The American Journal of Chinese Medicine, Vol. 36, No. 6, 1029–1039

Institute for Advanced Research in Asian Science and Medicine

The Efficacy of Electroacupuncture Therapy for Weight Loss Changes Plasma Lipoprotein A, Apolipoprotein A and Apolipoprotein B Levels in Obese Women

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Abstract: In the present study, we aimed to investigate the effects of electroacupuncture treatment on lipoprotein A, apolipoprotein A and apolipoprotein B levels in obese subjects. Fifty-eight women were studied in 3 groups as follows: 1) Placebo acupuncture ($n = 15$; mean age $= 41.47 \pm 4.61$, and mean body mass index {BMI} $= 33.43 \pm 3.10$); 2) Electroacupuncture (EA) ($n = 20$; mean age $= 40.55 \pm 5.30$, and BMI $= 35.65 \pm 3.84$) and 3) Diet restriction groups ($n = 23$; mean age $= 42.91 \pm 4.02$, and BMI $= 34.78 \pm 3.29$). EA was performed using the ear points, Hungry, Shen Men and Stomach the body points, Hegu (LI 4), Quchi (LI 11), Tianshu (St 25), Zusanli (St 36), Neiting (St 44) and Taichong (Liv 3) for 20 days. Intragroup comparisons were made by using paired samples t-test whereas intergroup differences were investigated by the two-way variation analysis and LSD test. There was a 4.7% ($p < 0.001$) weight reduction in patients with electroacupuncture application, whereas patients in diet restriction had a 2.9% ($p < 0.001$) weight reduction. There were significant decreases in lipoprotein A ($p < 0.05$) and apolipoprotein B ($p < 0.05$) levels in the EA compared to the control group and no changes in apolipoprotein A levels was observed in EA, diet and placebo acupuncture groups. EA therapy may be a useful approach for the treatment of obesity for both losing weight and lowing the risk factors for cardiovascular disease associated

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with obesity, since this application may decrease the plasma lipoprotein A and apolipoprotein B levels.

Keywords: Electroacupuncture; Weight Loss; Lipoprotein A; Lipoprotein B; Obesity.

Introduction

Obesity is one of the major health problems in industrialized countries. It is often associated with chronic diseases such as hyperlipidemia, hypertension, insulin resistance, type II diabetes, atherosclerosis, coronary heart diseases (De Fronzo and Ferrannini, 1991; Leonhardt *et al.*, 1999). Treatments for obesity include: diet restriction, regulation of physical activity, behavior treatment, pharmacotherapy, operation or acupuncture application or the combination of any of these methods (Cabıoglu and Ergene, 2005; Ernst, 1997; Richards and Marley, 1998).

Electroacupuncture (EA) results in weight loss in obese people when it is applied on particular points that are effective for obesity treatment (Cabıoglu and Ergene, 2005; Zhan, 1993; Sun and Xu, 1993). Acupuncture stimulates the auricular branch of the vagal nerve and raises serotonin levels. Both of these activities have been shown to increase tone in the smooth muscle of the stomach, thus suppressing appetite (Shiraishi *et al.*, 1995). Among other things, serotonin enhances intestinal motility. Acupuncture application increases the beta endorphin (BE) in the plasma and central nervous system by affecting lipid and carbohydrate metabolism (Vettor *et al.*, 1993; Fu, 2000). There is the benign regulatory effect of acupuncture on the lipid metabolism (Liu, 1990). It also controls stress (Mulhisen and Rogers, 1999) and depression (Ulett *et al.*, 1998) via endorphin (Richter *et al.*, 1983; Vettor *et al.*, 1993) enkephalin, serotonin and dopamine production.

Lipoprotein A {Lp(a)} is a LDL-like lipoprotein that has been associated with increased risk of coronary heart disease, stroke and restenosis (Scanu, 2001). Lp(a) is many times more potent than cholesterol in its patching ability and has a tendency to attract other Lp(a) particles. The aggregation of Lp(a) forms a plaque that leads to vascular occlusion. Lp(a) has the capacity to bind fibrin and membrane proteins of endothelial cells and monocytes. Lp(a) is a major and independent genetic risk factor for atherosclerosis and cardiovascular disease.

In recent years, there have been more studies showing the correlation between the levels of lipoprotein and peripheric artery diseases. General approach in these literatures is that the levels of apolipoprotein A and apolipoprotein B are high (16). Elevated levels of other lipids, including lipoprotein (a) and apolipoprotein A-1 and B are also now thought to be important indicators of heart disease risk. Apolipoprotein B may actually turn out to be a very accurate indicator of heart disease risk in women.

In this study, we aimed to investigate the effects of electroacupuncture and diet restriction on body weight and on the levels of serum Lp(a), apolipoprotein A and apolipoprotein B in obese women.

Materials and Methods

In this study, EA application was performed in a private acupuncture treatment clinic, and serum samples were studied at the laboratories of the Department of Physiology, the Meram Faculty of Medicine of Selcuk University.

Subjects

Fifty-eight women were studied in 3 groups as follows: 1) Electroacupuncture (EA) (n = 20; mean age = 40.55 ± 5.30, and BMI = 35.65 ± 3.84); 2) Diet restriction (n = 23; mean age = 42.91 ± 4.02, and BMI = 34.78 ± 3.29) and 3) Placebo electroacupuncture (n = 15; mean age = 41.47 ± 4.61, and mean body mass index {BMI} = 33.43 ± 3.10). No statistically significant differences were found in the mean values of age, height, body weight, and body mass index among these three groups (Table 1).

The Determination of Acupuncture Points

Acupuncture points were determined with a measure unit called "Personal Cun" which is used in traditional Chinese medicine and with an electronic detector that gives off a special light when it comes onto the point.

Selected Ear and Body Acupuncture Points

The Hungry, Shen Men and Stomach ear points and the Hegu (LI 4), Quchi (LI 11), Tianshu (St 25), Zusanli (St 36), Neiting (St 44) and Liv 3 body points were selected for the obesity treatment.

Table 1. Changes on Body Weight and in Serum Lipoprotein (a), Apoprotein A, Apoprotein B Levels in Subjects after the EA, Diet and Placebo EA Therapy

	EA Group	Diet Group	Placebo EA Group	p
Body Weight				
1st day	84.1 ± 5.6	83.4 ± 6.7	80.5 ± 1.7	0.001
20th day	80.2 ± 5.5**	81.0 ± 3.9	78.3 ± 1.7	
Lipoprotein (a)				
1st day	24.5 ± 2.7	26.0 ± 5.1	24.3 ± 5.5	0.05
20th day	22.1 ± 3.4*	24.7 ± 4.0	27.2 ± 4.7	
Apoprotein A				
1st day	159.3 ± 25.2	159.0 ± 22.0	160.1 ± 20.3	0.60
20th day	152.7 ± 14.4	150.8 ± 17.4	159.2 ± 24.2	
Apoprotein B				
1st day	108.7 ± 23.3	108.4 ± 14.6	112.0 ± 21.2	0.05
20th day	101.3 ± 21.3*	107.0 ± 15.6	118.6 ± 17.9	

*$p < 0.05$ — The statistical difference of the EA compared to placebo EA groups.
**$p < 0.001$ — The statistical difference of the EA compared to diet and placebo EA groups.

Ear points:

1. The Hungry ear point is located at the junction of the lines drawn horizontally from the apex tragus and vertically from the intertragic notch.
2. The Shen Men point is at one third of the lateral side of the upper edge of triangular fossa.
3. The Stomach point is at an area where helix crus terminates.

Body points:

1. The LI 4 point of the body point is located at the dorsal face of hand between first and second metacarpal bones and in the middle of the radial side of the second metacarpal bone (Fig. 1).
2. The LI 11 point is located between the Lu 5 (Chise) and the lateral epicondilus of the humerus at the end of transvers cubital line when the elbow is in flexion position. This

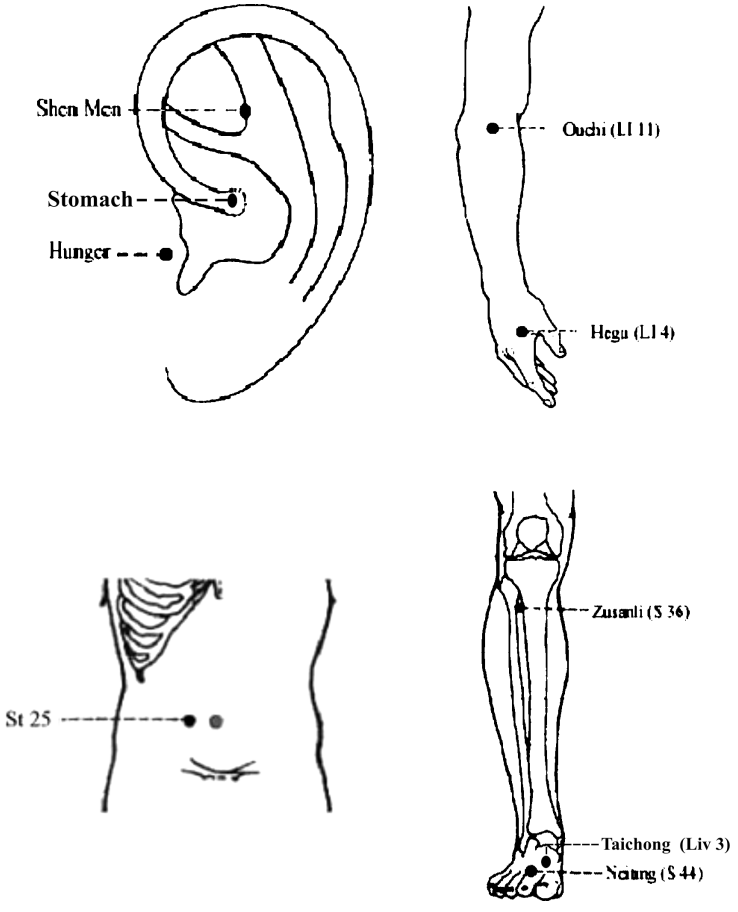

Figure 1. Schematic diagrams of the acupuncture points stimulated.

point is the most lateral point of the elbow transversal curve when the arm is in maximum flexion position (Fig. 1).
3. The St 25 point is 2 cun lateral of the umbilicus (Fig. 1).
4. The St 36 point is 3 cun below the patella lower edge and between the tibials anterior muscle and flexor digitorium communis muscle (Fig. 1).
5. The St 44 point is between the second and third phalanges on the foot and at the lateral and distal side of the second metatorsodigital joint (Fig. 1).
6. The Liv 3 point is in the depression distal to the junction of the first and second metatarsal bones, on the dorsal of the foot.

Electroacupuncture Application to Ear and Body Points

Ear and body EA application were performed daily for 30 min over 20 days, everyday at the same time from 8:30 am to 9:00 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 and Shen Men points on each ear. 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 alternanses. 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 patients were asked to stimulate the needles implanted at the acupoints by pressing them with their fingers before meals and when the patients felt hungry.

The 12 women in the control group were subjected to placebo. 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 in the EA group.

Diet Program

A 1,470 kcal diet was prepared for women in 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.

Weight and the 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) to the square of the corresponding heights (m^2).

Preparation of Samples

Blood samples (5 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 before breakfast. Blood samples were centrifuged at 1,000 rpm for 10 min. The supernatants were collected and kept in − 20°C until the analysis in the laboratory.

Determination of Serum Lipoprotein (a) Level

The Lp(a) test is based upon the reactions between Lp(a) and latex-covalently bound antibodies against Lipoprotein-A. This test is used with "Lp(a) Latex Autom" kit (Sentinel Ch., Milan, Italy). The mean value of the test was between 0 and 30 mg/dL.

Determination of Serum Apolipoprotein A1 and Apoprotein B Levels

Serum apolipoprotein A1 and apoprotein B levels were determined with "Olympus System Reagent 800" (Olympus Diagnostica GmbH, Lismeehan, Ireland). When a sample is mixed with buffer and antiserum solution, they will react with their specific anti-human antibodies to yield insoluble aggregates. The mean value of the test for apolipoprotein A1 and apoprotein B were between 73 and 169 mg/dL and 58 and 138 mg/dL, respectively.

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 value of less than 0.05 was considered to be statistically significant.

Results

Changes in Weight Loss

There was 4.7% weight reduction in patients with electroacupuncture application, whereas patients on diet restriction and placebo acupuncture had 2.9% and 2.8% weight reduction respectively (Table 1). The weight reduction in EA group ($p < 0.001$) was more significant than that of both the diet restriction and placebo acupuncture groups. No differences were found between the diet restriction and placebo acupuncture groups.

Lipoprotein (a), Apoprotein A and Apoprotein B Levels

There were significant decreases in lipoprotein (a) ($p < 0.05$) and apoprotein B ($p < 0.05$) levels in the EA group compared to the placebo acupuncture group and no changes in

apoprotein A levels in the EA, diet and placebo acupuncture groups. There were no differences on levels of lipoprotein (a) and apoprotein B in the diet group compared to the EA and placebo EA groups (Table 1).

Discussion

In this study, we observed weight loss along with decreases in lipoprotein (a) and apoprotein B levels in obese women by using ear and body electroacupunctures. There were no changes in lipoprotein (a) and apoprotein B levels in the diet and placebo EA groups. Our study suggests that EA application is more effective than placebo EA and diet therapy for weight loss in obese women. In the placebo EA group, the acupuncture needles were inserted superficially into selected body points that were not acupuncture points. Acupuncture points are different in the distribution of the somatosensory receptors and the number of free nerve endings. They are also the dense loci for nocireceptors, golgi-tendon receptors, Meissner corpuscles, Krause's end-bulbs and glomus-bodies (Kho and Robertson, 1997). It is expected that there are certain differences in the effects of inserting the needle between receptor dense acupuncture points and the other side of the body as not acupuncture points.

Qunli and Zhicheng (2005) investigated the therapeutic effects of ear acupuncture, body acupuncture and the combined use of both in the treatment of obesity. In the study, it was concluded that 5 kg weight loss was observed in 46% of test subjects with the combined use of ear and body acupunctures, in 35% with body acupuncture and in 27% with ear acupuncture for 24 days. In this study, there was 3.9 kg weight reduction in patients with electroacupuncture body and ear application for 20 days. They observed that the effects of body and body plus ear acupunctures were obviously superior to those of ear acupuncture alone and the effects of the combined use of ear and body acupunctures were better than those of body acupuncture alone.

Huang *et al.* (1996) observed a loss of 4.4 kg of body weight following an ear acupuncture application over 8 weeks in obese people. Diet and exercise programs were also included in addition to ear acupuncture. The diet program was planned to meet daily needs by calculating daily activity and other factors. The exercise program was planned to expend 300–500 kcal in one session 3 to 5 times weekly. While their study application was performed only to one ear on the selected ear points of Shen Men, Stomach, Triple Energy, Hungry every other session, we performed on the Hungry and Shen Men points alone but on both ears and additionally, we performed EA application to body points which they did not. We observed a 3.9 kg body weight loss with EA application only over a 20 day period without any exercise program. In this study we attribute more weight loss in a short period of time due to the use of both body and ear EA, the shorter interval between applications compared to the study of Huang and his colleagues.

Auricular and body acupuncture were effective in reducing obesity clinically (Cabıoglu and Ergene, 2005; Qunli and Zhicheng, 2005; Xu and Fei, 1985; Soong, 1975). Many overweight people are aware that diets can help with weight loss but have difficulty in suppressing their appetite. The principle involved behind the use of auricular and body acupuncture is to utilize the effects of stimulation of different meridian points effectively to restore normal

functions to the gastrointestinal tract (Mukaino, 1986; Asomoto and Takeshige, 1992). However, activation of satiety center in hypothalamus by means of auricular acupuncture is reported by Asomoto and Takeshige (1992) and this characteristic of auricular acupuncture may be helpful in controlling the intolerable hunger (Asomoto and Takeshige, 1992). Stomach and Hunger points were used to get fullness and satiety sensation (Huang *et al*., 1996). The stimulation of Hungry point creates an increase of fullness feeling and suppression of hunger feeling (Asomoto and Takeshige, 1992). Ear Hungry point was able to reduce the tone of the gastrointestinal tract (Jiang, 1986). The Shen Men point, which regulates the function of cerebral cortex, is used for tranquility and sedation (Mukaino, 1981; Wang and Kain, 2001). The stimulation of Stomach point regulates gastric functions (Li *et al.,* 1992). Stimulation of the LI 4 and LI 11 points has a regulatory effect on intestinal motility (Maciocia, 1989). The St 36 and St 44 points stimulate the satiety center in the ventromedial nucleus of hypothalamus and increase the fullness feeling (Zhao *et al.*, 2000). The stimulation of the St 36 point modulates the gastrointestinal motility causing an increase in bowel movement in people who have hypoactive gastrointestinal motility and an opposing decrease in people who have increased bowel motility (Li *et al.*, 1992). Furthermore, St 36 point has long been used to treat both diarrhea and constipation. Such normalization of colon function could be theoretically explained by impulses reaching the lumber plexus. The effects of such stimulation have been reported both in animals and humans (Li *et al.,* 1992; Jin *et al.,* 1992). In our study, we used EA application to the Hungry, Shen Men and Stomach points on both the ears and body points LI 4, LI 11, St 25, St 36, St 44 and Liv 3.

Lipoprotein (a) is a type of fat-protein molecule that resembles LDL cholesterol but has an additional piece to its structure called apoprotein (a). Lipoprotein (a) is a major component of the plaques found in the blood vessels of atherosclerosis patients. People with coronary artery disease exhibit elevated lipoprotein (a) (Scanu, 2001). Lp(a) exhibits trombojenic and aterojenic characteristics and may play a role in the treatment of damaged tissue (Hubinger *et al.*, 1997). Lp(a) is a major independent risk factor for cardiovascular disease especially coronary diseases (Angles-Cano, 1977). It was determined that increasing lipoprotein (a) along with LDL levels in blood raised the risk of arteriosclerosis (Hubinger *et al.*, 1997). The essential difference between Lp(a) and low density lipoproteins (LDL) is apolipoprotein A, a glycoprotein structurally similar to plasminogen, the precursor of plasmin, the fibrinolytic enzyme (Angles-Cano, 1977). Lip(a) contend to connect plasminogen receptors. Combining plasminogen receptors, Lip(a) blocks these receptors. Therefore, the more the quantity of lip(a) in plasma, the more the risk of thrombosis occurs. Whereas apoprotein A is the major protein of HDL, apoprotein B is the leading protein of lipoproteins except for HDL. Drexel *et al*. (1996) have determined that the levels of high plasma cholesterol, LDL cholesterol, triglyceride and apoprotein B are the factors that support periferic artery diseases.

Cabıoglu and Ergene (2005) applied body and ear acupunctures for 20 days to 22 women who had BMI between 30 and 40, and a 1425 Kcal diet program was given to 21 women under the same circumstances. In addition, there was a control group of 12 women. In the study associated with body weight, levels of the serum total cholesterol, triglyceride, HDL cholesterol and LDL cholesterol in obese women were examined. Hungry and Shen Men ear points, and the Hegu (LI 4), Quchi (LI 11), Tianshu (St 25), Zusanli (St 36), Neiting

(St 44) and Taichong (Liv 3) body points were used in acupuncture group. There was a 4.8% weight reduction in obese women with electroacupuncture application, whereas obese women in diet restriction had a 2.5% weight reduction. There were significant decreases in total cholesterol and triglycerides levels in EA and diet groups compared to the control group. Furthermore, there was a decrease in LDL levels in the EA group compared to those in the control group. In our study, there was a 4.6% weight reduction in obese women with electroacupuncture application, whereas obese women in diet restriction had a 2.8% weight reduction. Furthermore, there were significant decreases in lipoprotein (a) and apoprotein B levels in the EA compared to the control group. In both studies, we observed that there was approximately similar weight loss with the same method. Since the levels of total cholesterol, LDL cholesterol and triglyceride are high, it is thought that lip(a) and apoprotein B should be examined. In this study, we applied ear and body EA with 2 Hz current frequency by adding stomach point on the ear in obese woman. As our previous study, we examined weight loss as a result of this application and the levels of lip(a), apoprotein A and apoprotein B in serum. There was a 4.7% weight loss with EA for 20 days, we also observed a reduction in lip(a) and apoprotein B levels.

It has been determined that electroacupuncture application with different current frequencies causes the secretion of different endogen opioids. It has also been observed that low current frequency (2 Hz) electroacupuncture application increases the concentration of endomorphins, enkephalins and β-endorphin but high current frequency (100 Hz) electroacupuncture application increases the concentration of dynorphin in the central nervous system (Han *et al*., 1999; Cabioğlu and Ergene, 2006). The studies which showed the lipolytic activity of pro-opiomelanocortin products were performed as *in vivo* and *in vitro* studies on animals (Schwandt, 1985; Richter *et al.,* 1983; Richter and Schwandt, 1985). Richter *et al.* (1983) investigated the lipolitic activity of beta endorphin in the isolated fat cells of rabbits *in vivo*. It was determined that as a result of the effect of β-endorphin on fat cells, the levels of free fatty acid and glycerol were increased in the rabbit plasma. This effect was blocked by naloxone. Vettor *et al.* (1993) studied the lipolytic activity of β-endorphin in isolated human fat tissue. In their study, it was observed that BE application caused the increase of glycerol secretion from isolated fat cells, naloxone inhibited this effect.

Our results suggest that electroacupuncture therapy is an effective therapy for weight loss in obese women. The risk factors for cardiovascular diseases associated with obesity may be decreased since electroacupuncture application decreases the plasma lipoprotein (a) and apoprotein B levels.

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