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PRESS RELEASE

Effects of auricular acupressure combined with low-calorie diet on the leptin hormone in obese and overweight Iranian individuals

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ABSTRACT

Background Human leptin is a peptide hormone that is released from white adipocytes. The absence of leptin or its receptor leads to uncontrolled food intake, leading to obesity. In the present work, the effects of auricular acupressure combined with low-calorie diet on the leptin hormone level were investigated.

Methods Volunteers (n=86) with body mass indices (BMI) between 25 and 45 kg/m^2 were randomised into a case (n=43) or a control (n=43) group. Participants in each group received a low-calorie diet for 6 weeks. The case group was treated with auricular acupressure and the control group received a sham procedure. Plasma leptin levels, body fat mass, body weight and BMI were measured before and after treatment.

Results Participants who received auricular acupressure showed significant reductions in their plasma leptin levels (18.57%, $p<0.01$) as well as in their body fat mass (4%, $p<0.05$). These changes were not observed in the control group. The reduction in leptin was significantly greater in the acupressure group than the controls.

Conclusions Auricular acupressure combined with a low-calorie diet significantly reduced plasma levels of leptin. However, the mechanism of this reduction is not clear.

INTRODUCTION

Human leptin is a 167-amino-acid peptide hormone that is released from white adipocytes. Serum leptin concentration appears to reflect total body fat mass.[1 2] Leptin can also be synthesised by the brown adipose tissue, placenta, ovaries, skeletal muscle, stomach, mammary epithelial cells, bone marrow, pituitary and liver.[3 4] Stimulation of leptin receptors in the hypothalamic nuclei decreases fat storage and inhibits appetite through several mechanisms.[2 5–8] The absence of leptin or its receptor leads to uncontrolled food intake, leading to obesity.

Previous studies have shown that hyperphagia and obesity in mice or humans may be the outcome of mutations in leptin receptors or a consequence of inadequate leptin secretion by fat cells.[9] However, circulating levels of leptin do not decrease in people who are obese. This may be because plasma levels of leptin rise in proportion to the increased body fat mass.[10 11] Therefore, obesity may be associated with leptin resistance,[10 11] caused by defects at the level of leptin receptors or in post-receptor signalling pathways.[12] The combination of the two mechanisms could explain the obesity: first, the absence of leptin or its receptor leads to uncontrolled food intake in normal people;[2 5–8] second, the leptin resistance that is caused by defects in leptin receptors or the large increase in leptin signals in obese people.[10–12] However, obesity is now recognised as a low-grade inflammatory state that affects iron metabolic pathways through the iron regulatory peptide hepcidin.[13 14] Leptin, as a positive regulator of hepcidin, may improve iron metabolism disorders in obese individuals.[13 14] Also, mice with leptin or leptin receptor deficiencies are protected against the development of several inflammatory and autoimmune diseases. Thus, leptin appears to have a central role in immune response.[15] Although many complementary therapies have been proposed for the treatment of obesity, few are truly therapeutic.[16] Currently, the most popular treatments for obesity are diet, exercise, pharmacological agents, surgery and acupuncture.[17 18] Many overweight people are aware that diet can help them lose weight, but have difficulties suppressing their appetite.

In recent decades, the use of acupuncture has been growing.[18–25] It is claimed that acupuncture has useful long-term effects on body weight (BW) and appetite control;[26 27] however, some findings are in conflict with this claim.[28] Regardless, recent studies show

that exercise alone is not a good way to reduce weight through decrease in leptin and body fat mass.[29 30] Many studies have investigated the effects of acupuncture on weight loss and appetite control,[27 31–33] but only one study has reported the effects of auricular acupuncture alone on plasma levels of leptin hormone, body fat mass, body mass index (BMI) and BW in women.[24] Since blood leptin levels regulate the immune responses[15] and affect iron metabolic pathways through hepcidin expression,[13 14] leptin level regulation in obese people may play an important role in healthcare. Here, we examined the effects of combined auricular acupressure and low-calorie diet treatment on the plasma levels of leptin in overweight and obese people. If leptin levels could be controlled by auricular acupressure and low-calorie diet, it could be an alternative approach to reduce the complications of obesity.

MATERIALS AND METHODS

Study design and subjects

A total of 90 overweight or obese individuals were recruited from the nutrition clinic of Ghaem Hospital, Mashhad, Iran. They had received no other weight control treatments and had no medical or drug history within 3 months before their participation in the study. The participants were given verbal and printed information about the objectives of the study, were given time to discuss the study and were encouraged to ask questions. Those participants with diabetes, severe hypertension, heart disease, endocrine abnormalities, hepatocellular diseases, or those who were pregnant or refused to participate at any point, were excluded from the study (n=10). In all, 90 participants were eventually enrolled (16 men and 74 women) with an age range of 18–55 years and a BMI between 25 and 45 kg/m^2. All participants provided written informed consent, and all forms were filed in their medical records. The study was approved by the Ethics Committee of Mashhad University of Medical Sciences.

Anthropometric and biochemical assessments were performed before and after treatment. Subjects were enrolled in the study after checking for the inclusion/exclusion criteria, and were arranged into pairs matched by gender, age and BMI category. Using random numbers generated in Microsoft Excel (Redmond, Washington, USA), pairs were randomised into two equal groups (n=45).

One group designed as the case group received auricular acupressure with a low-calorie diet, and the other (control) received sham auricular acupressure with the same low-calorie diet. This report used the rounded BMI values of 25 and 30 as boundary values, 30>BMI≥25 as overweight and ≥30 as an index of obesity. The study design is shown in figure 1.

The participants were asked to follow a washout diet (isocaloric diet) for 2 weeks before starting the trial, and then consumed a low-calorie diet for 6 weeks. The low-calorie diet was observed on a 500 kcal energy deficit per day, below the individual's daily energy requirements. Resting energy expenditure was calculated with Harris and Benedict's equation,[34] and was used to determine the amount of food per day for each participant. The washout diet and the 6-week dietary programme for each participant were planned by a nutritionist based on the participant's energy expenditure. The participant's compliance was monitored every week.

Auricular acupressure treatment

Six standardised acupressure points were selected on the external ear according to the usual tenets of Chinese medicine and clinical experience: *Shenmen* (TF4), Stomach (CO4), hunger point, Mouth (CO1), centre of ear (HX1) and *Sanjiao* (CO17). Acupressure in the case group was applied with routine ear press plasters and seeds.[20] After sterilising the acupuncture points with 75% alcohol preparation pads, the acupuncturist applied the ear pressing plaster with the seed at the acupuncture points. Ear pressing plasters (*Vaccaria* ear seeds, Beijing Zhongyan Taihe Medicine, Beijing, China) were applied to acupuncture points on both ears in each treatment and were kept on the ear for 3 days.[20] All participants were requested to apply pressure to the auricular points before eating. Fresh pressing plasters were placed on the points twice a week for a total of 6 weeks.[20] The procedure was performed by an expert acupuncturist. No damage was observed in the acupressure treated auricles and no short-term adverse effects were reported.

Sham auricular acupressure in controls

The control group received sham auricular acupressure using placebo ear plasters without seeds (*Vaccaria* ear seeds). Hip (AH5), Spleen (CO13), Nose and Oesophagus (CO2) points were used for sham acupressure. The treatment procedure was the same as in the case group.

Anthropometric measurements

For all the patients, BW, BMI and body fat mass were measured with a body composition analyser Tanita BC-418 (Tanita, Tokyo, Japan) according to a standard protocol.[35] The participants' height was measured and recorded with a standard procedure. These variables were recorded at the beginning of the study and the day after the treatment ended. BMI was used to classify participants as overweight (25–29.9 kg/m^2) or obese (BMI≥30 kg/m^2). All measurements were recorded with a standard method between 7.00 and 8.00 am, after the participants had abstained from food since the previous evening.

Plasma samples

Blood samples were collected before and after treatment. Plasma was prepared within 2 h after blood collection and stored at −80°C until analysis. Haemolysed samples were excluded.

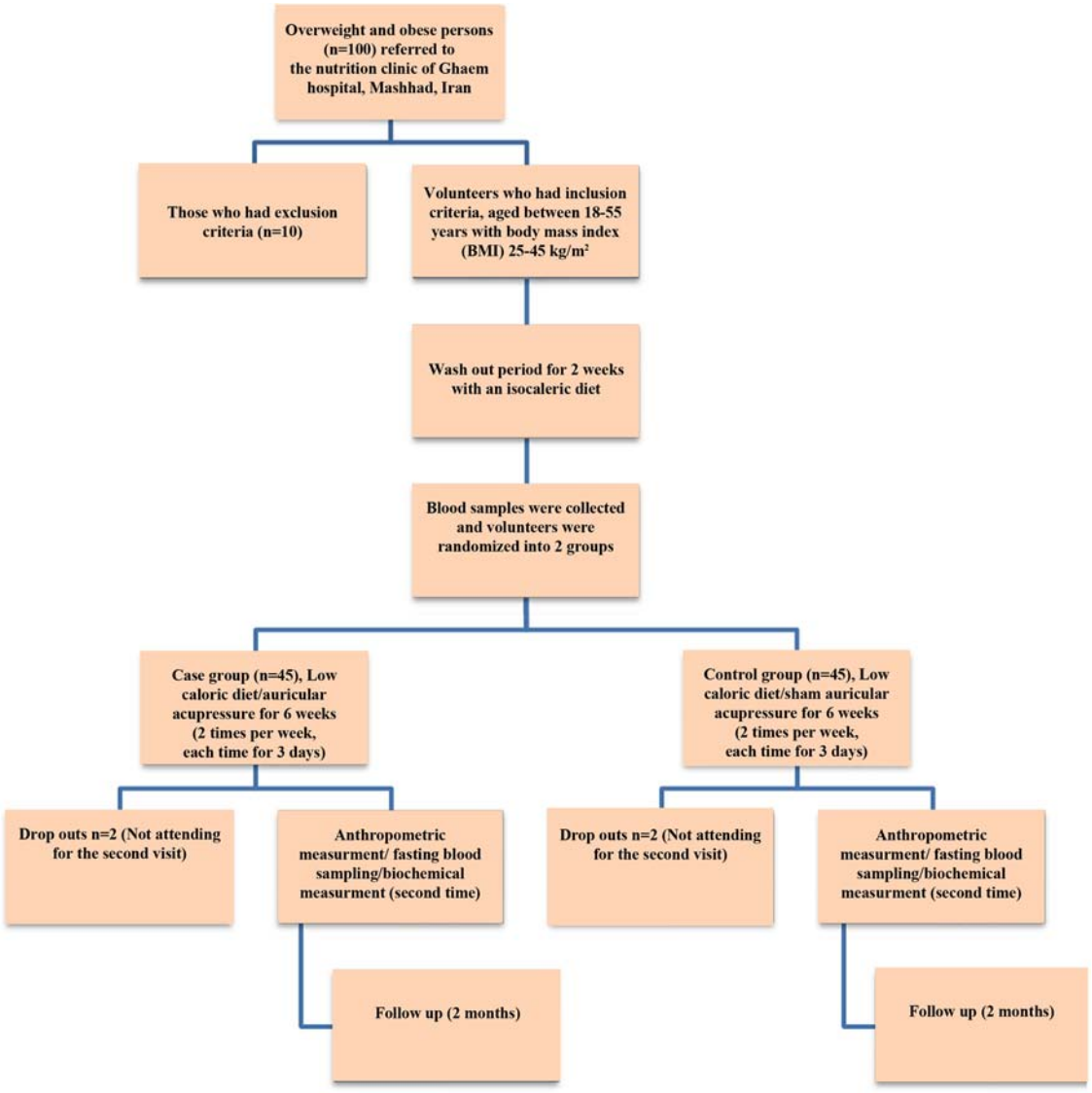

Figure 1 Flowchart related to the study design of this work.

Leptin hormone measurement

Leptin concentration in the plasma was measured in duplicate with a commercial sandwich ELISA kit (Human Leptin ELISA, Clinical range, Biovendor, Heidelberg, Germany).[36–38]

Statistical analysis

All statistical analyses were performed with SPSS software (V. 16, Chicago, Illinois, USA). The results are presented as mean±SD. Paired t tests were used to compare related samples and independent t tests were used to compare independent samples. In all cases, $p<0.05$ was considered as statistically significant.

RESULTS

In each group 43 participants completed the study and two dropped out at the end of the study due to personal reasons and long course of the treatment. There were no significant differences between the case and control groups when their mean values of age, sex, height, weight and BMI were compared (table 1). Likewise, body fat mass and plasma leptin levels did not differ significantly between the case and control groups before the treatment ($p>0.05$).

Volunteers in the case group showed significant reductions in their mean values of BW (3.4%, $p<0.01$) and BMI (3%, $p<0.01$) after treatment with combined low-calorie diet and auricular acupressure for 6 weeks. Mean values of BW (1.7%, $p<0.01$) and BMI (2%, $p<0.01$) also decreased significantly after 6 weeks of sham treatment. Although the percentage of weight loss in the case group was twice as great as in the control group, the difference was not statistically significant. Likewise, the total body fat mass did not differ significantly between the two groups.

Data in table 2 shows statistically significant reductions in the body fat mass and plasma levels of leptin

Table 1 Demographic data of case and control groups

Group	Sex	Number	Age, years	Height, m	BW, kg	BMI, kg/m²
Case	Women	37	37.62±9.49	1.58±0.06	81.20±11.64	32.23±3.92
	Men	6	37.00±7.61	1.69±0.06	88.95±9.44	30.80±1.40
	Total	43	37.57±9.26	1.59±0.06	81.89±11.58	32.11±3.78
Control	Women	37	37.54±9.83	1.58±0.05	79.13±9.64	31.85±3.97
	Men	6	38.40±9.83	1.67±0.07	90.44±6.26	32.60±1.41
	Total	43	37.65±9.71	1.59±0.06	80.50±9.96	31.94±3.75

Values are expressed as mean ±SD. Independent t tests were used to compare case and control groups and no significant differences were observed ($p>0.05$).
BMI, body mass index; BW, body weight.

Table 2 Effects of auricular acupressure on obesity indicators in overweight and obese participants

	Case group			Control group			
Variable	Pretreatment	Post-treatment	Within-group comparison (p value)	Pretreatment	Post-treatment	Within-group comparison (p value)	Comparison of the changes between case and control groups (p value)
Body weight, kg	81.89±11.58	79.09±11.84	0.001	80.50±9.96	79.08±10.51	0.001	0.1
BMI, kg/m²	32.11±3.78	31.12±3.73	0.001	31.94±3.75	31.28±3.90	0.001	0.6
Body fat mass, kg	31.21±7.90	29.90±7.74	0.01	30.63±7.00	30.02±7.40	0.2	0.2
Plasma leptin level, mg/m³	0.26.±0.14	0.21±0.13	0.001	0.23±0.12	0.22±0.13	0.4	0.007

Values are expressed as mean±SD. Paired t tests were used to compare variables before and independent t tests were used to compare the changes in values between case and control groups. No significant differences were observed between case and control groups at baseline ($p>0.05$).
BMI, body mass index.

in the participants treated with auricular acupressure for 6 weeks. The control group showed no significant changes in these variables after 6 weeks of sham treatment. There was a significant difference in plasma levels of leptin between the groups.

As summarised in table 3, a highly significant ($p<0.01$) positive correlation was observed between plasma levels of leptin and the obesity indices after the real and sham treatments used in this study.

DISCUSSION

Acupuncture has been found effective in weight loss and suppression of appetite in some studies[39 40] though not in all.[24] Secretion of leptin may be an important means by which the adipose tissue signals the brain that enough energy has been stored and intake of food is no longer necessary.[2] Thus, interfering with the leptin system is one of the possible mechanisms by which auricular acupuncture controls appetite and induces weight loss.[39] However circulating concentrations of leptin are elevated in obese people and rodents.[41] The high levels of leptin may result in leptin resistance which appears to be a major factor in obesity.[10–12] The main finding of this study is that acupressure reduced plasma levels of leptin in overweight and obese participants. Although Hsu *et al* studied the effects of auricular acupuncture on obese women, we included both genders. Also, they used auricular acupuncture alone as a main treatment[24] but volunteers in our study were subjected to auricular acupuncture and a low-calorie diet. In another method of acupuncture Kang *et al* employed body electroacupuncture and ear point tapping and pressing treatment for subjects in the case group.[39] This method of treatment also showed to be effective in reducing serum leptin levels and BW.

Another finding of our study was that acupressure caused a decline in total body fat mass of subjects in the case group. This decrease is in line with our results regarding the decreased levels of plasma leptin in the case group. Notably, adipose tissue is the major source of circulating leptin; therefore, lowering effects of acupressure on plasma leptin may be due to a decrease in the body fat mass. This notion is also supported by the positive correlation we observed between plasma levels of leptin and body fat mass. However, the exact mechanism of the reducing effects of acupressure on the plasma leptin remains to be elucidated. Further research will also be needed to understand the mechanism by which auricular acupressure affects body fat mass.

Blood leptin levels regulate the immune responses[15] and affect iron metabolic pathways through hepcidin expression.[13 14] Indeed, hepcidin may play an important role in hypoferremia of inflammation in obese people. The expression of hepcidin is regulated by iron homeostasis, hypoxia, inflammation and leptin. Thus, obesity

Table 3 Correlation between the changes in plasma leptin concentrations and obesity indices after auricular acupressure

Changes		Leptin hormone levels		
		Case	Control	Case and control
Body fat mass	r	0.466	0.175	0.345
	p Value	0.003	0.234	0.002
Body weight	r	0.576	0.493	0.573
	p Value	0.001	0.001	0.001
Body mass indices	r	0.573	0.565	0.596
	p Value	0.001	0.001	0.001

$p<0.05$ was considered significant, $p<0.01$ was considered highly significant.
r, Pearson's correlation coefficient.

treatment with combined treatment in obese people may have an important role in reduction of hepcidin and hypoferremia compensation through leptin decline. Therefore, in our study, we just evaluated the level of leptin after combined treatment and our interest has now shifted to the identification of hepcidin and iron metabolism changes in future studies.

Although participants in the case group showed a reduction in mean values of their BW and BMI after 6 weeks of treatment, but the difference between these mean values and those of subjects in the control group did not achieve significance. These findings are not consistent with those of other investigators who found significant changes in BW after acupuncture.[24 28] The discrepancy may be in part because of the differences in demographic and anthropometric characteristics of the participants and in the duration of treatment. It would be of interest to test other auricular acupressure points in the future studies. We also recommend that similar studies be conducted in a metabolic clinic setting to better control other variables such as dietary intake (matched across case and control participants), and to include a third group that receives only diet treatments.

Conclusions

In participants who received a combination of low-calorie diet and auricular acupressure, plasma leptin levels decreased more than in participants who received sham auricular acupressure. Our results are consistent with the notion that auricular acupressure combined with dietary modification is a beneficial complementary treatment for reducing body fat mass. However, these findings should be reconfirmed by other studies with larger sample sizes and with a third group treated with a low-caloric diet alone.

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Contributors AAO, BZ, GF, MS, MGM, PM, SD, MD: designed and coordinated the study and wrote the manuscript. MGM, MN, MS: dietician and food advice. SD, MD, HA, PA: recruitment of subjects and collection of data. SD, MD, PM: carried out statistical analysis and the main writing process of the manuscript; were also responsible for the performance of the biochemistry laboratory resistance test analysis, and were involved in clinical and laboratory data collection. BZ, HA: acupuncturists. MRP, MHD, MS, MGM: took part at their respective clinical centres and were involved in interpretation of the data; also participated in the review of the final manuscript.

Competing interests None.

Patient consent Obtained.

Ethics approval The study was approved by the Ethics Committee of Mashhad University of Medical Sciences.

Provenance and peer review Not commissioned; externally peer reviewed.

Summary points

- Acupressure is sometimes used to assist weight loss.
- A 6 week course of acupressure reduced leptin levels significantly compared with sham acupressure.

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