Plasma nesfatin-1 level in obese patients after acupuncture: a randomised controlled trial

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ABSTRACT
Background Nesfatin-1 is an anorexigenic hormone suggested to regulate obesity.
Objective To investigate the relationship between nesfatin-1 level and anthropometric and metabolic parameters in obese patients, and examine the change in plasma nesfatin-1 level after acupuncture treatment.
Methods 64 obese adult patients without diabetes and 58 normal weight control subjects were enrolled in this study. The obese patients were randomly divided into an acupuncture plus diet group (n=32) and a diet only group (n=32). Measurements were repeated after 45 days.
Results Body mass index (BMI), waist and hip circumferences, serum insulin, lipoprotein and insulin resistance measures were significantly higher, and plasma nesfatin-1 level was significantly lower, in obese patients than in normal weight controls. In addition, negative correlations were found between plasma nesfatin-1 level and BMI, waist and hip circumferences. Weight reduction in participants after acupuncture and diet restriction was 7.0% and 4.3%, respectively. Plasma nesfatin-1 level increased from 2.75±1.16 to 3.44±1.28 ng/mL and from 2.86±1.07 to 3.23±1.06 ng/mL in acupuncture and diet groups, respectively; the difference was significant, p<0.05.
Conclusions Plasma nesfatin-1 level is reduced in obese adults, and is increased after acupuncture. The beneficial effect of acupuncture on obesity is associated with increased plasma nesfatin-1 level.

INTRODUCTION
Nesfatin-1 is a new hypothalamus nucleobindin 2 (NUCB2)-derived peptide that is distributed in the appetite-controlling hypothalamic nuclei, such as arcuate nuclei, paraventricular nuclei, supraoptic nuclei and lateral hypothalamic areas. Moreover, nesfatin-1 has been detected in the stomach, pancreatic endocrine islets, testis and adipose tissue in rodents.1 The mRNA expression of nesfatin-1 is significantly decreased by fasting and significantly increased in the hypothalamus on re-feeding.2 Furthermore, systemically administered nesfatin-1 inhibits appetite and body weight gain in rodents, suggesting that it is an anorexigenic hormone.3 Interestingly, fasting plasma levels of nesfatin-1 were significantly lower in patients with acute myocardial infarction.4

Nesfatin-1 is a newly discovered anorexigenic hormone, and whether it is involved in the development of clinical obesity is not clear. The nesfatin-1/NUCB2 ratio in cerebrospinal fluid/plasma is negatively associated with body mass index (BMI), body weight, fat mass and cerebrospinal fluid glucose level.5 In addition, plasma nesfatin-1 level has been shown to be negatively correlated with BMI in non-obese men.6, 7 However, the circulating nesfatin-1 level was higher in mice fed a high-fat diet and showed a positive correlation with BMI in humans.8 Therefore, further studies are needed to clarify the role of nesfatin-1 in obesity.

The suggestion that acupuncture, a traditional Chinese treatment, is effective and safe for the treatment of obesity has attracted attention recently.9 Although the mechanism by which acupuncture helps to reduce obesity is not clear, several studies suggest that it increases the neural activity associated with the ventromedial nuclei of the hypothalamus, raises the tone of the smooth muscle of the stomach and decreases circulating leptin and insulin levels.10 Since nesfatin-1 is an anorexigenic protein newly associated with loss of appetite, we hypothesised that it might be involved in the effects of acupuncture.
acupuncture on obesity. In this study, we compared plasma nesfatin-1 levels of obese patients and normal weight controls, investigated the relationship between nesfatin-1 level and anthropometric and metabolic parameters in obese patients and examined the change in plasma nesfatin-1 level after acupuncture treatment in obese patients.

**MATERIALS AND METHODS**

**Study population**

Sixty-four obese adults (33 men and 31 women) were recruited from the outpatient obesity clinic at Qingdao Municipal Hospital. Only patients with a BMI $\geq 28$ and age $> 18$ years were included in the study. Patients with diabetes or impaired glucose tolerance and postmenopausal women were excluded. Fifty-eight control subjects of normal weight (BMI $< 24$ kg/m$^2$) were recruited among patients who underwent routine physical examination at Qingdao Municipal Hospital. Anthropometric and metabolic parameters were measured in both obese and control subjects, and blood samples were taken. The 64 obese patients were randomly divided into an acupuncture group (n=32) and diet group (n=32). Patients in the diet group were treated with dietary adjustment only, continuously for 45 days, and those in the acupuncture group were treated with acupuncture plus dietary adjustment every day (7 days a week) for 45 days. The study was approved by the institutional ethics committee, and written informed consent was obtained from all subjects before the study.

**Measurements**

Data on demographic factors, BMI, waist circumferences (WC), hip circumference, waist-to-hip ratio (WHR) and systolic and diastolic blood pressures were recorded at the beginning and end of treatment. Height was measured to the nearest 0.5 cm and body weight was measured with a digital scale to the nearest 0.1 kg. BMI (weight in kg over height in m$^2$) was calculated. Blood samples were collected for the measurement of total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), glucose, insulin and nesfatin-1 levels. Venous blood was collected in the morning from a forearm vein of subjects after overnight fast into EDTA tubes containing 500 kallikrein inactivation unit aprotinin to prevent digestion of peptides by proteases. Serum and plasma were separated and stored at $-80^\circ$C for further analysis. Plasma nesfatin-1 level was measured using a commercial enzyme-linked immunosorbent assay kit (Phoenix Pharmaceuticals, Belmont, California, USA), with a measurement interval of 0.78–50 ng/mL. Blood glucose, TC, TG, HDL, low-density lipoprotein (LDL) levels were measured by standard laboratory methods. Insulin level was measured using an electrochemiluminescence immunoassay. The homeostasis model of assessment-insulin resistance index (HOMA-IR) was calculated for each patient using the formula: fasting glucose (mmol/L) $\times$ fasting insulin (IU/mL)/22.5.

**Acupuncture**

Body acupuncture was performed for 30 min. The acupuncture needles were 5 cm long, with a 0.22 mm diameter (Huanqiu Acupuncture Medical Appliance, Suzhou, China). Electrical stimulation was given by a pulse generator (HANS Electrostimulator, Beijing, China) using 0.05 ms pulse width at 2 Hz frequency at 3 V with bidirectional square waves. Zhongwan (CV12) was connected to Tianshu (ST25) on one side, Guanyuan (CV4) was connected to ST25 on the other side, Zusanli (ST36) was connected to Sanyinjiao (SP6), and Fenglong (ST40) was connected to Yiningguan (SP9) on the same side.

**Diet protocol**

A diet of 1400 kcal was given to the subjects in both the diet and acupuncture groups, which provided the minimal calorie intake over their basal metabolism. The diet programme was explained to the patients before the study, and macronutrient energy distribution was 25–30% fat, 50–55% carbohydrate and 15–20% protein.

**Statistical analysis**

Statistical analysis was performed using SPSS V20.0 (SPSS Inc). The Kolmogorov–Smirnov Z test showed that some data were not normally distributed. Therefore, a Student t test for normally distributed data and Mann–Whitney test for non-normally distributed data were used. Data were presented as means±SD. Correlations between variables were analysed with Pearson’s coefficient. A p value of $<0.05$ was considered statistically significant.

**RESULTS**

**Baseline clinical and laboratory characteristics**

The clinical and laboratory features of the subjects are shown in table 1. The mean age was 37±15.6 years for obese patients and 34±8.3 years for the control group (p=0.11). There were no significant differences in age and gender distribution between the two groups. We found significant differences in BMI, WC, hip circumference, WHR and glucose, serum insulin, LDL cholesterol (LDL-C) and HOMA-IR between the two groups (p<0.05), but no significant differences in TC, TG, HDL cholesterol (HDL-C) levels, systolic and diastolic blood pressures (p>0.05) (table 1).

**Plasma nesfatin-1 levels at baseline**

Plasma nesfatin-1 level was significantly lower in obese patients (2.81±1.75 ng/mL) than in normal weight controls (3.78±2.36 ng/mL) (p=0.009) (figure 1). Serum nesfatin-1 level showed no significant difference between women and men in all subjects (3.56 ±2.41 ng/mL vs 2.96±1.70 ng/mL, p=0.120).
Correlation analysis showed a negative correlation between plasma nesfatin-1 level and BMI, WC and WHR in the obese group (figure 2). We found no correlation between plasma nesfatin-1 level and glucose, insulin and HOMA-IR in the obese group. In the control group no correlation between plasma nesfatin-1 level and any measured parameter was found (data not shown).

Comparison of the acupuncture and diet groups

Three subjects left before the end of the study for personal reasons and thus 61 participants completed the study. Acupuncture was given to 31 subjects (17 male, 14 female) aged 38±2.7 years and with a BMI of 35.2±4.04, while the diet programme alone was provided to 30 subjects (16 male, 14 female) aged 36±4.2 years and with a BMI of 34.4±3.18. We found that the gender, age, basal levels of anthropometric parameters, lipid profiles did not differ significantly between the acupuncture and diet groups at baseline (p>0.05, see online supplementary table S1).

Body weight was reduced from 74.6±7.5 kg and 75.6±8.3 kg in acupuncture and diet groups before treatment, to 69.4±6.1 kg and 72.3±7.5 kg after treatment, respectively, representing 7.0% and 4.3% weight reductions. Plasma nesfatin-1 level was increased in the acupuncture group from 2.75±1.16 ng/mL to 3.44±1.28 ng/mL, and in the diet group from 2.86±1.07 ng/mL to 3.23±1.06 ng/mL; the difference was significant (p<0.05). Compared with pretreatment values, the mean body weights in both the diet and acupuncture groups were decreased significantly (p<0.05), and plasma nesfatin-1 levels in both groups were increased significantly (p<0.05). The differences between groups in body weight and nesfatin-1 level were significant (p<0.05) (figure 3).

DISCUSSION

Nesfatin-1 is a multifunctional metabolic hormone in the hypothalamus and adipocytes which is related to reduced food intake and body weight, regulates gastrointestinal function and insulin secretion. A recent study reported that nesfatin-1 has a remarkably prolonged effect on body temperature, suggesting the role of nesfatin-1 as a new anorexigenic factor and modulator of energy balance.13

Only a few previous studies have investigated the relationship between nesfatin-1 level and anthropometric and metabolic parameters in obesity, and the results are inconsistent. Ramanjaneya et al67 found significant positive correlation between BMI and nesfatin-1 level in human obese subjects. Saldanha et al14 also found positive correlation between BMI and nesfatin-1 level in overweight subjects. However, Abaci et al investigated the relation of serum nesfatin-1 level with metabolic and clinical parameters in 37 obese and 31 healthy children, and found that the serum nesfatin-1 level was significantly lower in the obese group than in controls. In addition, in obese subjects nesfatin-1 was negatively correlated with the BMI. Moreover, Tsuchiya et al reported that fasting nesfatin-1 level was significantly lower in subjects with high BMI than in non-obese subjects, and the peripheral concentration of nesfatin-1 showed a negative correlation with BMI, percentage of body fat, body fat weight and blood glucose. In patients with non-alcoholic fatty liver disease, serum nesfatin-1 level in the obese group was significantly lower than in the non-obese group.16

In this study we found a lower plasma level of nesfatin-1 in obese people than in normal weight controls. Our study is the first to investigate the...
relationship between plasma nesfatin-1 level and anthropometric and metabolic parameters in obese adults. We found that the plasma nesfatin-1 level was negatively correlated with BMI, WC and WHR. These data support a negative association between nesfatin-1 level and metabolic parameters. However, we did not match food intake, daily activity and other factors for the obese adults and normal weight controls. This is a limitation of this study and further studies are needed to confirm the role of reduced nesfatin-1 level in the pathophysiology of obesity. In addition, perhaps owing to inadequate sample size, we found no correlation between nesfatin-1 level and insulin level.

There is some evidence that acupuncture is effective for the treatment of obesity. It has been suggested that acupuncture increases the feeling of fullness and motivation for a low-calorie diet. Lin et al. found that electroacupuncture caused an improvement in insulin resistance and lowered secreted insulin in type 2 diabetes mellitus. In our study we observed significant increases in plasma nesfatin-1 level in the acupuncture group compared with the diet group. Nesfatin-1 has been reported to induce satiation (reduction of meal size) as well as satiety (decreased meal frequency associated with prolonged intervals between meals), delay gastric emptying in animals and suppress gastroduodenal motility in mice. Therefore, we propose that the effect of acupuncture on weight loss is partly due to an increased circulating nesfatin-1 level.
In conclusion, we found that the nesfatin-1 level was decreased in obese subjects, and negative correlations were found for plasma nesfatin-1 level with BMI, WC and WHR. The beneficial effect of acupuncture on obesity is associated with increased plasma nesfatin-1 level.

Acknowledgements This study was supported by grants from the National Natural Science Foundation of China (81200175), Qingdao Technology Development Project (2012-1-3-1-(2)-nsh) and Qingdao Shiman Research Development Fund (2011-3-104-yy).

Contributors YG, HuD: conceived and designed the experiments. YG, MX, WS: performed the experiments. XY: analysed the data. HoD: contributed reagents/materials/analysis tools.

Funding National Natural Science Foundation of China.

Competing interests None.

Patient consent Obtained.

Ethics approval Institutional ethics committee.

Provenance and peer review Not commissioned; externally peer reviewed.

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*Acupunct Med* 2014 32: 313-317 originally published online May 9, 2014
doi: 10.1136/acupmed-2014-010530

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