Is There a Relationship between Obstructive Sleep Apnea Syndrome Severity and Nesfatin-1?

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Key Words

Obstructive sleep apnea syndrome · Apnea hypopnea index · Body mass index · Nesfatin-1

Abstract

Background: Obstructive sleep apnea syndrome (OSAS) and obesity frequently occur together. The relationship between increased appetite and obesity is well known; however, despite existing knowledge about the relationship between OSAS and obesity, it is not fully understood. **Objectives:** This study aimed to evaluate the relationship between OSAS and the appetite-suppressing hormone nesfatin-1 independent of body mass index (BMI). **Methods:** A total of 134 cases were included in the study; 102 with OSAS (OSAS group) and 32 healthy controls (control group). All cases underwent polysomnography, and nesfatin-1 levels were determined. **Results:** Nesfatin-1 levels were significantly lower in the OSAS group compared to the control group ($3,776.5 \pm 204.8$ and $4,056.2 \pm 101.5$ pg/ml, respectively; $p < 0.001$). In addition, there was a statistically significant negative correlation between nesfatin-1 and the apnea hypopnea index ($r = -0.543$; $p < 0.001$). The statistically significant relationship persisted after adjusting for confounding intergroup factors such as

age, gender and BMI ($p < 0.001$). In the OSAS group, there was a statistically significant correlation between nesfatin-1 and neck circumference ($r = -0.304$; $p = 0.02$) but not between nesfatin-1 and BMI and waist circumference. There was no statistically significant difference in nesfatin-1 levels between the sexes. **Conclusion:** OSAS patients have lower nesfatin-1 levels compared to controls, and a greater nesfatin-1 deficit corresponds to an increased severity of OSAS and an increased neck circumference. Replacement therapy may be a potential treatment for obese OSAS patients who have lower nesfatin-1 levels, which may have additional benefits through appetite suppression and weight loss.

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Introduction

Obstructive sleep apnea syndrome (OSAS) is a condition which causes recurrent respiratory dysfunctions (apnea, hypopnea) during sleep due to obstruction in the upper respiratory passages. OSAS can affect several body systems and is a major health concern. It affects approximately 2–4% of the population [1]. Several risk factors, including obesity, have been associated with an increased

prevalence of OSAS in the general population [2], and it has been reported that 50–77% of obese patients are diagnosed with OSAS [3]. Especially, central obesity has been shown to be a potent risk factor for the presence and progression of sleep apnea [4]. Despite evidence linking obesity and central adiposity with sleep apnea, a considerable variability exists in the prevalence of OSAS, even among those who are markedly obese [4].

In addition to central obesity, increased adiposity around the pharynx may have an effect on the severity of OSAS by narrowing the upper airway, increasing neck circumference and fat deposition [5], increasing airway collapsibility [6] and increasing continuous positive airway pressure requirements [7]. Weight loss may have an effect by decreasing airway collapsibility, likely due to reductions in mechanical loads or improvements in pharyngeal neuromuscular control. These mechanisms may be related to alterations in humoral factors, including ghrelin, adiponectin and leptin, which have been linked to changes in body weight and regional adiposity [8]. However, their association with obesity may differ, e.g. ghrelin increase shows a correlation with the amount of weight loss, whereas the other factors can modulate the loss of weight from visceral and subcutaneous fat stores [9]. Nesfatin-1, a recently discovered hormone [10], is a member of those humoral factors, but its effect on weight loss has not yet been investigated extensively, and the effect on OSAS severity is unknown.

This study aimed to evaluate whether or not a relationship exists between nesfatin-1 and OSAS severity.

Methods

Study Population

A total of 151 consecutive cases who applied to the outpatient clinic of our sleep center with complaints related to sleep-related breathing disorders and diagnosed as OSAS, who had not been previously treated, were included in the study between September 2012 and September 2013. Forty-nine of them were excluded due to a diabetes history, smoking history, presence of thyroid dysfunction or use of medications for weight loss. A total of 32 healthy controls were included in the study after ruling out OSAS with polysomnography [apnea hypopnea index (AHI) <5]. OSAS was defined as an AHI ≥ 5 and excessive daytime sleepiness [11]. The participants were divided into two groups, consisting of OSAS cases ($n = 102$) and healthy controls ($n = 32$).

Measurements

After an initial clinical evaluation, a questionnaire was used to obtain information about a history of snoring, witnessed apnea and excessive daytime sleepiness. The Epworth Sleepiness Scale was used to evaluate sleepiness. A detailed physical examination

was carried out and anthropometric measurements, including neck and waist circumference, height, weight and body mass index (BMI), were obtained.

Blood Sampling and Biochemical Investigations

Blood samples for nesfatin-1 were drawn in the morning around 9 a.m. from a forearm vein at the end of an overnight fasting period of at least 8 h. Tubes with a 2-ml capacity containing EDTA were used for collecting blood. The blood was carefully and immediately (in a few seconds) transferred from these tubes to centrifuge tubes which contained aprotinin (0.6 TIU/ml of blood) and immediately stored on ice. Before centrifugation, the centrifuge tubes were gently rocked several times to inhibit the activity of proteinases. After the centrifugation process at 4,000 rpm for 10 min at 5°C, plasma was obtained. The separated plasma was stored in a –80°C freezer until the time of assay. Plasma nesfatin-1 levels were measured using a commercial ELISA kit (No. E90242Hu ELISA kit, USCN Life Science, Wuhan, PR China). Some previous studies in the literature have used the ELISA method for measuring nesfatin-1 peptide levels.

A venous blood sample was obtained, and thyroid-stimulating hormone (TSH) and free thyroxine 4 (FT4) levels were analyzed by the chemiluminescence method using an ACS-180 hormone analyzer system. The normal range for TSH is 0.49–4.6 mIU/l, while for FT4, it is 0.6–1.8 ng/dl.

Sleep Study

Full polysomnography monitoring was performed using the Compumedics E-series Sleep System (Compumedics Sleep, Melbourne, Vic., Australia). Electroencephalography (EEG), electro-oculography, electromyography and electrocardiography were performed simultaneously. Surface electrodes were used to record EEG channels, right and left electrooculographies and submental electromyography. Ventilatory flow either at the nose or at both the nose and the mouth was measured with airflow. Respiratory movements of the chest and abdomen, as well as the body position, were monitored by inductive plethysmography bands. Arterial oxygen saturation was measured transcutaneously with a finger oximeter. Apnea was defined as continuous cessation of airflow for ≥ 10 s, and hypopnea was defined as at least 50% reduction of airflow for ≥ 10 s with an oxygen desaturation of $\geq 3\%$ or an EEG arousal from sleep. Apneas were classified as obstructive, central or mixed according to standard criteria of the American Academy of Sleep Medicine [11].

All patients were informed about the study and provided consent to participate. The study was planned according to the ethics guidelines of the Declaration of Helsinki, and the study protocol was approved by the Local Ethics Committee (B.30.2.ATA.0.01.00/133).

Statistical Analysis

Statistical analysis was performed using SPSS for Windows (version 19.0; SPSS Inc., Chicago, Ill., USA). Student's *t* test was used to compare parameter values between the groups. The relationship between nesfatin-1 and AHI, neck circumference, BMI and waist circumference was evaluated by Pearson's correlation test. The relationship between each group and nesfatin-1 was examined using logistic regression, adjusting for confounding factors (age, gender and BMI for each group and age, gender and AHI for BMI). Statistical significance was accepted as a *p* value <0.05.

Table 1. Demographic characteristics of the study groups

	OSAS group (n = 102)	Control group (n = 32)	p value
Age, years	52.0±11.9	47.8±14.4	0.100
BMI	33.4±6.1	31.0±4.6	0.042
NC, cm	40.6±4.2	36.5±4.2	0.012
WC, cm	89.8±7.4	86.0±5.2	0.008
ESS	10±4	3±2	0.001

Values are means ± standard deviations. NC = Neck circumference; WC = waist circumference; ESS = Epworth Sleepiness Scale.

Table 2. Laboratory findings of the study groups

	OSAS group (n = 102)	Control group (n = 32)	p value
Nesfatin-1, pg/ml	3,776.5±204.8	4,056.2±101.5	<0.0001
TSH, mIU/l	1.49±0.97	1.29±0.46	0.254
FT3, ng/dl	3.25±0.62	3.12±0.43	0.278
FT4, ng/dl	0.91±0.15	0.89±0.15	0.720
AMS	89.4±3.7	94.0±1.4	<0.001
SMS	85.8±5.6	92.3±1.6	<0.001
MD	7.3±3.9	2.8±0.75	<0.001

Values are means ± standard deviations. AMS = Awake mean oxygen saturation; SMS = sleeping mean oxygen saturation; MD = sleeping mean oxygen desaturation.

Results

A total of 134 subjects, including 102 (76.1%) OSAS patients (64 male and 38 female) and 32 (23.9%) controls (22 male and 10 female), with a mean age of 52 ± 11.9 and 47.8 ± 14.4 years, respectively, were included in the study. Demographic characteristics and laboratory findings of the groups are shown in tables 1 and 2.

Nesfatin-1 levels were 3,776.5 ± 204.8 pg/ml in the OSAS group and 4,056.2 ± 101.5 pg/ml in the control group, and this difference was statistically significant ($p < 0.0001$). Furthermore, a statistically significant negative correlation was found between nesfatin-1 and AHI ($r = -0.543$; $p < 0.0001$). After adjusting for age, gender and BMI, the correlation between AHI and nesfatin-1 remained statistically significant ($p < 0.0001$). The relationship between nesfatin-1 and AHI is shown in figure 1.

Fig. 1. The relationship between nesfatin-1 and AHI.

In the OSAS group, there was a statistically significant correlation between nesfatin-1 and neck circumference ($r = -0.304$; $p = 0.02$) but not between nesfatin-1 and BMI and waist circumference. The relationship between nesfatin-1 and neck circumference, BMI and waist circumference is shown in figure 2. There was no statistically significant difference in nesfatin-1 levels between the sexes. Nesfatin-1 levels for males and females were 3,765 ± 205 versus 3,796 ± 206 pg/ml in the OSAS group ($p = 0.457$) and 4,044 ± 117 versus 4,084 ± 50 pg/ml in the control group ($p = 0.308$), respectively.

Discussion

The most significant finding in this study was that nesfatin-1 levels were lower in OSAS patients compared to healthy controls, and they were negatively correlated with OSAS severity (AHI). After controlling for age, BMI and sex, the negative correlation between the nesfatin-1 level and OSAS severity still remained significant. Although BMI, neck circumference and waist circumference were higher in OSAS patients compared to healthy controls, only neck circumference showed a significantly negative correlation with nesfatin-1 levels. The study also revealed that there was no significant difference in nesfatin-1 levels between the sexes.

Fig. 2. The relationship between nesfatin-1 and neck circumference (a), BMI (b) and waist circumference (c).

In a recent study investigating nesfatin-1 levels in OSAS patients, it was found that nesfatin-1 levels are lower in severe OSAS patients compared to moderate OSAS patients, and nesfatin-1 levels were inversely correlated with OSAS severity according to AHI [12]. The study also found a negative correlation between nesfatin-1 levels and BMI, waist-hip ratio and homeostasis model assessment of insulin resistance [12]. Although we found the same relationship of nesfatin-1 levels with OSAS severity, we could not find the same relationship between nes-

fatin-1 levels and BMI. We also found that nesfatin-1 levels are negatively correlated with neck circumference but not with waist circumference.

Although the chicken-and-egg question concerning obesity and OSAS still remains unanswered, it is conceivable that OSAS and obesity may interact and potentiate their detrimental consequences. In OSAS, hypoxia of adipocytes could play an important role in the metabolic disturbances associated with obesity [13, 14]. In addition, OSAS and obesity share common mechanisms such as

increased sympathetic activity, oxidative stress and inflammatory activity [15, 16]. Although central obesity has been shown to be a potent risk factor for the presence and progression of sleep apnea, OSAS prevalence shows considerable variability, even among those who are markedly obese [4]. Thus, increased adiposity around the pharynx in addition to central obesity may explain this variability. In addition, high calorie intake, which is associated with humoral factors, such as leptin and nesfatin-1, may affect metabolic regulation, and these mechanisms may also influence the regulation of upper airway patency, which is highly associated with OSAS severity [5–7].

Nesfatin-1 was first described in 2006 by Oh-I et al. [10] as being a satiation-inducing molecule found in the hypothalamus with 82 amino acids and a 9.7-kDa molecular mass. Studies have reported that nesfatin-1 inhibits food intake independent of leptin but acts through a mechanism linked to melanocortin receptors 3 and 4 [10, 17]. Although the mechanisms of nesfatin-1 in relation to obesity are not fully understood, it is believed to interact with other anorexigenic molecules, especially leptin or melanocortin [10, 18]. It has been reported in the literature that nesfatin-1 is secreted by the neurons in areas of the brain associated with the regulation of energy balance [10, 19, 20]. Its appetite regulation effect is independent of many transmitter systems but is related to the melanocortin system [10, 18, 21]. In the current study, it was found that nesfatin-1 levels were lower in OSAS patients compared to controls, and lower nesfatin-1 levels correlated with increased OSAS severity. This relationship was still apparent after correcting for confounding factors such as age, gender and BMI. Due to the role of nesfatin-1 in satiety, lower nesfatin-1 levels result in increased ap-

petite, which increases neck circumference and subsequently increases the severity of OSAS.

In a study on the relationship between nesfatin-1 and BMI as an obesity criterion, leptin, preptin and acylated ghrelin levels increased with a higher BMI, whereas desacylated ghrelin decreased and nesfatin-1 showed no clear relationship to BMI [22]. In another study investigating BMI and nesfatin-1 in relation to gender and stress, a slight positive relationship was found, although it was inconsistent [23]. In the current study, we found no relationship between BMI and nesfatin-1 levels nor between BMI and OSAS severity. Our findings suggest that fat distribution is more important than weight gain or central obesity. Although expression of nesfatin-1 seems to depend on sex and nesfatin levels are higher in males [24–26], we could not find any difference between the sexes. This issue also needs thorough investigation.

The results of our study show that nesfatin-1 levels were lower in cases with severe OSAS. Thus, a replacement therapy with nesfatin may have a role in the management of OSAS in the future. However, a limitation of our study was that it only investigated the association of OSAS with nesfatin but not with other adipokines, which would be useful for clarifying the complex pathophysiology of energy balance mechanisms in OSAS patients.

In conclusion, nesfatin-1 levels may be lower in OSAS patients compared to the rest of the population, and nesfatin-1 levels negatively correlate with OSAS severity and neck circumference. Nesfatin-1 levels of OSAS patients can be measured, and nesfatin-1 might be supplemented if necessary. Therefore, nesfatin-1 may be suitable as an additional treatment for OSAS, suppressing appetite and leading to weight loss.

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