the subjects are shown in Table 1. No differences were found between the groups in terms of these parameters.

Changes in Weight Loss

A 5% decrease in body weight was observed in the EA group (mean body weight before therapy, 83.4 ± 6.6 kg versus mean body weight after therapy, 79.3 ± 6.6 kg). A 3% decrease in body weight was observed in the diet restriction group (mean body weight before therapy, 83.5 ± 6.7 kg versus mean body weight after therapy, 81.0 ± 6.7 kg). A 2.8% decrease in body weight was observed in the placebo EA group (mean body weight before therapy, 81.3 ± 2.3 versus mean body weight after therapy, 79.1 ± 2.2). An increase in the weight loss was observed when weight loss in the EA group ($p < 0.000$) was compared to that in the diet restricted and placebo EA groups using the Tukey HSD test.

Serum Insulin, C-Peptide and Glucose Levels in EA, Diet and Placebo EA Groups

There were increases in serum insulin ($p < 0.001$) and c-peptide levels ($p < 0.000$) in the women treated with EA compared to those in the placebo EA and diet restriction groups. A decrease was observed in the glucose levels ($p < 0.01$) in both the EA and diet restriction groups compared to those in the placebo EA group. However, there was no difference in the glucose levels between the EA and diet restriction groups.

Table 1. Characteristics of Subjects

	Placebo EA	EA	Diet	P
Age	41.8 ± 4.6	42.1 ± 4.4	42.9 ± 4.3	0.74
Height	1.57 ± 0.0	1.59 ± 0.0	1.58 ± 0.0	0.21
Body Mass Index	33.2 ± 3.5	35.9 ± 3.6	34.7 ± 2.7	0.07

EA; electroacupuncture.

Table 2. Changes in Serum Insulin, C-Peptid and Glucose Levels in Subjects after the Placebo EA, EA and Diet Therapy.

	Placebo EA Group	EA Group	Diet Group	P
Body weight				
1st Day	81.3 ± 2.3	83.4 ± 6.6	83.5 ± 6.7	
20th Day	79.1 ±2 .2	79.3 ± 6.6[**]	81.0 ± 6.7	0.000
Insulin (μIU/ml)				
1st Day	12.4 ± 7.8	10.8 ± 4.9	13.4 ± 5.8	
20th Day	11.8 ± 8.1	14.9 ± 9.7[**]	10.3 ± 4.4	0.001
C-peptid (ng/ml)				
1st day	3.3 ± 1.4	3.01 ± 1.2	3.0 ± 0.7	
20th Day	2.8 ± 1.4	3.9 ± 1.9[**]	2.5 ± 0.8	0.000
Glucose (mg/dl)				
1st Day	102.3 ± 10.3	106.3 ± 9.3	108.1 ± 22.8	
20th Day	100.1 ± 10.5	96.3 ± 7.9[*]	98.1 ± 20.7[*]	0.01

[*]$p < 0.05$ The statistical difference of the EA and diet compared to placebo EA groups.
[**]$p < 0.00$ The statistical difference of the EA compared to diet and placebo EA groups.

Discussion

It has been reported that acupuncture application in obesity treatment is effective in weight loss (Zhan, 1993), affecting emotional factors such as stress (Richards and Marley, 1998), appetite (Shiraishi *et al.*, 1995), intestinal motility (Maciocia, 1989) and metabolism.

Zhao and his colleagues (2000) investigated irritability of the satiety center in the ventral medial nucleus of the hypothalamus by applying EA to rats. EA was applied to one side of the body on one day and the other side on the alternate day using the body acupuncture points St 36 and St 44 for 5 minutes at one session a day over 12 days. They determined an increase in irritability of the satiety center in the ventral medial nucleus of the hypothalamus. Although the rats were left free in the consumption of food and water, weight loss at a rate of 6.5% was observed. In our study, a rate of 5.0% weight loss was also observed in the EA application group on the St 36 and St 44 body acupuncture points in combination with different body points over 20 days. We propose that these points used in our studies might contribute to weight loss by causing a sense of fullness in subjects.

Huang and his colleagues (1996) observed a 4.4 kg loss of body weight through EA on the ear acupuncture points of the Shen Men, Stomach, Sanjiao and Hungry. This

application was applied to one ear in one session and the other ear in the next session once a week over 8 weeks on obese subjects. Diet and exercise programs were also added to this application. The diet program was planned for daily needs, calculating daily activities and other factors, whereas the exercise program was planned towards the consumption of 300–500 kcal at each session 3 to 5 times a week. In our study, EA was applied only to the Hungry and Shen Men points on both ears and in addition, to the body points LI 4, LI 11, St 36 and St 44. As a result, we observed a 4.1 kg weight loss in the EA application group. We presume that this pronounced weight loss in a shorter time might be associated with the use of both body and ear acupuncture points and the increased frequency of the sessions.

In our study, although the same calorie regimen was given to EA, diet restriction and placebo EA groups, it was observed that the body weight loss (4.1 kg) of EA application was more effective compared to that in the diet restriction and placebo EA.

Liu *et al.* (1993) investigated the levels of norepinephrine, epinephrine and glucose in plasma and the changes of cortisol levels in plasma and saliva following acupuncture treatment for weight loss in obese subjects. Acupuncture was applied to 10 men and 58 women over a month. A significant weight loss was observed in these subjects by means of methods used in Traditional Chinese Medicine. Furthermore, the levels of norepinephrine, epinephrine, cortisol in plasma and the level of cortisol in saliva were found to increase, whereas plasma levels of glucose decreased. In our study, we similarly found decreasing glucose levels and weight loss following EA therapy.

Chang and his colleagues (1999) also investigated the changes in plasma levels of beta endorphin and insulin in diabetic rats after EA application. The EA was applied to the Ren 12 and Ren 4 points for 30 minutes by a pulse generator at a 15 Hz frequency at 10 mV. In that study, they used 8 type II diabetic rats, 7 streptozotocin-induced type I diabetic rats, 8 genetic type I diabetic rats and 8 normal rats. The increase in plasma levels of beta endorphin and insulin in normal and type II diabetic rats was observed after EA application. The increase in the plasma insulin level triggered by EA could be blocked by naloxone, a beta endorphin inhibitor. These results suggest that increasing beta endorphin level in plasma following EA application might cause an increase in insulin release from pancreatic B cells. In our study, we observed weight loss and an increase in the plasma insulin level as a result of EA application on obese non-diabetic subjects.

In the study reported by Shapira and his colleagues (2000), changes in plasma insulin and glucose levels in type II diabetic Psammomys obese rats were observed after EA application. The EA application was performed using 80 mA at a 15 Hz electrical current, and the sessions were performed every other day for 30 minutes over 3 weeks. Type II diabetic obese rats (n = 29) were allocated into 3 groups as follows: (1) EA application on the abdominal region using specific points (n = 11); (2) EA application on the back region using nonspecific points (n=9); and (3) control group (n = 9). Abdominal EA application was performed on the Zhongwan (Ren 12) and Guanyuan (Ren 4) points. While an increase was determined in the plasma insulin levels with EA application on the abdominal and back regions compared to those of the control group, no difference was observed in the plasma insulin level between the two EA groups. Furthermore, a decrease in plasma glucose level was observed after abdominal EA application. The EA application

performed on specific abdominal acupuncture points caused a hypoglycemic effect without any weight loss in these obese rats. Similar to these findings, we determined an increase in plasma insulin level and a decrease in glucose level after EA application using different points specifically selected for the treatment of obese subjects.

Bruni and his colleagues (1979) determined that beta endorphin was also secreted from islets of the Langerhans of the pancreas in addition to the central nervous system. Surprisingly, beta endorphin concentrations were found to be higher in the pancreas than in the central nervous system. Mu, delta and kappa opioid receptors were observed in the pancreas, liver and brain (Khawaja *et al.*, 1990). It has been reported that EA applied at different frequencies could cause the release of endogenous opioids to activate specific receptors (Chen *et al.*, 1992).

The attempt to treat diabetes through acupuncture and herbal medicine has been recorded in Chinese literature (Feng *et al.*, 1997; Hu, 1995), but only a few studies on the efficacy of these methods have been published (Mo *et al.*, 1996; Chen *et al.*, 1994). Acupuncture application is effective in the treatment of type II diabetes and it also may be effective in the prevention of complications of diabetes (Hu, 1995). The effect of acupuncture on type II diabetes has been addressed in experimental and clinical studies (Shapira *et al.*, 2000; Chen and Wei, 1985). In the animal study, it has been observed that EA application activates the glucose-6-phosphatase (Hu, 1995). It has been determined that EA application decreases the blood glucose levels by increasing insulin synthesis in the pancreas and receptors on the target cells (Hu, 1995). Most type II diabetic patients are obese. The resistance to insulin in type II diabetics is a secondary phenomenon that occurs in the case of being overweight. We observed a reduction in the serum glucose levels in the diet group receiving the 1450 kcal diet program corresponded to basal metabolic levels. Thus, this suggests that weight loss in these subjects as a result of low daily calorie intake may cause a decrease in insulin resistance (Weir *et al*., 1994). This correlation between resistance to insulin and obesity has not completely been understood. Further investigations are needed to understand the underlying mechanisms of the effects of EA on diabetes mellitus.

Preproinsulin is converted to proinsulin in the endoplasmic reticulum of B cells of the pancreas by the microsomal enzymes. The proinsulin in the Golgi apparatus is ultimately converted to insulin and C-peptide. The increase in plasma levels of insulin and C-peptide after EA might be due to EA stimulation of insulin synthesis in pancreatic B cells (Shoelson and Halban, 1994). It appears that an increase in plasma levels of beta endorphin following EA application may induce the release of insulin.

Our results suggest that EA therapy is an effective method in the treatment of obesity. EA therapy also helps serum glucose levels to decrease through the increase of serum insulin and C-peptide levels. Additional research is needed to further explain the therapeutic effects of EA on obesity and diabetes.

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