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The Effect of Acupuncture and Diet on Serum Leptin and Nesfatin-1 Levels in Overweight/Obese Individuals
Aşırı Kilolu/Obez Bireylerde Akupunktur Ve Diyetin Serum Leptin Ve Nesfatin-1 Düzeylerine Etkisi

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Abstract
Acupuncture applications and diet are frequently used in obesity treatment. We aimed to investigate the changes in serum leptin and nesfatin-1 levels measured before and after acupuncture and diet in overweight/obese individuals in the presented study. This interventional study was carried out on 90 overweight/obese patients. Participants were divided into three groups: Group 1 (Acupuncture), Group 2 (Diet), and Group 3 (Those who did not take any attempts to lose weight- control group). Serum leptin and nesfatin-1 levels were measured before and after the intervention.

The 30th days leptin values were significantly decreased in the acupuncture group (**p=0.040**) while they were significantly increased in the control group (**p=0.039**). It was detected that the nesfatin-1 values were significantly increased in both acupuncture **(p=0.032)** and diet groups **(p=0.017).** Also, body weights significantly decreased both in acupuncture **(p=0.032)** and diet groups **(p<0.001).** Our results support the effects of acupuncture treatment on appetite hormones. Further research on the mechanisms of endogenous and exogenous actions of the recently discovered hormones, leptin and nesfatin-1, are needed.

Keywords: Acupuncture, Diet, Leptin, Nesfatin-1, Obesity.

Özet
Akupunktur uygulamaları ve diyet obezite tedavisinde sıklıkla kullanılmaktadır. Sunulan çalışmada aşırı kilolu ve/veya obez bireylerde akupunktur ve diyet öncesi/sonrasında ölçülen serum leptin ve nesfatin-1 düzeylerindeki değişiklikleri araştırmayı amaçladık. Bu müdahele çalışması 90 kilolu/obez hasta üzerinde yapıldı. Katılımcılar üç gruba ayrıldı: Grup 1 (Akupunktur), Grup 2 (Diyet) ve Grup 3 (Kilo vermek için herhangi bir girişimde bulunmamış olanlar- kontrol grubu). Müdahale öncesi ve sonrası serum leptin ve nesfatin-1 düzeyleri ölçüldü. Akupunktur grubunda 30. gün leptin değerleri anlamlı olarak azaldı (p=0.040), 3. grupta ise anlamlı olarak arttı (p=0.039). Nesfatin-1 değerlerinin hem akupunktur (p=0.032), hem de diyet (p=0.017) gruplarında anlamlı olarak arttığı saptandı. Ayrıca vücut ağırlıkları hem akupunktur (p=0.032) hem de diyet gruplarında (p<0.001) anlamlı olarak azaldı. Sonuçlarımız akupunktur tedavisinin iştah hormonları üzerindeki etkilerini desteklemektedir. Yakın zamanda keşfedilen leptin ve nesfatin-1 hormonlarının endojen ve ekzojen etkilerinin mekanizmaları hakkında daha fazla araştırmaya ihtiyaç vardır.

Anahtar Kelimeler: Akupunktur, Diyet, Leptin, Nesfatin-1, Obezite.

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Introduction

Obesity is a global health problem that has spread rapidly all over the world in recent years. Besides, obesity raises the risk of health problems and adversely affects the quality of life and life expectancy.[1] The European Health Interview Survey reported that 36.9-56.7% of all women and 51-69.3% of all men were overweight or obese between 2008 and 2009.[2]

According to the Turkish diabetes epidemiology study (TURDEP-I) study, the prevalence of general obesity in Turkish adults was reported to be 22.3% between 1997and 1998. This rate was 30% for women and 13% for men.[3] Also, in the TURDEP-II study (2010), the overall prevalence of obesity among Turkish adults reached 31.2%. In the last 12 years, the prevalence of obesity has increased by 34% in women and 107% in men.[1,3,4]

The current treatment of obesity includes nutritional modifications, exercise, medications, acupuncture, surgical methods, and the combinations of these methods.[5]

Acupuncture based on traditional Chinese medicine since ancient China is a treatment method based on the stimulation of special ear or body points to regulate energy excess or deficiency. Most recently, acupuncture has been become a more widespread treatment method of obesity due to reasons such as ease of use and having almost no side effects.[5,6]

Leptin is a polypeptide consisting of 167 amino acids, which induces weight loss. The ob gene product leptin is an adipocyte tissue hormone and was first cloned from the adipose tissue in 1994 by Friedman (Rockefeller University). It has been determined that leptin limits weight gain by reducing food intake, and it circulates in plasma at levels that parallel the amount of body fat.[7]

Nesfatin-1, a satiation-inducing molecule found in the hypothalamus with 82 amino acids and a 9.7-kDa molecular weight, was first discovered in 2006 by Oh-I et al. Although the mechanisms of nesfatin-1 in relation to obesity have not been fully elucidated, it has been considered to interact with other anorexigenic molecules, in particular leptin or melanocortin.[8]

Leptin induces weight loss by both decreasing appetite and food consumption and increasing the activity and heat production.[7] Nesfatin-1, on the other hand, is useful in the physiological control of feeding behavior. It reduces the food intake by controlling the peristalsis of the gastrointestinal tract and therefore controls the body weight.[8]

Many non-pharmacological methods have been tried for obesity management and acupuncture has gained importance in the treatment of obesity with its promising results. The results of the literature review indicated that more research is needed on the mechanism of action of newly discovered hormones. In this study presented in this context, we examined the effects of acupuncture and diet on serum leptin and nesfatin-1 levels in overweight/obese subjects.

Materials and Methods

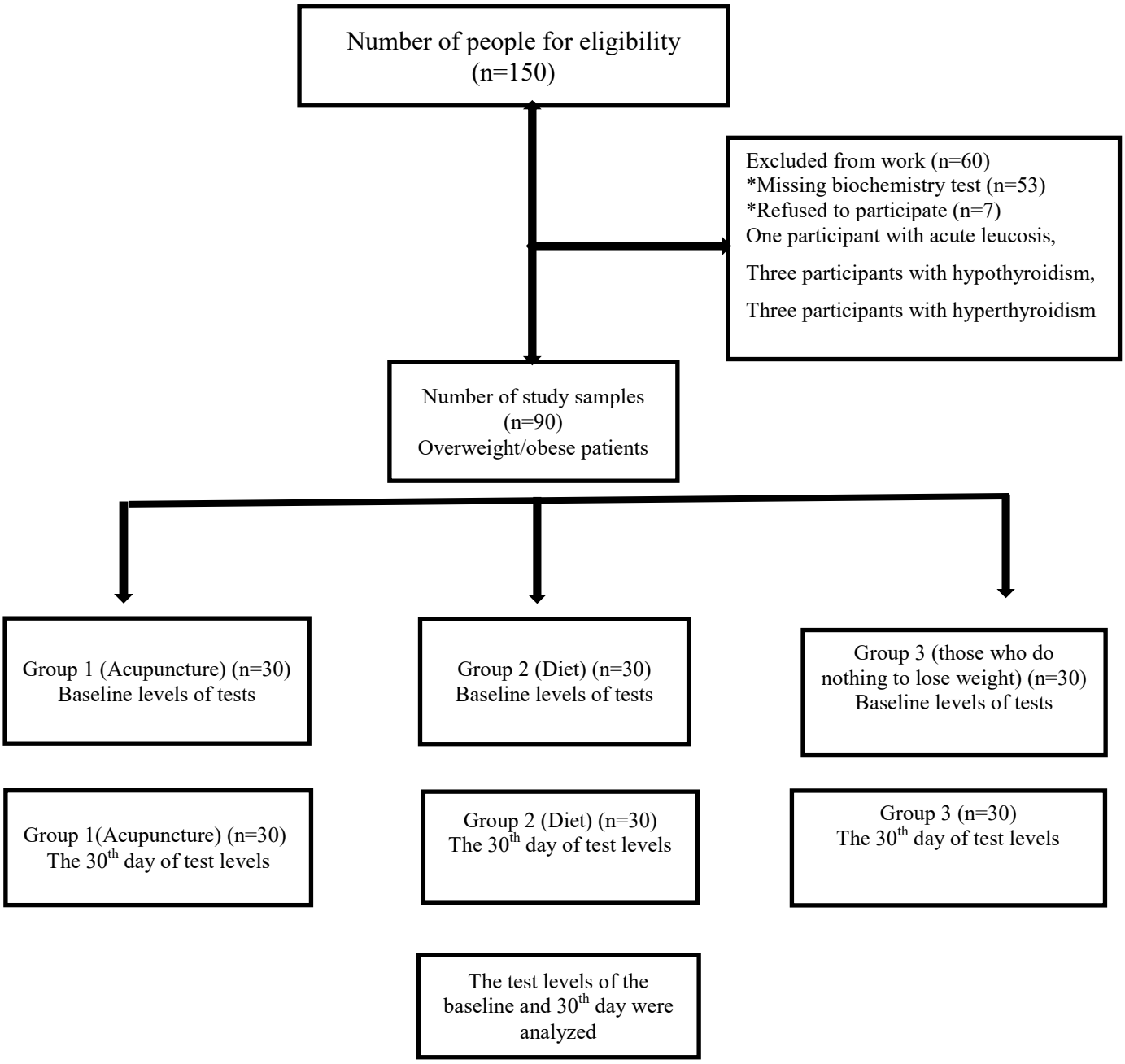
The type, place, and population of the study

This intervention study was carried out in the family medicine outpatient clinic between February 2018 and March 2018. A total of 150 participants were included in the study. One participant with acute leucosis, one participant with hypothyroidism, three participants with hyperthyroidism, and 55 participants who did not want to give a control blood sample were excluded from the study. Hence, the study was completed with 90 participants.

Sample and sampling method

In our study, the number of subjects included in the study was calculated using $n=t^2.p.q/d^2$ formula because the number of individuals in the universe was not known. According to this calculation, 90 women were included in our study.Individuals were assigned randomly to groups with equal chances. Based on random number tables, the participants were enrolled in the study after checking for the inclusion criteria, and were arranged into pairs matched by gender and body mass index (BMI) category. Using random numbers, pairs were randomly divided into three equal groups (n=30). The groups consisted of 30 individuals. The first group included the participants who received acupuncture (Group 1: Acupuncture group), the second group included those who applied to have a diet program (Group 2: Diet group), and the third group included those who did not take any attempts to lose weight (Group 3: Control group). Participants in Group 1 were referred to the Department of Traditional and Complementary Medicine for losing weight. Measurements were repeated after 30 days. The patients had information about the intervention. Seven patients were excluded as they stated that they would not continue the study.

WORKING FLOW CHART



Exclusion criteria

Patients who had a pregnancy, acute coronary artery disease, diabetes mellitus, liver and renal failure, depression, anxiety, severe psychotic disorders, malignancy, immunodeficiency, obstructive sleep apnea, and chronic obstructive pulmonary disease were excluded from the study. Those with body mass index (BMI) below 25 kg/m^2 were not included in the study.

Ethical considerations

Ethical approval for the study was obtained from the Meram Medical Faculty Ethics Committee (Approval date: 14.07.2017, number: 2017/998).

The participants were duly informed, and written and oral consent was obtained according to the principles of the Helsinki Declaration.

Anthropometric measurements

Height, weight, and waist circumferences of the participants were measured and recorded. Participants' height and weight were measured in a standing position with a standard stadiometer (Seca GmbH & Co. KG., Hamburg, Germany) after they were asked to take off their shoes and thick clothing. The BMI was calculated with the formula (BMI)=weight (kg)/height (m^2). The participants were categorized according to the BMI levels as thin (<18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2), and obese (30 and over kg/m^2).

Evaluation of body composition

A Bioelectrical Impedance Analysis device (Tanita InnerScan Body Composition Monitor, Tokyo, Japan) was used to measure and evaluate the body composition of the participants. Body fat mass, total body water, bone mass, muscle mass, basal metabolic rate (BMR), and visceral fat rating of participants were determined.

Content and application of the diet program

Daily energy requirements were calculated based on age, gender, weight, and height of the diet group. A personalized weight-loss plan was prepared by the nutrition and dietetics department. Care was taken to ensure that the energy content of the diet was not less than the rate of individuals resting energy expenditure (REE), in other words, basal metabolic rate (BMR). No additional products or substances were used for losing weight. After calculating the daily energy requirements (kilocalories), care was taken to ensure that the dietary contents were in compliance with the requirements in terms of carbohydrate (gr), protein (gr), fat (gr), vitamins, and minerals. The daily energy distribution was composed of 55-60% carbohydrates, 12-15% protein, 25-30% fat, and special diet programs were prepared for each individual. The contents of the diet consisted of foods in the healthy food pyramid.

Collection of blood samples

Blood samples after 12-hours fasting were collected at the beginning and end of the study from all participants. Blood samples were centrifuged at 4°C and 1000xg for 10 minutes by using a cooled centrifuge (Hettich Rotina 46R, Hettich Zentrifugen, Tuttlingen, Germany). The supernatant serum was separated and stored at a -80°C deep freezer (New Brunswick UC50, New Brunswick Scientific, New Jersey, USA) for leptin and nesfatin-1 analysis. Before and after the intervention, fasting blood sugar (FBS), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c) values were measured on the day of blood collection in each patient. Serum samples were stored at -80 °C in a New Brunswick U570 (New Brunswick Scientific, New Jersey, USA) refrigerator until leptin and nesfatin-1 levels were measured.

Serum leptin and nesfatin-1 levels were measured by enzyme-linked immunosorbent assay (ELISA) according to the procedures supplied by the Elabscience, China (E-EL-H0113 and E-EL-H2373, respectively).

Serum samples were used to determine leptin concentration, which was measured using a commercial Human Leptin ELISA Kit (Elabscience Biotechnology Co., Wuhan, China) according to the manufacturer's protocol (the intra-assay coefficients of variation (CV) and inter-assay CV were <5,59% and <6,21%, respectively). Serum samples were used to determine nesfatin-1/NUCB2 concentration, which was measured using a commercial Human Nesfatin-1 ELISA Kit (Elabscience Biotechnology Co., Wuhan, China) according to the manufacturer's protocol (the intra-assay CV and inter-assay CV were <5,54% and <6,73%, respectively). The human nesfatin ELISA Kit was particularly specific to nesfatin-1/NUCB2.

Determination of acupuncture points

There are different application points for acupuncture therapy. These points can be determined by different systems. In the traditional Chinese medical approach, which is the origin of acupuncture, "personal cun" was used as a measurement unit. The length between the phalanges of the middle finger of the patient gives the patients' personal cun. In traditional acupuncture, cun measurements are used to determine the locations of acupuncture points. On the other hand, parallel with the developments in technology, electronic devices based on the electrical properties of acupuncture points was developed in recent years. The point finder electrodes of these devices produce signals when they pass over an acupuncture point.[9] In this study, both the traditional cun measurement and acupuncture point detection with the electronic Agiskop DT (Agiscop DT point detector, Sedatelec, Lyon, France) device were used.

Acupuncture application points

Body and auricular acupuncture application were used in this study. The acupuncture points were cleaned with alcohol and then dried with clean cotton. Shen Men, and stomach ear points were selected for auriculotherapy. The following traditional Chinese medicine points were used for body acupuncture: Quchi (LI-11), Nei Guan (P6), ZuSanLi (St36), Neiting (St44), Taichong (Liv 3), and Yin-Tang.

Acupuncture therapy

Body acupuncture therapy was administered as two sessions per week for four weeks. Each treatment session lasted approximately 20 minutes. In these treatment sessions, sterile stainless steel acupuncture needles for single-use (0.25x50 mm, 0.25x25 mm, and 0.22x13 mm, Hua Long, Chiana) were used for body acupuncture points applying the electroacupuncture method. Auricular acupuncture was administered as one session per week for four weeks. Acupuncture points in each session were determined by the Agiskop DT device. Semi-permanent ear acupuncture needles (0.24x1.5 mm, Hua Long, Chiana) were used in each session.

Statistical analysis

Statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS, version 20). As descriptive statistics, mean and standard deviation were used for continuous variables, frequency and percentage were used for the categorical variables. Chi-square test was used to compare the categorical data. The One-Way ANOVA (for normally distributed variables) test was used to compare more than two independent groups. The paired samples t-test (for normally distributed variables) was used to compare the differences between paired samples. Results were evaluated at a 95% confidence interval, a significance level of $p<0.05$.

Results

The sociodemographic characteristics of the participants in the study were shown in Table 1. Table 2 summarized the comparison of leptin and nesfatin-1 values measured on the 0th and 30th day. There was no difference among the three groups regarding nesfatin-1 measured at the beginning and end of the study (p=0.966[ab]), (p=0.200[ac]), (p=0.122[bc]). On the other hand, the before and after intervention nesfatin-1 values were increased in the diet **(p=0.017[b])** and acupuncture groups **(p=0.032[a])** (Table 2).

Table 1. *Sociodemographic characteristics of the participants*

	Acupuncture		**Diet**		**Control**		**Total**	**χ^2**	**p***
	n	%	n	%	n	%			
Gender									
Male	15	34,1	14	31,8	15	34,1	44	0,089	0,956
Female	15	32,6	16	34,8	15	32,6	46		
Education status									
≤High school	12	23,5	16	31,4	23	45,1	51	8,729	**0,013**
> High school	18	46,2	14	35,9	7	17,9	39		
Employment status									
Employed	22	48,9	14	31,1	9	20,0	45	11,864	**0,003**
Unemployed	8	17,8	16	35,6	21	46,7	45		
Marital status									
Married	29	38,7	23	30,7	23	30,7	45	7,140	**0,028**
Single	1	6,7	7	46,7	7	46,7	15		

* Chi-square test was used.

Table 2. *Comparison of leptin and nesfatin-1 values measured on the 0th and 30thday*

	Acupuncture[a]	**Diet[b]**	**Control[c]**		
	Mean±SD	**Mean±SD**	**Mean±SD**	**F**	**p***
Leptin (ng/ml)					
Baseline	5.2±0.7	5.1±0.8	4.7±0.9	3102.112	0.527[ab] 0.157[ac] 0.719[bc]
30thday	4.5±1.1	4.8±1.2	5.0±0.9		
p**	**0.040**	0.177	**0.039**		
Nesfatin-1 (ng/ml)					
Baseline	21.6±12.6	23.3±16.2	22.3±10.4	284,740	0,966[ab] 0,200[ac] 0,122[bc]
30thday	26.8±16.5	27.7±18.2	19.2±9.1		
p**	**0.032**	**0.017**	0.149		

p*: One-way ANOVA, p**: Paired Samples t-test, SD: Standard deviation

There wasn't a relationship between the three groups in terms of leptin values measured at the beginning and on the 30th day (p= 0.527[ab], 0.157[ac], and 0.719[bc], for Group 1, 2, and 3, respectively). The baseline day-30 leptin were decreased in the acupuncture group **(p=0.040[a])** while they increased in the normal group **(p=0.039[c])**.
There was no difference between the three groups concerning FBS, total cholesterol, TG, HDL-c values measured at the baseline and day 30 (p>0.05) (Table 3). Conversely, a significant correlation was found between the acupuncture and control groups **(p=0.002[ac]),** as well as the diet and normal groups **(p=0.016[bc])** regarding LDL-c values.

Table 3.*Comparison of biochemical parameters measured on the 0th and 30thday*

	Acupuncture[a]	**Diet[b]**	**Control[c]**		
	Mean±SD	**Mean±SD**	**Mean±SD**	**F**	**p***
FBS (mg/dl)					
Baseline	103.3±21.9	95.4±8.7	101.1±18.2	3019.928	0.299[ab] 0.820[ac] 0.646[bc]
30thday	100.3±29.3	92.3±10.5	97.1±17.5		
p**	0.484	0.099	0.172		
T. Cholesterol (mg/dl)					
Baseline	200.8±41.8	187.9±31.1	220.1±39.7	2865,994	0,991[ab] **0,006[ac]** **0,004[bc]**
30thday	191.1±37.9	189.7±40.8	223.0±37.5		
p**	0.055	0.710	0.584		
TG (mg/dl)					
Baseline	207.7±181.6	122.1±61.2	165.7±110.9	211.563	0.104[ab] 0.959[ac] 0.180[bc]
30thday	179.2±128.3	124.2±61.2	171.8±108.2		
p**	0.215	0.833	0.743		
LDL-c (mg/dl)					
Baseline	117.7±32.9	114.8±28.6	139.8±35.6	1473.585	0.781[ab] **0.002[ac]** **0.016[bc]**
30thday	109.4±32.1	115.2±32.8	139.4±34.5		
p**	0.106	0.919	0.936		
HDL-c (mg/dl)					
Baseline	47.5±12.4	48.7±9.3	49.8±15.8	1151.107	0.999[ab] 0.435[ac] 0.454[bc]
30thday	48.8±13.9	48.9±7.6	54.4±26.0		
p**	0.235	0.800	0.254		

p*: One-way ANOVA, p**: Paired Sample t-test, SD: Standard deviation, FPS: Fasting plasma sugar, TG: Triglyceride, LDL-c: Low-density lipoprotein cholesterol, HDL-c: High-density lipoprotein cholesterol

There wasn't a direct relationship between the three groups regarding body weights measured on days 0 and 30 (p>0.05). However, body weights significantly decreased in the acupuncture and diet groups from day 0 to day 30 **(p=0.032[a]) (p<0.001[b])** (Table 4).
Same as the body weight results, there was no direct relationship between the three groups concerning BMI (p>0.05). The BMI values of the patients were statistically decreased in the diet group **(p<0.001[b])** (Table 4).
Considering the metabolic age values measured on day 0 and 30, a significant relationship was found between diet and control groups **(p=0.016[bc])**. Although the metabolic age values slightly decreased in the acupuncture and diet groups, this decrease was not significant (p=0.08) (Table 4).
The total fat values were reduced in acupuncture **(p<0.001[a])** and diet groups **(p<0.001[b]).** Similarly, also the visceral fat mass values were reduced in the acupuncture **(p<0.001[a])** and diet groups and (**p=0.048[b]**)**.** In the diet group, the fat ratios on the last day of the study was significantly reduced **(p=0.023[b]).** Muscle mass values of the patients measured on day 0 and 30 in the acupuncture group were significantly decreased **(p=0.022[a]).** Lastly, the total body water values were significantly increased in the acupuncture **(p=0.023[a])** and diet groups **(p=0.047[b])** (Table 4).

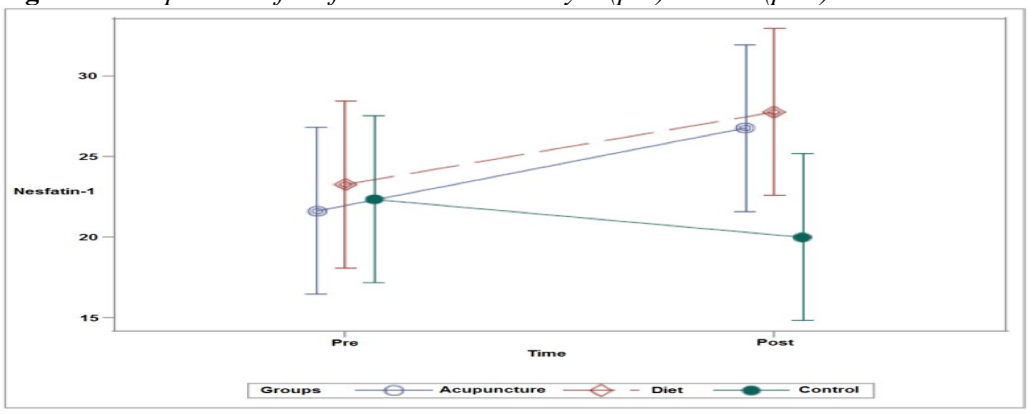
Table 4. *Comparison of body compositions measured on the 0th and 30th day*

	Acupuncture[a]	Diet[b]	Control[c]		
	Mean±SD	**Mean±SD**	**Mean±SD**	**F**	**p***
Body weight (kg)					
Baseline	91.9±17.5	90.5±16.2	90.3±18.5	2460.783	0.996[ab] 0.963[ac] 0.935[bc]
30th day	88.5±16.3	88.1±16.7	89.7±18.8		
p**	**0.032**	**<0.001**	0.130		
BMI (kg/m²)					
Baseline	31.5±5.2	31.3±5.2	31.4±4.9	3952.010	0.960[ab] 0.750[ac] 0.894[bc]
30th day	30.2±3.9	30.6±5.0	31.1±4.7		
p**	0.059	**<0.001**	0.129		
Metabolic age (year)					
Baseline	55.5±18.3	45.3±12.4	53.5±12.0	1529.836	0.112[ab] 0.701[ac] **0.016**[bc]
30th day	51.3±10.7	45.1±11.9	53.7±12.9		
p**	0.168	0.662	0.771		
Total fat mass (kg)					
Baseline	30.1±9.6	29.9±9.6	30.6±10.8	725.500	0.980[ab] 0.819[ac] 0.708[bc]
30th day	29.0±9.8	28.5±9.5	30.7±11.8		
p**	**<0.001**	**<0.001**	0.131		
Visceral fat mass (kg)					
Baseline	10.5±4.2	8.7±4.3	10.8±4.9	458.838	0.310[ab] 0.836[ac] 0.109[bc]
30th day	10.1±3.9	8.4±4.2	10.7±4.6		
p**	**<0.001**	**0.048**	0.639		
Fat ratio (%)					
Baseline	33.4±8.0	33.2±8.3	33.7±8.2	1486.462	0.984[ab] 0.908[ac] 0.827[bc]
Day 30th	32.8±8.7	32.5±8.2	33.7±7.9		
p**	0.121	**0.023**	0.961		
Muscle mass (kg)					
Baseline	57.2±13.1	56.7±13.1	55.5±10.4	1935.379	0.997[ab] 0.994[ac] 0.982[bc]
30th day	56.3±12.6	56.6±13.0	56.0±10.6		
p**	**0.022**	0.742	0.394		
Total body water (%)					
Baseline	47.1±4.9	47.5±5.5	46.8±5.2	7502.957	0.961[ab] 0.923[ac] 0.793[bc]
30th day	47.5±5.2	47.9±5.5	47.0±4.9		
p**	**0.023**	**0.047**	0.499		

p*: One-way ANOVA, p**: Paired Samples t-test, SD: Standard deviation, BMI: Body mass index

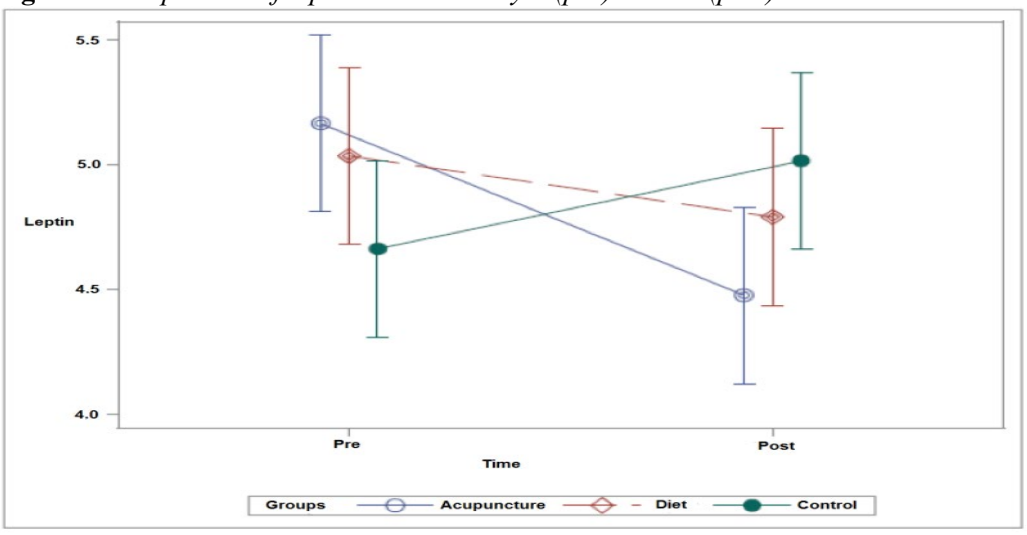
When the nesfatin-1 values before and after acupuncture were evaluated, the level of nesfatin-1 was decreased in the control, while it increased in the acupuncture and diet groups (Figure 1).

Figure 1.*Comparison of nesfatin-1 values on day 0 (pre) and 30 (post).*



Additionally, when pre-and post-measurements of leptin values were evaluated, the level of leptin was decreased in the acupuncture and diet groups, while it increased in the control group (Figure 2).

Figure 2.*Comparison of leptin values on day 0 (pre) and 30 (post).*



Discussion

To the best of our knowledge, this study is one of the most important studies in the literature comparing the effects of diet and acupuncture on appetite hormones and obesity. In the presented study, approximately 3.4 kg and 2.4 kg weight loss was observed in the acupuncture and diet groups, respectively. Qingfu et al. applied general body acupuncture treatment to 110 obese patients as one session per week for three months. They observed approximately 5.0 kg body weight loss.[10] In Cabioglu et al.'s study, there was a 4.5% weight reduction in the patients with acupuncture application, whereas patients on diet restriction had a 3.1% weight reduction.[11]

In a study performed by Considine et al., serum leptin levels were compared between control and obese participants and found as 7.5±9.3 ng/ml and 31.3±24.1 ng/ml, respectively.[12] In another study, some changes in the levels of appetite hormones were evaluated in 20 female patients with normal weight and obesity. The researchers found that serum leptin levels were higher in the obese compared to the controls.[13]

Weigle et al. reported a reduction in both weight and serum leptin in obese patients on a 700 kcal diet for three months.[14] In the study by Cabioglu et al., 20 female patients were given a diet of 1400 kcal, while 20 patients were given acupuncture for 20 days. In the same study, a 33.4% decrease was found in serum leptin levels in the acupuncture group and a 15.1% decrease was found in the diet group, with a 5.4% weight loss in the acupuncture and 2.8% in the diet group.[11] In the presented study, a weight loss of 3.7% was found in the acupuncture group and 2% in the diet group with a 13% decrease of serum leptin levels in the acupuncture and a 6% decrease in the diet group were determined. The 30th day leptin values were significantly decreased in the acupuncture group while they were significantly increased in the control group.

The difference between studies may probably be because of the limited study period and the differences in the diet programs. Daily acupuncture treatment and the 1400 kcal diet program may be the reasons for the differences. In a

study conducted by Özkan et al., participants were divided into five groups according to their BMI values and serum nesfatin-1 levels of each group were compared.[15]
Anwar et al. compared the fasting serum nesfatin-1 of 40 healthy and 40 obese adults in their study, and they detected that serum nesfatin-1 levels were higher in the obese compared to controls.[16] In another study, the level of nesfatin-1 was lower in obese participants than in normal participants.[17]
Deniz found a negative relationship between nesfatin-1 and BMI.[18] Similar to the aforementioned study, in a different study conducted on 50 adults, no relationship was found between BMI and nesfatin-1.[19] In the presented study, the levels of nesfatin-1 at the end of the study were increased from baseline both in the acupuncture and diet groups, while it was decreased in the normal weight group. A negative relationship was found between nesfatin-1 and BMI.
In the study performed by Guo, in obese and normal weight individuals, a negative relationship was found between BMI and nesfatin-1, hip and waist circumferences. After acupuncture and dietary restriction, the body weight of the participants decreased, while the levels of nesfatin-1 increased statistically.[20] Similarly, in the presented study, nesfatin-1 values were found to be increased by 24% in the acupuncture group and 18% in the diet group.
Unlike the results obtained in the present study, in Kara's study, leptin levels decreased in the group who lost weight with diet. In contrast, obestatin and nesfatin1 levels are not affected by weight loss.[21]
There was no difference between the groups concerning FBS, total cholesterol, TG, HDL-c values at the baseline and day 30. Conversely, a correlation was found between the acupuncture and control**,** as well as the diet and control regarding LDL-c values. In a study performed by Cabioglu, there were decreases in total cholesterol and triglyceride levels in acupuncture and diet groups compared to the controls. Furthermore, there was a decrease in LDL levels in the acupuncture group compared to the controls. No significant changes could be found in HDL levels among the three groups.[22]

Limitations of the study
This study group is not entirely representative of the Turkish population. The most important limitations of our study were the small group of participants and the short follow-up period. The fact that other appetite-related peptides (ghrelin and orexin-A) were not evaluated in the present study may be another limitation.

Conclusion and Recommendation
The results of this study support the effect of acupuncture treatment on appetite hormones. In addition to participants who were receiving diet therapy, the significance of the weight loss in the acupuncture group suggests that acupuncture can be used as an adjunct method for the treatment of obesity. Both the literature review and the results of this study have shown that there is a need for further research on the mechanisms of endogenous and exogenous actions of the recently discovered hormones, leptin and nesfatin-1. Also, further randomized controlled large-scale studies are required for supportive acupuncture therapy in the treatment of obesity. Nesfatin-1 appears to be a hope-promising target for the development of an effective medication in the treatment of obese individuals.

Contribution to the Field
The results of this study can give an idea about the endogenous and exogenous mechanisms of action of the newly discovered hormones, leptin and nesfatin-1. We think that this study will contribute to the literature by comparing the effects of diet and acupuncture on appetite hormones and obesity.

Ethical Aspect of the Research
All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Ethical approval for the study was obtained from the Necmettin Erbakan University Medical Faculty Ethics Committee (Approval date: 14.07.2017, number: 2017/998). Theparticipants were duly informed, and written and oral consent was obtained according to the principles of theHelsinki Declaration.

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