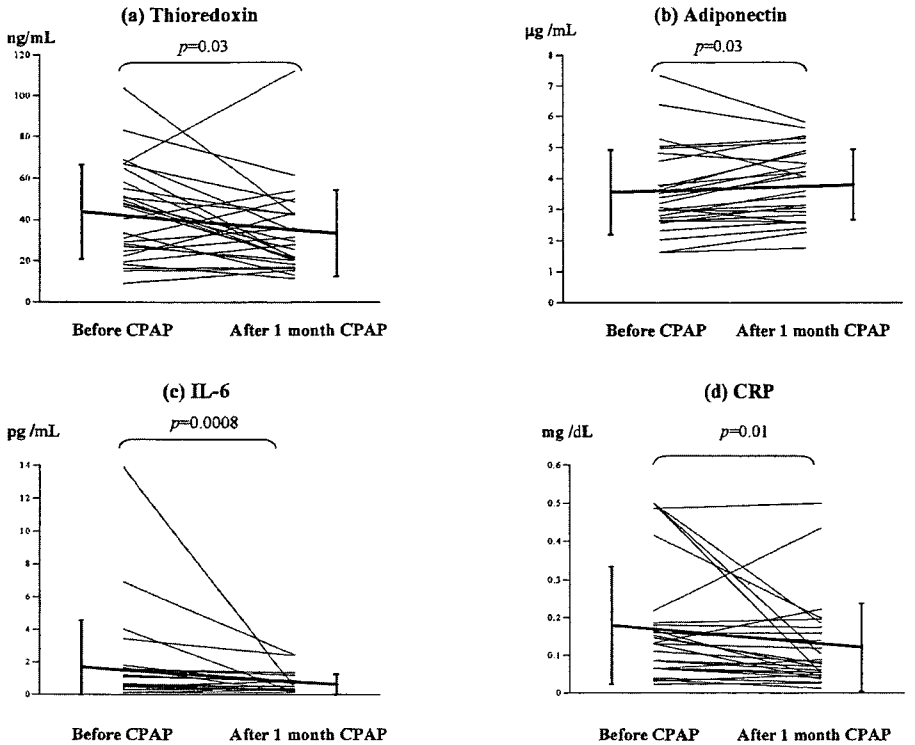


**FIG. 6. Changes in the plasma thioredoxin, adiponectin, IL-6, and serum CRP levels after 1 month of nasal CPAP use in the OSA treatment group.** Nasal CPAP treatment significantly reduced the plasma thioredoxin level but increased the plasma adiponectin level. Nasal CPAP treatment significantly reduced the plasma IL-6 and serum CRP levels. OSA, obstructive sleep apnea; IL-6, interleukin 6; CRP, C-reactive protein; CPAP, continuous positive airway pressure.

0.27) between the 2 days of measurement (mean interval, 39.4 days) during which nasal CPAP treatment was not provided (see Table 3, Fig. 7).

## DISCUSSION

In the OSA patient group ($n$ 41), the plasma TRX level, a marker of oxidative stress, was significantly increased before nasal CPAP treatment, but the plasma level of adiponectin, an adipocytokine, was significantly reduced. The plasma TRX level ($n$ 53: the 41 OSA subjects and the 12 non-OSA subjects) was positively correlated with RDI ($p$ 0.001) and percentage of time with $Sao_2$ 90% ($p$ 0.002). Plasma TRX was strongly related to OSA independent of BMI, age, and current smoking habit ($p$ 0.04; $R$ 0.38). After nasal CPAP treatment, the plasma level of TRX decreased, as did the levels of other cardiovascular parameters, such as serum CRP and plasma IL-6, whereas the plasma level of adiponectin increased.

TRX expression is induced by oxidative stress, and this protein scavenges reactive oxygen radicals directly or together with TRX-dependent peroxiredoxin. Moreover, TRX is released from cells in the presence of oxidative stress, and the plasma/serum TRX levels are good markers of oxidative stress (12, 22). Several studies showed that the TRX level ranges from 10 ng/ml to 30 ng/ml among normal subjects and that it is 40 ng/ml in patients with oxidative stress (20, 21, 35, 36). In the present study, the plasma TRX level was significantly higher in the 41 OSA subjects before nasal CPAP therapy than in the 12 non-OSA subjects (41.0 24.4 ng/ml *vs.* 23.9 14.7 ng/ml; $p$ 0.02). Our results strongly support that OSA patients are subjected to hypoxia-induced oxidative stress every night. Some studies (3, 5) showed that patients with OSA have decreased antioxidant capacity. Indeed, a high concentration of TRX, such as 1,000 ng/ml, would be needed to scavenge reactive oxygen radicals completely and have antiinflammatory effects (24, 25). The mean TRX level of the OSA patients in this study was 41 ng/ml. Therefore, the TRX level in OSA patients may be insufficient to act as an antioxidant protein. Recently, Svatikova *et al.* (37) found that healthy OSA patients without any other comorbidities do not manifest evidence of higher oxidative stress by measuring the levels of oxidized products such as thiobarbituric acid–reactive substances, oxidized low-density lipoprotein, and isoprostanes, contrary to previous reports (3, 5, 15, 32, 41). Oxidative stress in OSA patients may be demonstrated more clearly by measuring the level of an antioxidant protein such as TRX. The plasma TRX level was positively correlated with both RDI and the percentage of time with $Sao_2$ 90%. The plasma TRX level was strongly related to OSA, independent of BMI, age, and current smoking habit. Plasma TRX may be a good marker of oxidative stress in OSA patients and may

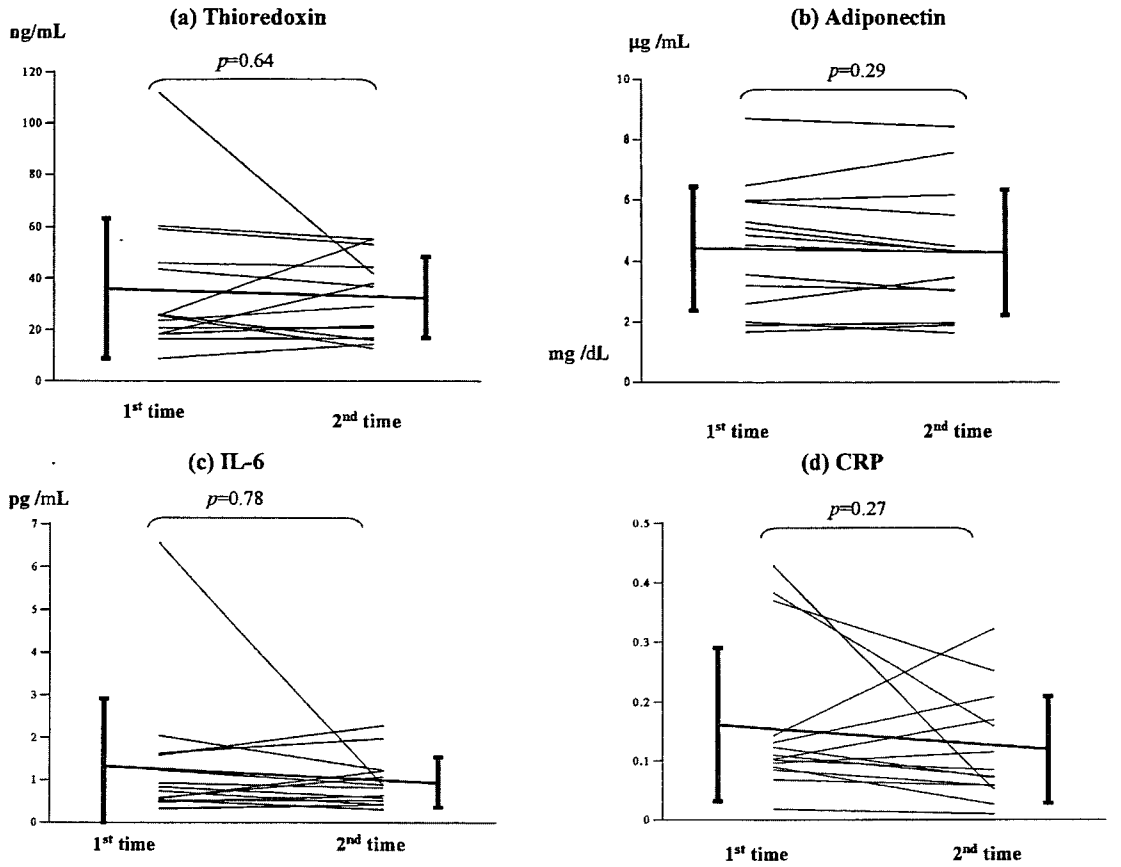


**FIG. 7. Changes in the plasma thioredoxin, adiponectin, IL-6, and serum CRP levels between the 2 measurement days (mean interval, 39.4 days) during which nasal CPAP treatment was not provided in the OSA untreated group.** The plasma thioredoxin, adiponectin, IL-6, and serum CRP levels did not significantly differ between the two measurement days. OSA, obstructive sleep apnea; IL-6, interleukin 6; CRP, C-reactive protein; CPAP, continuous positive airway pressure.

be a sensitive barometer of the effectiveness of nasal CPAP treatment. Because the number of subjects in our study was small, further studies are needed to investigate the precise role of TRX in OSA.

In this study, the plasma adiponectin level was significantly reduced in the OSA treatment group before nasal CPAP treatment, and it increased after nasal CPAP treatment for 1 month. The plasma adiponectin level was negatively correlated with the plasma TRX level. Adiponectin is an adipocyte-specific cytokine. In clinical studies, a low adiponectin level has been associated with insulin resistance (10), atherosclerosis (18), and cardiovascular diseases (28). Moreover, the increased oxidative stress in patients with obesity has a significant impact on the low adiponectin level (7). OSA is clearly associated with obesity and is also linked to the risk of insulin resistance (11) and cardiovascular diseases (34). However, the linkage between OSA and adiponectin has been equivocal (9, 40). This ambiguity may be due to the complexity of regulation of adiponectin. For example, obesity itself results in a low adiponectin level (2), as well as in our data: the plasma adiponectin level in this study was negatively correlated with BMI ($p$ 0.02; $r$ 0.32).

In addition to plasma TRX and adiponectin levels, we measured the levels of inflammatory markers that have been considered to be elevated in oxidative stress. It has been reported that the levels of inflammatory markers such as CRP and IL-6 were elevated in patients with OSA, and they were reduced by nasal CPAP therapy (33, 42). In our data, the CRP level was significantly elevated, although the IL-6 level was not significantly elevated in the untreated OSA patients. However, when CPAP was administered to OSA patients, the CRP and IL-6 levels significantly decreased. A recent study (8) showed that CRP in OSA patients may be associated with obesity rather than with OSA itself. In our data, the difference in CRP level between the OSA and non-OSA subjects disappeared after adjusting for BMI. The inflammatory pathway can be initiated by oxidative stress (14). Because many confounding factors participate in the inflammatory pathway, it is difficult to show a clear association between OSA and inflammation. However, considering that the plasma TRX level was positively correlated with the plasma IL-6 and serum CRP levels and that CPAP treatment improved the serum CRP and plasma IL-6 levels, oxidative stress may be one mechanism of inflammation in OSA patients. Oxidative stress markers such as TRX are directly as-

sociated with the pathogenesis of OSA and may be more sensitive markers of OSA than inflammatory markers.

This study has some limitations. The first limitation was that a significant difference in BMI was found between the non-OSA group and the OSA patient group. The serum CRP level is increased in patients with obesity, whereas the plasma adiponectin level is decreased (2, 10). In this study, the plasma TRX level was not correlated with BMI. In addition, BMI was not a significant variable in the multiple regression analysis with plasma TRX level as the dependent variable. Therefore, the difference in BMI between the non-OSA group and the OSA patient group would not have significant effects on the plasma TRX level.

Another limitation of this study is that polysomnography was not performed in the non-OSA volunteers. The volunteers were not heavy snorers. It was recently reported that the best agreement between AHI and 3%ODI values was found among individuals with AHI values 15, where the difference between the estimated AHI and 3%ODI values was only -0.4 among 49 subjects (26). Therefore, although sleep-disordered breathing in the non-OSA volunteers in this study was measured by oximetry and not polysomnography, this would not have a significant effect on the overall results.

The last limitation is that the effects of nasal CPAP were not examined in a randomized, placebo-controlled design because of the difficulty in implementing placebo nasal CPAP treatment under the official medical insurance system in Japan. However, in the OSA-untreated group, who did not receive nasal CPAP treatment, the levels of the mediators did not change significantly during the interval between the measurement points. Therefore, we could reveal the effects of nasal CPAP.

In conclusion, we demonstrated that the plasma TRX level was elevated in patients with OSA independent of BMI and comorbidities, but that it is reduced by nasal CPAP. TRX has the potential to be a good marker to evaluate oxidative stress in OSA patients and to monitor the effectiveness of CPAP therapy.

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## ABBREVIATIONS

AHI, apnea hypopnea index; BMI, body mass index; CPAP, continuous positive pressure; CRP, C-reactive protein; IL-6, interleukin-6; nCPAP, nasal CPAP; OSA and ObA, obstructive sleep apnea; 3%ODI, 3% oxygen desaturation index; RDI, respiratory disturbance index; $Sao_2$, arterial oxygen saturation; TRX, thioredoxin.

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Address reprint requests to:
*Kazuo Chin*
*Department of Respiratory Medicine*
*Kyoto University Graduate School of Medicine*
*54 Shogoin Kawahara-cho, Sakyo-ku*
*606-8507 Kyoto, Japan*

*E-mail:* chink@kuhp.kyoto-u.ac.jp

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# Acylated ghrelin level in patients with obstructive sleep apnoea before and after nasal CPAP treatment

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# Acylated ghrelin level in patients with obstructive sleep apnoea before and after nasal CPAP treatment

Ken-ichi Takahashi, MD[a], Kazuo Chin, MD, PhD[a], Takashi Akamizu, MD, PhD[b], Satoshi Morita, MD, PhD[c], Kensuke Sumi, MD,PhD[a], Toru Oga, MD, PhD[a], Hisako Matsumoto, MD, PhD[a], Akio Niimi, MD, PhD[a], Tomomasa Tsuboi, MD, PhD[d] Shunichi Fukuhara, MD, PhD[c], Kenji Kangawa, PhD[b,e], Michiaki Mishima, MD, PhD[a]

[a]Department of Respiratory Medicine, Kyoto University Graduate School of Medicine, 54 Shogoin Kawahara-cho, Sakyo-ku, 606-8507, Kyoto, Japan,
[b]Department of Experimental Therapeutics, Translational Research Center, Kyoto University, 54 Shogoin Kawahara-cho, Sakyo-ku, 606-8507, Kyoto, Japan,
[c]Department of Epidemiology and Health Care Research, Kyoto University Graduate School of Medicine, Yoshida Konoe-mach, Sakyo-ku, 606-8501, Kyoto, Japan,
[d]Minami-Kyoto Hospital, National Hospital Organization, Naka-Ashihara, Joyoh-shi, 610-0113, Kyoto, Japan,
[e]Department of Biochemistry, National Cardiovascular Center Research Institute, 5-7-1 Fujishiro-dai, Suita, 565-8565, Osaka, Japan.

**Authors' contributions:** K. Takahashi conceived of the study, participated in its design, acquisition, and interpretation of data, and drafted the manuscript. K. Chin conceived of the study and participated in its design and interpretation of data. T. Akamizu supervised methods of sample measurements. S. Morita reviewed data analysis. K. Sumi participated in acquisition of data. T Oga, H Matsumoto, A Niimi and T Tsuboi contributed to data interpretation. S Fukuhara reviewed data analysis. K Kangawa supervised methods of sample measurements. M Mishima contributed to data interpretation.

**The institution which this work was performed:** Kyoto University Hospital of Medicine, Kyoto, Japan

**Correspondence to:** Kazuo Chin
Department of Respiratory Medicine, Kyoto University Graduate School of Medicine, 54 Shogoin Kawahara-cho, Sakyo-ku, 606-8507 Kyoto, Japan.
Tel: +81-75-751-3852 Fax: +81-75-751-3854 Email: chink@kuhp.kyoto-u.ac.jp

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## ABSTRACT

**Background:** Patients with newly diagnosed obstructive sleep apnoea (OSA) were reported to have had recent weight gain before its diagnosis. Ghrelin stimulates food intake and increases weight gain. Plasma ghrelin is decreased in obese and increased in lean individuals. Of the two circulating forms of ghrelin, acylated and unacylated, the former is thought to be essential for the biological activity of ghrelin.

**Methods:** The plasma levels of the two forms of ghrelin were measured in 21 OSA patients (mean, 46.2 sleep disordered events/hour) before and after 1 month of nasal CPAP (nCPAP) treatment, and were compared with those in 14 untreated OSA patients and 13 individuals without OSA.

**Results:** Although the BMI was significantly higher in the 21 OSA patients than in the non-OSA group, the baseline acylated (11.4 ± 5.86 vs. 7.19 ± 3.80 fmol/mL, $p$=0.03) and unacylated (84.2 ± 50.6 vs. 48.3 ± 23.2 fmol/mL, $p$=0.02) ghrelin levels were higher in the OSA patients. The total ghrelin level was positively correlated with the number of sleep disordered breathings ($p$=0.002). After 1 month of nCPAP treatment, the acylated ghrelin level significantly decreased ($p$=0.02) while the unacylated ghrelin level did not ($p$=0.09).

**Conclusions:** Treatment of OSA may play an important role in the management of obesity in these patients by reducing the acylated ghrelin level.


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**Key words:** ghrelin, growth hormone, nasal continuous positive airway pressure, obese, obstructive sleep apnoea

**Short Title:** acylated ghrelin in sleep apnoea

## INTRODUCTION

Obstructive sleep apnoea (OSA) is highly prevalent among adults worldwide. The association of OSA with serious morbidity and mortality has raised public health concerns.[1] There is strong evidence that obesity, which also significantly affects morbidity and mortality and whose incidence has increased dramatically in developed countries, is a causal factor of OSA. Patients with newly diagnosed OSA were reported to have had recent significant weight gain prior to its diagnosis,[2,3] and after commencement of treatment, most patients were reported to have no change in weight.[4] However, the origin of this phenomenon has not been clarified.

Ghrelin, a 28-amino-acid peptide, exhibits a variety of biological activities, including stimulation of growth hormone (GH) release, food intake and weight gain.[5,6] Ghrelin levels are decreased in obese individuals and increased in lean individuals.[7,8] A portion of ghrelin molecules possesses a unique fatty acid modification, an n-octanoylation, at Ser 3. Of the two circulating forms of ghrelin, acylated and unacylated (desacyl), the acylated form is thought to be essential for the biological activity of this protein. Recently, however, desacyl ghrelin was reported to influence both cell proliferation and adipogenesis.[9,10,11,12] We previously isolated the acylated form of ghrelin from the rat stomach[5] and reported the plasma levels of acylated ghrelin and desacyl ghrelin in healthy volunteers.[7] Harsch et al.[13] measured the total ghrelin levels in OSA patients by radioimmunoassay (RIA). Ulukavak et al.[14] examined the ghrelin levels in OSA patients by enzyme-linked immunosorbent assay (ELISA). In the former report, there was a higher ghrelin level in the sleep apneics versus controls. However, there was no significant difference between the two groups in the latter report. In addition, they did not measure the levels of the two forms of ghrelin separately. To better understand the role of ghrelins, it is important to measure the plasma levels of both acylated ghrelin and desacyl ghrelin separately by more sensitive ELISAs.

**Based on reports about the body weight changes before and after nasal**

**continuous positive airway pressure (CPAP) treatment,[2-4] we hypothesized that both the acylated and desacyl ghrelin levels in OSA patients are elevated and that they decrease by CPAP therapy.** Therefore, we measured the plasma levels of acylated and desacyl ghrelin in patients with OSA before and following nasal CPAP treatment and in healthy volunteers without OSA. **Leptin is an adipocytokine whose level appears to be increased in obese individuals.[15] Leptin levels were reported to be increased in OSA patients and to decrease with CPAP therapy.[16,17] In other reports, the blood leptin level did not change significantly before and after nasal CPAP treatment.[13,18] In the present study, we additionally measured GH and leptin levels to investigate the characteristics of ghrelin secretion in patients with OSA.** This study was approved by the medical ethics committee of our institute and all patients and subjects in the study groups provided written informed consent.

## METHODS

### Subjects

#### OSA Treatment group

The OSA Treatment group included 21 male patients with OSA (mean [± SD] age, 52.5 ± 8.7 years). Blood samples were obtained from these patients before and after 1 month of nasal CPAP treatment. Polysomnography in the OSA patients was performed by the previously-reported method[15,19] before CPAP treatment. The number of episodes of apnoea and hypopnoea per hour, i.e., the apnoea and hypopnoea index (AHI), was 46.2 ± 14.7 events/hour, **arousal index was 37.4 ± 16.2 events/hour** and the body mass index (BMI) was 28.8 ± 3.75 kg/m$^2$ (Table 1). Apnoea was defined as a complete cessation of airflow at the nose and mouth that lasts for ≥ 10s. Hypopnoea was defined as a decrease in thoracoabdominal motion of ≥ 50% that lasts for ≥ 10s and which was associated with a fall in the baseline $SaO_2$ of ≥ 3%. All AHI values were expressed as the number of episodes of apnoea and hypopnoea per hour over the total

sleep time. The BMI of all 21 subjects was over 25 kg/m$^2$, which is defined as obese in Japan. The total sleep time, AHI, lowest arterial $O_2$ saturation, and % time of arterial $O_2$ saturation<90% during sleep were calculated in each patient. Patients with an AHI of more than 20 events per hour were candidates for nasal CPAP therapy. The patients underwent CPAP titration manually and received CPAP treatment with adequate pressure (9.56 ± 2.01 cm$H_2O$) on the first night. Thereafter, they received CPAP therapy nightly for 1 month at home before revisiting our outpatient clinic. **We calculated the average daily time of usage of the CPAP machine which was based on a reading of the time counter in each nasal CPAP machine. The average time of usage of the CPAP machine in the 21 OSA patients was 5.5 ± 1.3 (range 3.8 – 8.9) hours per day.**

**OSA Untreated group**

Fourteen other OSA patients (age, 53.2 ± 9.1 years; AHI 43.7 ± 19.1 events/hour; BMI 27.9 ± 3.0 kg/m$^2$) were placed in the OSA Untreated group, and were matched in age, BMI and AHI with the OSA Treatment group. The patients in the OSA Untreated group underwent polysomnography more than one month after their first visit to the outpatient clinic. Blood samples were taken in the morning twice at a 1-month interval. **CPAP therapy was started in these patients after the two blood samples were obtained.**

**Non-OSA healthy volunteer group**

The Non-OSA group was comprised of 13 volunteers (mean age, 48.1 ± 12.0; BMI 24.5 ± 2.99 kg/m$^2$) who were matched in age with the two OSA groups (Table 1). However, their BMI was significantly lower than that of the two OSA groups. They were not heavy snorers, and it was confirmed that they did not have sleep disordered breathing by oximetry. A blood sample was obtained from the subjects in the Non-OSA group.

**In all of the volunteers, the arterial oxygen saturation was continuously monitored during sleep with a pulse oximeter (Pulsox-24; Minolta, Osaka, Japan)**

**over two consecutive nights. The severity of sleep apnea in the volunteers was quantified by the 3% oxygen desaturation index (3%ODI), which was the number of oxygen desaturations of 3% or more below the baseline level per hour during sleep. Subjects who had a 3%ODI of less than 5 were diagnosed as not having OSA. The 3%ODI of the 13 volunteers was 3.26 ± 1.02 (range, 1.43 – 4.89) and 12 of the 13 subjects showed no desaturations below 90%. One subject showed a lowest $SpO_2$ value of 88% and % time of 0.5% of desaturation below 90% during sleep.**

**The rates of co-morbidities (hypertension, diabetes mellitus, hyperlipidemia) and current smoking habit were not significantly different between the Non-OSA group and each of the two OSA groups (Table 1).** All OSA and Non-OSA subjects in this study received the same medical regimen beginning 1 month before the start of this study and throughout the study period. Because only the BMI was significantly lower in the Non-OSA group than in the OSA groups, multiple linear regression analysis was performed.

The respiratory disturbance index (RDI)[20] was defined as (a) AHI in the OSA patients and (b) 3%ODI in the Non-OSA group.

**Blood samples**

Blood samples were drawn at 8:00 am after a fast beginning at 8:00 pm the previous night. Blood samples were immediately centrifuged at 3000 **rpm** at 4°C for 10 minutes and the plasma was separated. Then, 1N HCl (10% volume of plasma volume) was immediately added to the plasma sample. All samples were stored at -80°C until assay. The levels of the acylated and desacyl forms of ghrelin and GH were measured by the previously reported method.[7] **Leptin levels were measured by radioimmunoassay.**

**Data analysis**

The unpaired *t*-test was used to compare the OSA groups with the Non-OSA group. Differences between the measurements made at the two time points were compared with the paired *t*-test. Because obesity would be expected to affect the plasma ghrelin levels, multiple linear regression analysis was performed for BMI to look for independent associations with OSA. These calculations were performed using StatView software for Windows (Version 5.0; Abacus Concepts, Berkeley, CA). A $p$ value of $<0.05$ was considered significant.

## RESULTS

### Effect of OSA on ghrelin levels

It was reported that the blood ghrelin level was higher in lean individuals than in obese individuals.[7,8] While the Non-OSA group had leaner body than the OSA Treatment group in this study as demonstrated by the significantly lower BMI in the former group, we found that the acylated ghrelin level was significantly higher in the OSA Treatment group before CPAP therapy than in the Non-OSA group (11.4 ± 5.86 vs. 7.19 ± 3.80 fmol/mL, $p = 0.03$). The desacyl ghrelin level was also significantly higher in the OSA Treatment group than in the Non-OSA group (84.2 ± 50.6 vs. 48.3 ± 23.2 fmol/mL, $p = 0.02$). There was no significant difference in the ratio of acylated to unacylated ghrelin levels between the two groups (0.17 vs. 0.15, $p = 0.67$). After adjustment for BMI, the differences in the acylated ($p = 0.007$) and desacyl ($p = 0.01$) ghrelin levels between the OSA Treatment group and the Non-OSA group became more significant (Figure 1). Moreover, even after adjustment for all variables including age, BMI, rate of current smoking and co-morbidities, the acylated ($p = 0.003$) and desacyl ($p = 0.03$) ghrelin levels were significantly higher in the OSA Treatment group than in the Non-OSA group.

**In our previous study,[7] the acylated and desacyl ghrelin levels of 16 males (age 34.7 ± 7.1 years, mean BMI 23.4 ± 3.0 kg/m$^2$) were 10.9 ± 6.1 and 49.1 ±**

**23.5 fmol/mL, respectively. The BMI of the group in the previous report was similar to that of the non-OSA group in the present study ($p$= 0.29) and the ghrelin levels of the group in the previous report[7] were also similar to those of the non-OSA group in this study (acylated: $p$=0.10, desacyl: $p$=0.99). The desacyl and total ghrelin levels of the OSA patients in the present study were significantly higher than those of the normal subjects in the previous study ($p$=0.01, $p$=0.01, respectively), although there was no significant difference in the acylated ghrelin level ($p$=0.83).**

**Relationships between various parameters before nasal CPAP treatment and ghrelin levels**

The relationships between various parameters such as BMI, RDI, or the percentage of time with $SaO_2$<90% and ghrelin levels were analyzed among the 48 subjects (OSA Treatment, n = 21; OSA Untreated, n = 14; Non-OSA, n = 13). The total ghrelin level (acylated plus desacyl) and desacyl ghrelin level were positively correlated with RDI (total: $p$ = 0.002 and desacyl: $p$= 0.003), although the acylated ghrelin level was not ($p$ = 0.14). Lowest arterial $O_2$ saturation was not significantly correlated with the total ghrelin level ($p$ = 0.27), acylated ghrelin level ($p$ = 0.25), nor the desacyl ghrelin level ($p$= 0.31). The % time of arterial $O_2$ saturation<90% was not correlated with the total ghrelin level ($p$ = 0.33), the acylated ghrelin level ($p$ = 0.86) nor the desacyl ghrelin level ($p$= 0.29).

**Effect of nasal CPAP treatment on ghrelin levels**

The BMI did not significantly change after 1 month of nasal CPAP treatment (28.8 ± 3.75 vs. 28.8 ± 3.66 kg/m$^2$, $p$ = 0.58). There were no significant changes in the acylated ($p$ = 0.25) and desacyl ($p$ = 0.24) ghrelin levels after 3 or 4 days of nasal CPAP treatment. The acylated ghrelin level significantly decreased after 1 month of nasal CPAP usage (11.4 ± 5.86 to 9.08 ± 4.79 fmol/mL, $p$ = 0.02), while the desacyl ghrelin

level tended to decrease (84.2 ± 50.6 to 65.1 ± 41.3 fmol/mL, $p = 0.09$) (Figure 2). After one month of nasal CPAP treatment, the differences in the acylated and desacyl ghrelin levels between the OSA patients and Non-OSA subjects were not significant.

**Growth Hormone level in OSA patients and effect of nasal CPAP treatment**

There was no significant change in the GH level ($p = 0.33$) after 3 or 4 days of nasal CPAP treatment. The GH level in the OSA Treatment group was significantly lower than that in the Non-OSA subjects, but it increased significantly after 1 month of nasal CPAP therapy (Figures 1,2). Among the 14 untreated OSA patients, no significant changes in the acylated ghrelin level, desacyl ghrelin level, and GH level (acylated ghrelin: $p = 0.97$, desacyl ghrelin: $p = 0.59$, GH: $p = 0.51$) were noted at a 1-month interval.

**Leptin level in OSA patients and effect of nasal CPAP treatment**

**Leptin levels were measured in 14 of the 21 OSA patients in the OSA Treatment group. Among the 14 patients, only the acylated ghrelin level tended to decrease after 1 month of nasal CPAP usage (p=0.08) but not significantly. The leptin level in the 14 OSA subjects was not significantly different from that in the Non-OSA subjects and did not change after one month of CPAP treatment (Table 2).**

## DISCUSSION

In this study, we found that both the plasma acylated and desacyl ghrelin levels were significantly higher in the OSA patients than in the non-OSA subjects. In addition, among the 48 subjects (OSA Treatment, n = 21; OSA Untreated, n = 14; Non-OSA, n = 13), the RDI before nCPAP treatment was significantly correlated with both the total ghrelin level and desacyl ghrelin level. After one month of nCPAP treatment, the acylated ghrelin level significantly decreased. The elevated acylated ghrelin level in

OSA patients may be one factor that explains the recent body weight gain in OSA patients who are newly diagnosed with OSA. It was reported that the BMI of OSA patients did not change after nCPAP treatment.[4] Therefore, the reduction in acylated ghrelin level with nCPAP therapy in the OSA patients may explain the constancy of the BMI in OSA patients after nCPAP treatment.

The mechanism through which the ghrelin level becomes elevated in OSA patients was not fully investigated in this study. **In this study, the RDI (acylated ghrelin: $p = 0.14$, desacyl ghrelin: $p = 0.003$, total ghrelin: $p = 0.002$), but not desaturation (arterial O2 saturation<90%:% of time) (acylated ghrelin: $p = 0.86$, desacyl ghrelin: $p = 0.29$, total ghrelin: $p = 0.33$), before nasal CPAP treatment was significantly associated with the ghrelin level. It was reported that ghrelin has anti-inflammatory and anti-oxidant effects.[21,22,23] OSA has been associated with inflammation, endothelial dysfunction and increased oxidative stress,[24,25] which are generated by the repetitive episodes of nocturnal hypoxaemia and reoxygenation.[26] Intermittent hypoxemia in OSA patients may increase ghrelin levels. It was also reported that the neural branch of the sympathetic nervous system could directly stimulate ghrelin secretion.[27] Studies on patients with OSA have shown that these patients have a high level of sympathetic nerve activity.[28,29,30] The elevated sympathetic nerve activity in OSA patients may increase ghrelin secretion. The acylated ($p = 0.14$, $R = -0.30$), desacyl ($p = 0.51$, $R = 0.14$) and total ($p = 0.72$, $R = 0.074$) ghrelin levels were not correlated with the arousal index. Although we did not evaluate the sympathetic nerve activity in OSA patients in this study, the intermittent hypoxia (RDI) might have more significant effects on the ghrelin levels than sympathetic nerve activity (arousal index). Recently, it was reported that ghrelin stimulates GH release and that its secretion is stimulated by a reduced GH level.[31] The GH level was decreased in the untreated OSA patients, and it increased after CPAP therapy in this study as well as in a previously-reported study.[32] The**

**reduced GH level may stimulate ghrelin secretion in OSA patients. GH secretion is closely related to slow wave sleep (SWS) and the reduced GH level in OSA patients is probably due to loss of SWS.[32] CPAP therapy may improve sleep quality and increase GH secretion in OSA patients, which in turn may decrease ghrelin levels.**

**After one month of nasal CPAP treatment, the acylated ghrelin level in the OSA patients in this study significantly decreased and the ghrelin levels were similar to those in the Non-OSA subjects. These results may suggest the presence of "ghrelin resistance" in OSA patients. The high ghrelin levels before nasal CPAP treatment may indicate the presence of "ghrelin resistance" and one month of nasal CPAP treatment may not have been sufficient to improve "ghrelin resistance" completely whereas it was sufficient to reduce acylated ghrelin secretion.**

Harsch et al.[13] and Ulukavak et al.[14] previously reported ghrelin levels in patients with OSA. In the former study, the ghrelin levels were measured by RIA, and in the latter study the ghrelin levels were measured by ELISA as in the present study. Harsch et al.[13] reported that the baseline plasma ghrelin level was significantly higher in OSA patients. The unit of measurement in Harsch's study differed from that in our study as well as from that in Ulukavak's study. RIA measurement can not distinguish acylated ghrelin from desacyl ghrelin and inactive fragments of ghrelin. Ulukavak et al.[14] reported that there was no significant difference in ghrelin levels between OSA patients and normal controls. After 2 days of nasal CPAP treatment, the total ghrelin level significantly decreased in Harsch's study. In the present study, a significant decrease in acylated ghrelin level was found not after 3 or 4 days, but after one month of nasal CPAP treatment. Neither of the two previous studies measured the levels of the two forms of ghrelin. The present study is the first to measure the levels of the two forms of ghrelin; in addition, we believe that our results are reliable because we used a new direct ELISA assay[7] in this study. However, since there are several differences in the results

among the three studies including this study, additional studies are needed.

**Leptin is a circulating hormone produced by adipocytes whose plasma level is increased in obese individuals. Leptin induces a complex response involving control of body weight and energy expenditure.[15] The ghrelin levels tended to decrease after 1 month of nasal CPAP usage but not significantly (p=0.08). However, the leptin level did not change after one month of CPAP treatment (*p*=0.80). The reported leptin levels before and after nasal CPAP treatment were controversial.[14,16] In the present study, the number of patients in whom the blood leptin levels were measured, was small (n=14). Therefore, the difference in blood leptin level before and after nasal CPAP treatment might not be significant. This study suggests that the acylated ghrelin level may be a more sensitive marker in OSA patients than the leptin level, and would be one of the markers that show the effectiveness of nasal CPAP therapy. However, further studies are needed to investigate whether the acylated ghrelin level might become a sensitive marker of CPAP.**

One major limitation of this study was that there was a significant difference in BMI between the OSA patients and non-OSA subjects. **However, ghrelin levels are ordinarily lower in obese individuals than in lean individuals.[8] Therefore, if we had a BMI-matched control group, the differences in ghrelin levels between the OSA patients and the controls would be more significant. Indeed, in addition to a significant decrease in acylated ghrelin level after one month of nasal CPAP treatment without significant body weight change, the differences in the acylated ghrelin and desacyl ghrelin levels between the OSA Treatment group and Non-OSA group became more significant after adjustment for BMI: the p-value of the difference in acylated ghrelin level between the OSA Treatment group and Non-OSA group changed from 0.03 to 0.007 and that of the difference in desacyl ghrelin level changed from 0.02 to 0.01 after adjustment for BMI.** Secondly, we

should have measured the amount of physical daytime activity in the OSA patients because ghrelin exhibits a variety of biological activities. In the future, a study that investigates the ghrelin levels, BMI and biological activities of OSA patients is warranted.

Significant OSA is present in 40% of obese individuals, and 70% of OSA patients are obese.[33,34] Therefore, studies on the relationship between OSA, obesity and the management of body weight in OSA patients are necessary. The results of our study suggest that the plasma level of ghrelin may have significant effects on the body weight of OSA patients before and after treatment.

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## Conflict of Interest Statement

We declare that none of the authors have a conflict of interest in relation to this work.