The American Journal of Chinese Medicine, Vol. 34, No. 1, 1–11

Institute for Advanced Research in Asian Science and Medicine

Changes in Serum Leptin and Beta Endorphin Levels with Weight Loss by Electroacupuncture and Diet Restriction in Obesity Treatment

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Abstract: This study aims to investigate the role of changes in leptin and beta endorphin (BE) levels in weight loss following electroacupuncture (EA) application in obesity treatment. EA was applied to 20 females who were 41.45 ± 4.71 years old and had a body mass index of 36.00 ± 2.66; and a diet program was applied to 20 females who were 42.30 ± 4.35 years old and had a body mass index of 34.90 ± 3.21. There was a 4.5% weight reduction in the patients with EA application, whereas patients on diet restriction had a 3.1% weight reduction. A decrease of loss of body weight was observed in the EA group ($p < 0.000$) when compared against the diet restricted group. A decrease of serum leptin levels ($p < 0.000$) and an increase in the serum BE ($p < 0.05$) levels were observed in the EA group compared to the diet restricted group. In this study, reduced serum leptin levels paralleling to weight loss were observed in the EA group. Furthermore, it is thought that in the EA applied group, increasing serum BE level probably enhanced the lipolitic activity which may have caused weight loss in obese people by mobilizing energy stores. It may be considered that the EA application with diet restriction in obesity treatment is more effective than the diet restriction alone.

Keywords: Electroacupuncture; Obesity; Weight Loss; Leptin; Beta Endorphin.

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Introduction

Obesity is the overloading of fat depots in the body. It is considered a problem of energy imbalance. If the energy intake is higher than consumption, the fat ratio in body mass and the body weight increase. Obesity is one candidate for becoming the most prevalent health problem in this century (Palou *et al.*, 2000) and it is already the biggest problem in many developed countries (Leonhardt *et al.*, 1999). Genetic, neurological, cultural, psychological and endocrine disorders are some of the main causes of obesity (Lyznicki *et al.*, 2001).

Treatments for obesity include diet modification, physical activity, behavioral therapy, pharmacotherapy, surgery intervention, acupuncture application or combination of these applications. When a low calorie diet is applied to obese subjects, a reduction occurs in the body fat mass (Zuti and Golding, 1976). As a result, weakness and psychiatric symptoms may develop because of the negative nitrogen balance (Huang *et al.*, 1996). Diet alone does not contribute to the reduction of appetite (Richards and Marley, 1998; Ernst, 1997). The reduction of body weight by obese people using aerobic exercises alone, weight is usually regained after giving up the program (Smith, 1976). The use of appetite suppressant drugs or surgery intervention may not be effective in the long term treatment of obesity (Huang *et al.*, 1996).

Acupuncture is one of the oldest, well known traditional Chinese treatment methods. It is accepted that the most important mechanism of the acupuncture is its effect on endogenous opioids. It has long been known that electroacupuncture (EA) application has an analgesic effect on the applicants. Although there are many studies on the analgesic effect of acupuncture, studies on the effect of EA on metabolism are very rare. It is known that the EA application on obese people leads to some reduction of their body weight (Zhan, 1993; Sun and Xu, 1993). Ear acupuncture application controls the feeling of hunger (Richards and Marley, 1998; Asomoto and Takeshige, 1992) and it is reported that increased beta endorphin (BE), which is one of the endogenous opioids in plasma, following EA application affects the lipid and carbohydrate metabolisms (Jean-Baptiste and Rizack, 1980; El-Tayeb *et al.*, 1985; Richter *et al*., 1987).

Leptin plays a role in appetite, energy balance and regulation of neuroendocrin and immune functions (Ahima and Flier, 2000; Inui, 1999). Leptin exists mainly in fatty tissue but also exists in low concentrations in placenta, skeletal muscle and brain, stomach and mammalian epithelial tissues (Ahima and Flier, 2000). Leptin is synthesized by ob genes, and often creates its effect in conjunction with its specific receptors in the brain. The formation and secretion of leptin is mainly controlled by the fatty tissue. The plasma leptin levels parallel body weight and body fatty mass. Leptin normalizes the decreased metabolic rate and leads to reduction in the appetite (Inui, 1999).

The aim of this study was to investigate the weight loss of obese people with EA and diet restriction and to study changes of serum BE and leptin levels following these applications.

Materials and Methods

This study was carried out between September 2000 and September 2003. Each 20 days period three or four patients were taken to the study. EA application was performed in a private acupuncture treatment clinic. Serum samples were studied at the laboratories of the Department of Physiology, Meram Faculty of Medicine, Selcuk University.

Subjects

Forty volunteer women were taken into the study. The study included women aged between 35 and 50 with a body mass index (BMI) between 30 and 40. Necessary information was given to all volunteers prior to the application. Subjects were divided into two equal groups: an acupuncture group and a diet group. Forty women were studied in two groups as follows: (1) EA (n = 20; mean age = 41.4 ± 4.7, body weight = 94.6 ± 10.0 kg and BMI = 36.0 ± 2.6) and (2) Diet restriction (n = 20; mean age = 42.3 ± 4.3, body weight = 85.0 ± 8.5 kg and BMI = 34.9 ± 3.2) (Table 1).

The Determination of Acupuncture Points

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

Selected Ear and Body Acupuncture Points

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

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 placed at one-third of the lateral side of the upper edge of trianguler fossa.

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

The LI 4 point is located at the dorsal face of hand between the first and second metacarpal bones and in the middle of the radial side of the second metacarpal bone (Fig. 1). The LI 11 point is located between the Lu 5 (Chise) and the lateral epicondyle of the humerus at the end of transverse cubital line when the elbow is in flexion position. This point is the most lateral point of the elbow transversal curve when the arm is in maximum flexion position (Fig. 1). The St 25 point is 2 cun lateral of the umbilicus (Fig. 1). The

Table 1. The Age, Body Weight, Height and BMI of Electroacupuncture and Diet Groups

	EA Group	Diet Group
Age	41.4 ± 4.7	42.3 ± 4.3
Body Weight (kg)	94.6 ± 10.0	85.0 ± 8.5
Height (m)	1.61 ± 0,04	1.57 ± 0,04
BMI	36.0 ± 2.6	34.9 ± 3.2

The values are given with mean ± SD.

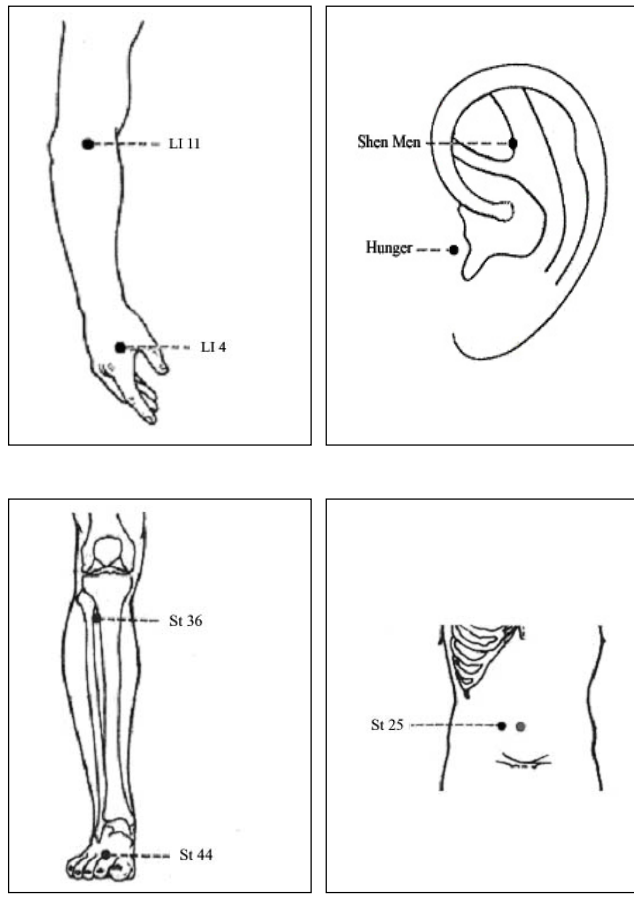

Figure 1. Schematic diagrams of the acupuncture points stimulated.

St 36 point is 3 cun below the patella lower edge and between the tibials anterior muscle and flexor digitorium communis muscle (Fig. 1). 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).

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 ventral medial nucleus of hypothalamus and increases the fullness feeling (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 an opposing decrease in people who have increased bowel motility (Li *et al.*, 1992).

Electroacupuncture Application

Body and ear EA applications were performed for 30 minutes for 20 days between 8:00 am and 8:30 am. Body EA was performed every day and ear EA was performed every other day. After EA application, permanent ear needles were placed on the Hungry points. The body acupuncture needles were 5 cm long and the ear acupuncture needles were 3.5 cm long, with a 0.22 mm diameter. Electrical stimulation was given by a pulse generator for 0.05 ms at 2 Hz frequency at 3 V with positive and negative alterations in square wave form. A Biothron instrument was used for this application. 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.

Diet Program

A diet of 1400 kcal was given to the subjects in both the diet and EA groups that provided the minimal calorie intake over their basal metabolism. Subjects continued their routine daily activities as before. The diet program was explained to the patients prior to the study, their full consent was taken and calorie intake continuously controlled every day for the EA group during the study procedure.

Weight and the Height of the Subjects

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

Preparation of Samples

Prior to the acupuncture application, diet program and after both applications, 6 ml intravenous blood samples were collected from the subjects in the morning between 8.00 and 9.00 am. Blood samples were centrifuged at 1000 rpm for 10 minutes. The supernatants were obtained and stored at −80°C for further analyses.

Serum Leptin Levels

The serum leptin levels were determined with the radioimmunoassay method (RIA, Linco Research Inc.). In this method, the result is obtained by the measurement of the anti-leptin antibody which reacts with human leptin. Expected values are between 5.6 and 29.3 ng/ml. The method consisted of the measurement of specific antiserum against antigens by RIA, containing radioactive antigen, separation of antigen-bound antibodies from non-bound antibodies and the determination of radioactivity.

Serum Beta Endorphin Levels

The serum BE levels were determined by the immunoradiometer method, using a BE Elisa immunoassay kit (EIA, Phoenix Pharmaceuticals Inc.). The working procedure of the kit was the measurement of antibodies labeled with ^{125}I reacting against BE. Following measurement with the kit, the mean levels of BE was determined to be 33 pg/ml in serum and 29 pg/ml in plasma.

Statistical Analysis

The statistical analyses were performed with the SPSS for Windows program. The differences between groups and between values before and after each group were calculated as mean ± standard deviation. Independent samples t-test were used in the statistical analyses. $p < 0.05$ was considered to be statistically significant.

Results

Weight Loss with EA Applications and Diet Program

Mean body weight decreased 4.5% in the EA group and 3.1% in the diet restricted group. A decrease of loss of body weight was observed in the EA group ($p < 0.000$) when compared against the diet restricted group by the independent samples t-test (Table 2).

Serum Leptin Levels after EA Applications and Diet Program

When the serum leptin levels ($p < 0.000$) of the pre- and post-EA and diet restricted groups were compared by the independent samples t-test, a decrease of serum leptin levels were observed in the EA group according to the diet restricted group. Serum leptin levels were decreased from 26.2 ± 6.4 ng/ml to 17.4 ± 5.5 ng/ml and from 23.4 ± 7.3 ng/ml to 20.9 ± 6.6 ng/ml in the EA and diet restricted groups, respectively (Table 2).

Table 2. The Body Weight, Leptin and Beta Endorphin Levels of Electroacupuncture and Diet Groups

	EA Group	Diet Group	T	P
Body Weight (kg)				
1st Day	94.6 ± 10	85.0 ± 8.5		
20th Day	90.4 ± 10†	82.4 ± 8.4	11,45	0,000
Leptin (ng/ml)				
1st Day	26.2 ± 6.4	23.4 ± 7.3		
20th Day	17.4 ± 5.5†	20.9 ± 6.6	4,31	0,000
Beta Endorphin (pg/ml)				
1st Day	16.4 ± 3.8	2.1 ± 6.8		
20th Day	21.4 ± 4.5*	20.8 ± 7.8	2,57	0,014

The values are given with mean ± SD. $^{*}p < 0.05$ according to the diet group, $^{\dagger}p < 0.000$ according to the diet group.

Serum BE Levels Following EA Application and Diet Program

When the serum BE levels of the pre- and post-EA applications and diet programs were compared by the independent samples t-test, an increase in the serum BE ($p < 0.05$) levels were observed in the EA group according to the diet program group. Serum BE levels were increased from 16.4 ± 3.8 pg/ml to 21.4 ± 4.5 in the EA group (Table 2).

Discussion

It has been reported that acupuncture application in obesity treatment is effective in weight loss (Ernst, 1997; Lei, 1988) by modifying appetite (Shiraishi *et al*., 1995) and emotional factors (Mulhisen and Rogers, 1999).

Zhao *et al*. (2000) also determined that when EA application was performed on the Zusanli (St 36) and Neiting (St 44) points, using rats with experimental obesity, the irritability of the satiety center in the ventral medial nucleus of hypothalamus increased. As we used the same points, we noticed a similar effect, so we think that EA application to these points causes satiation and leads to less food intake and a corresponding reduction in the body weight of our subjects.

Sun and Xu (1993) applied acupuncture treatment to the ear and body points to 110 obese patients. In their study, small seeds used as one method in traditional Chinese acupuncture, were placed on the ear points of Stomach, Mouth, Esophagus, Shen Men, Lung and Endocrine of a single ear every 3 to 5 days, and on the opposing ear in the subsequent session. In our study, we performed EA application on the Hungry and Shen Men points on both ears. In addition while Sun and Xu observed 5.0 kg body weight loss by applying acupuncture once on the body acupuncture points of St 25, St 36, Sanyinjiao (Sp 6), P6 and Fenglong (St 40) every 3 to 5 days over a 3-month period, we observed a body weight loss of 4.2 kg over a 20-day time period by EA application to the LI 11, LI 4,

St 36 and St 44 points and by acupuncture application to the St 25 point in addition to the ear points. We think that the greater weight loss of subjects in a shorter period of time was due to application of ear acupuncture every other day and body acupuncture every day and not at 3- to 5-day intervals. In addition, we presume that these results are related to the EA which we applied.

Huang *et al.* (1996) observed a loss of 4.4 kg of body weight following an ear acupuncture application over 8 weeks to obese people. In addition to ear acupuncture, diet and exercise programs were also included. 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 three to five times weekly. While in their study application was only to one ear on the selected ear points of Shen Men, Stomach, Triple Energy, Hungry every other session, we used application to only the Hungry and Shen Men points but on both ears and additionally, we performed EA application to body points which Huang *et al*. did not use. We observed a 4.1 kg body weight loss with only EA application over a 20-day period without any exercise program. The reasons for this result we attribute to the use of EA, the shorter interval between application and the application to both body and ear points, notwithstanding that Huang and his colleagues employed more ear points and included a diet and an exercise program.

In our study, there was a 4.5% weight reduction in the patients with EA application and diet restriction, whereas patients on diet restriction alone had a 3.1% weight reduction. We realized that the ear and body EA applications combined with diet restriction were more effective for body weight loss than the diet restriction alone.

We have not found any published studies reporting a change in BE levels in the serum and central nervous system in obese people with acupuncture application. Increase in BE both in the central nervous system and plasma with EA application has been observed in humans (Jin *et al.*, 1996) and animals (Takeshige *et al.*, 1993; Pan *et al.*, 1996; Petti *et al*., 1998). It has been particularly observed that there is a strong relationship between an acupuncture-induced analgesic effect and the existence of BE in brain tissue (Ullet *et al.*, 1998) and that the analgesic effect of EA application is more effective than that of acupuncture application (Wang *et al.*, 1992). We similarly observed that BE levels in serum in obese people increased following EA application and EA application is more effective for weight reduction in cases of obesity. It has been observed that different endogenous opioids are released with EA application at varying frequencies. It was reported that the BE concentration in the central nervous system increased with a lower frequency (2 Hz) EA application (Ullet *et al.*, 1998; Han *et al.*, 1999). We similarly observed an increase in serum BE levels following EA application at 2 Hz frequency (Han *et al.*, 1999).

When BE was applied to isolated rabbit fat cells, free fatty acids and glycerol levels were found to be increased in the plasma that could be blocked by naloxone (Richter *et al.*, 1983). BE has been also shown to increase glycerol secretion from the isolated fatty cells (Vettor *et al.*, 1993). In these studies, the lipolitic activity of BE has been observed in animals (Richter *et al.*, 1987) and in humans (Vettor *et al.*, 1993). BE is more effective on glucagon secretion than insulin in obese people. This results in a reduction in the plasma insulin-glucagon molar rate (Giugliano *et al.*, 1988 and 1991; Cozzolino *et al.*, 1996).

We presume that the increased plasma BE levels, following EA application, mobilizes energy depots with both lipolitic effects and glucagon levels, therefore increasing effects that contribute to weight reduction.

There are no reports of the changes of leptin levels in serum and the central nervous system following acupuncture application; but changes of the serum leptin levels with weight reduction by diet modification program only. Weigle *et al*. (1997) observed a 21% weight reduction and a reduction of 76.3% in the plasma leptin levels in male subjects with a 700 kcal diet over 95 days, while we produced a 5.43% weight reduction in the EA group female subjects over 20 days and a 2.8% weight reduction in the 1400 kcal diet group. Our observed 33.4% decrease in the serum leptin levels in the EA group and a 15.1% decrease in the diet group showed a parallel to the study of Weigle and his colleagues concerning weight reduction and the reduction of serum leptin levels. In a different study, when plasma leptin levels were determined in normal and obese people, they were 7.5 ± 9.3 ng/ml and 31.3 ± 24.1 ng/ml, respectively (Considine *et al.*, 1996). Following a 800 kcal diet program on obese people, four individuals lost 10% of their body weight in 8 weeks and three in 12 weeks, while the serum leptin levels decreased 53% and the ob RNA concentration in fat tissue decreased 38%. In our study, a weight reduction of 5.4% in the EA group and 2.8% in the 1400 kcal diet group with a 33.4% reduction of serum leptin levels in the EA group and a 15.1% reduction in the diet group over 20 days showed that decreases in the serum leptin levels paralleled weight reduction as observed in the other study. This is similar to the serum leptin levels decrease concurrent with weight loss in animals and humans that other studies have observed (Maffei *et al.*, 1995; Kolaczynski *et al.*, 1996).

In conclusion, we observed decreasing serum leptin levels paralleling weight loss in the EA group. Along with this, we propose that an increase in serum BE levels likely increases lipolitic activity, contributing to weight loss in cases of obesity. It may be considered that the EA application with diet restriction in obesity treatment is more effective than the diet restriction alone.

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