Abbreviations: AHI, apnoea-hypopnoea index; L-PGDS, lipocalin-type prostaglandin D synthase.

Figure 3.

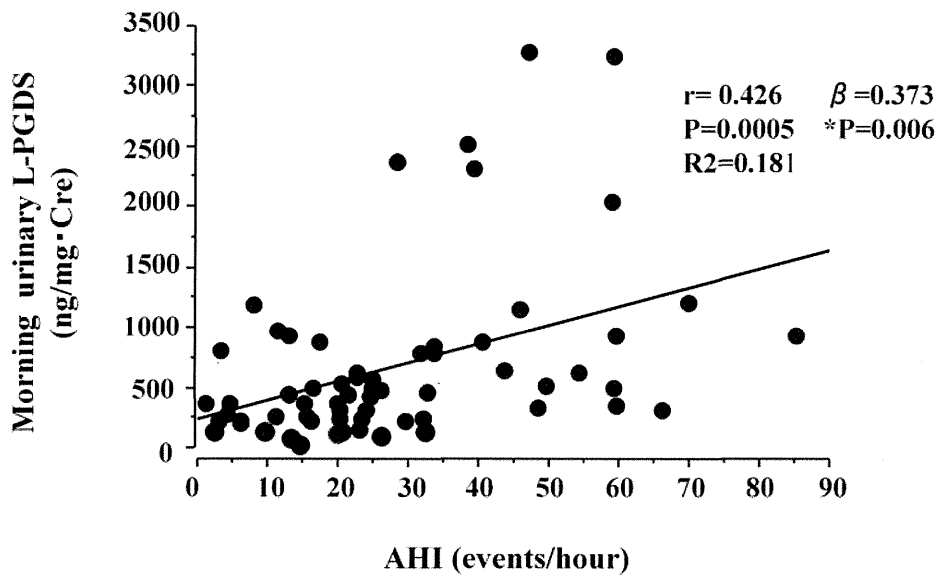


Figure 4.

Change in morning urinary L-PGDS concentrations (A) and RH-PAT index (B) before and after 2 days of CPAP. Individual data are presented.

Abbreviations: L-PGDS, lipocalin-type prostaglandin D synthase; CPAP, continuous positive airway pressure; RH-PAT, reactive hyperemia peripheral arterial tone.

Figure 4.

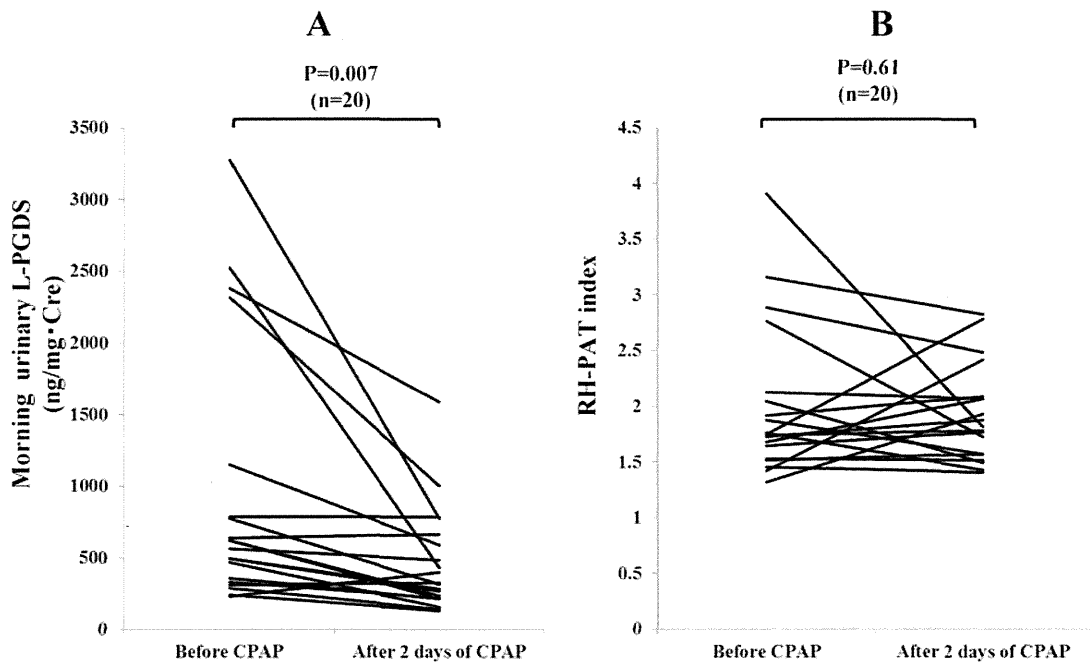
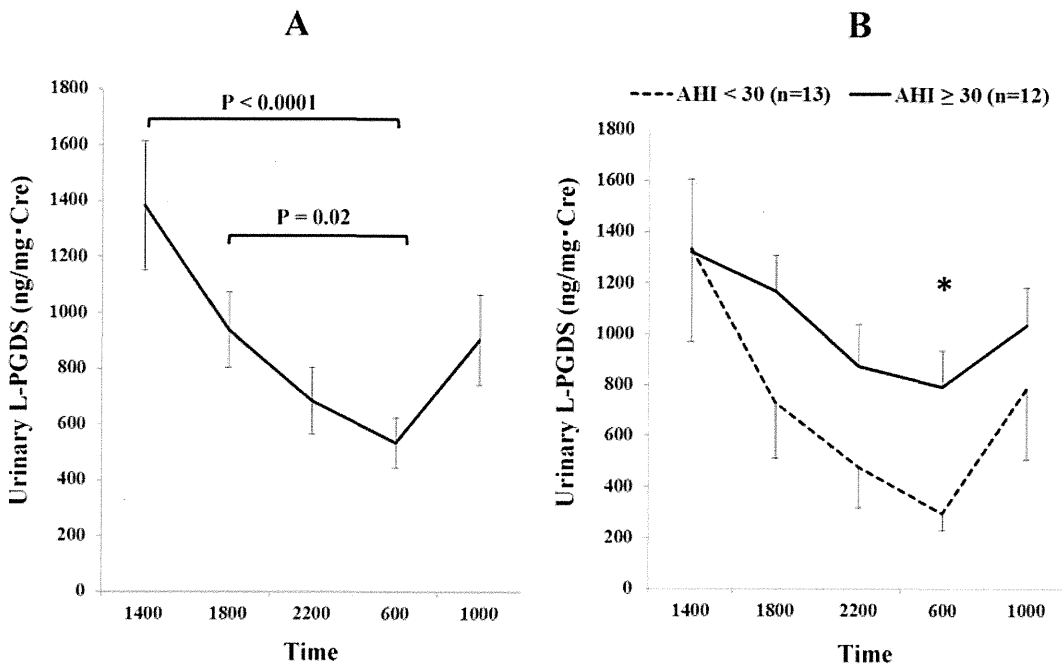


Figure 5.

Urinary L-PGDS concentrations in 25 study patients at 4-hour intervals within a day (except midnight) (A). Figure 3-B shows the comparison of circadian variations in urinary L-PGDS between patients with AHI $\geqq$ 30 (n=12) and patients with AHI < 30 (n=13). Data are shown as mean ± standard error (SE). Single asterisk indicates $p < 0.01$ between patients with AHI $\geqq$ 30 and those with AHI < 30.

Abbreviations: L-PGDS, lipocalin-type prostaglandin D synthase; AHI, apnoea-hypopnoea index.

Figure 5.


A
B
P < 0.0001
P = 0.02
AHI < 30 (n=13)
AHI ≥ 30 (n=12)
Urinary L-PGDS (ng/mg·Cre)
Time
1400
1800
2200
600
1000
*

Original Research | March 2013

Association Between Sleep Apnea, Sleep Duration, and Serum Lipid Profile in an Urban, Male, Working Population in Japan

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Abstract

Background: Dyslipidemia is often comorbid with obstructive sleep apnea (OSA), but few population-based studies have investigated their relationship. Short sleep duration is associated with hypertension and diabetes; however, its association with dyslipidemia is not well known. We investigated relationships among OSA, sleep duration, and the lipid profile in a community-based study.

Methods: We measured the respiratory disturbance index (RDI) and sleep duration by a type 3 portable device and actigraph in 275 men in a Japanese company. Fasting blood parameters were obtained from periodic inspection data.

Results: According to Japanese criteria, 143 subjects had dyslipidemia. Percent sleep time of oxygen saturation as measured by pulse oximetry (SpO_2) < 90% and prevalence of severe OSA were greater and sleep duration and mean SpO_2 during sleep were lower in subjects with dyslipidemia than in those without. Univariate analysis showed that the RDI was positively correlated with serum triglyceride (TG) levels ($\rho = 0.20$, $P < .01$), and sleep duration was negatively correlated with serum total cholesterol (TC) levels ($\gamma = -0.13$, $P = .03$) and serum low-density lipoprotein cholesterol levels ($\gamma = -0.12$, $P = .04$). Stepwise multiple regression analysis revealed that TG was correlated with RDI ($\beta = 0.14$, $P = .02$), BMI ($\beta = 0.20$, $P < .01$), and alcohol intake ($\beta = 0.20$, $P < .01$), and that TC was correlated with sleep duration ($\beta = -0.13$, $P = .03$), age ($\beta = 0.15$, $P = .02$), and waist/hip ratio ($\beta = 0.15$, $P = .02$).

Conclusions: Short sleep duration was associated with TC levels and RDI was positively associated with TG levels among working-aged men in an urban Japanese company. Correcting the status of OSA and/or short sleep duration might improve the lipid profile and cardiovascular consequences.

Obstructive sleep apnea (OSA) is characterized by recurrent episodes of apnea and hypopnea during sleep, which cause intermittent hypoxia and frequent arousals. Many studies have shown that OSA is a risk factor for hypertension, cardiovascular consequences,[1] diabetes mellitus (DM),[2,3] and metabolic syndrome.[4,5] A relationship between OSA and lipid metabolism was shown in animal models, in which intermittent hypoxia aggravated lipid metabolism.[6,7]

In human studies, most of which were clinic based, dyslipidemia (DL) was found more frequently in patients with OSA than in

subjects without OSA. However, it is still controversial whether OSA correlates independently with the serum lipid profile or what components of the lipid profile are associated with OSA.[4,9-15]

Similar to findings for OSA, a large number of studies demonstrated that short sleep duration is associated with hypertension,[16,17] cardiovascular consequences,[18] DM,[19,20] and metabolic syndrome.[21,22] On the other hand, the results of studies about relationships between sleep duration and the serum lipid profile were inconsistent.[21-27] It should be noted that all but one of these studies evaluated sleep duration by questionnaire, which is unacceptable as an objective measurement.[28]

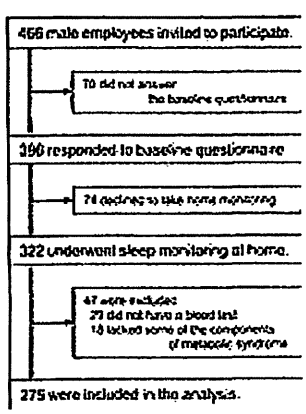
Thus, the relationships between lipid metabolism and OSA or sleep duration are not well known. We previously reported that sleep duration was significantly shorter in subjects with severe OSA than in those with mild or no OSA.[29] To our knowledge, however, the associations among sleep duration, OSA, and lipid metabolism have not been evaluated simultaneously. We hypothesized that OSA and sleep duration affect the lipid profile independently of other confounding factors. To test this hypothesis, we analyzed data from a cross-sectional epidemiologic health survey of middle-aged male employees in Japan, in whom we measured sleep duration and the respiratory disturbance index (RDI) by portable home-monitoring devices.[30]

Materials And Methods

Study subjects were male employees of an urban wholesale company in Japan as described elsewhere in detail.[30] All of the male employees were invited to participate in the study between January 2004 and December 2005. Of the 466 male subjects who were invited to participate, 396 answered the baseline questionnaire (85.0% of eligible subjects).

Of the questionnaire responders, 322 (69.1%) underwent monitoring with home-based portable monitors. There were no significant differences in characteristics (such as age, BMI, Epworth sleepiness scale scores, and numbers of current smokers, habitual snorers, alcohol drinkers, and subjects with hypertension) between eligible subjects and participants.[30] Of those 322 employees, 47 were further excluded from the analysis either because blood parameters were not measured (n = 29) or because some of the components of metabolic syndrome, such as blood parameters and waist circumference, were not determined (n = 18) (Fig 1). There was no shift worker in this population. The prevalence of DL, distribution of OSA severity, and mean duration of weekly sleep did not differ significantly between the included subjects and the excluded subjects ($P = .69$ and 0.85 by χ^2 tests and $P = .63$ by unpaired t test, respectively).

Figure 1. Flowchart of study subjects.



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We analyzed data on the remaining 275 subjects regarding the relationships among the lipid profile, sleep duration, and OSA. Written informed consent was obtained from all subjects. The study protocol was approved by the University Graduate School and Faculty of Medicine Ethics Committee, institutional review board approval number E-37.

Trained research staff performed measurements of weight, waist circumference, and BP. BP was decided based on the average of

the last three of seven measurements made after the subjects rested a few minutes in a sitting position. Height and fasting blood parameters, including fasting blood glucose (FBG), serum triglyceride (TG) levels, serum total cholesterol (TC) levels, and serum high-density lipoprotein cholesterol (HDL-C) levels, were obtained retrospectively from periodic inspection data. When TG levels were < 400 mg/dL, serum low-density lipoprotein cholesterol (LDL-C) levels were calculated by the Friedewald formula (LDL-C = TC − HDL-C − TG/5).

According to diagnostic criteria of DL for Japanese people,[31] DL was defined by LDL-C ≥ 140 mg/dL, HDL-C < 40 mg/dL, and/or TG ≥ 150 mg/dL, or by the current use of lipid-lowering drugs. Hypertension was defined by BP ≥ 140/90 mm Hg or by the current use of antihypertensive drugs. DM was defined by FBG ≥ 126 mg/dL or by use of glucose-lowering drugs.

Each subject wore an actigraph (Actiwatch AW-Light; Koninklijke Philips Electronics N.V.) for 7 days to estimate sleep-wake time[32] and a type 3 portable monitor (PM) (Morpheus; Teijin Pharma Ltd, which is the same as Somté; Compumedics Ltd) for two nights at home as an alternative to polysomnography in the diagnosis of OSA.[33] Sleep duration was estimated from analysis of actigraphy tracings in conjunction with a sleep diary. RDI (number of apnea and hypopnea episodes per hour of the analyzed time) was calculated from data acquired from the actigraph and the PM. PM records were inspected visually and scored by at least two medical doctors specialized in respiratory medicine. Apnea (cessation of breathing for at least 10 s) and hypopnea (> 50% reduction in the amplitude of nasal pressure or respiratory effort associated with > 3% reduction in oxygen saturation as measured by pulse oximetry (SpO_2) for ≥ 10 s) were scored while assessors were blinded to other information except for sleep-wake time by actigraphy. Data without SpO_2 values, illegible recordings, and data from recordings of < 2 h were excluded from analysis. When data from both recorded nights were available, records from the second night were analyzed further. No OSA and mild, moderate, and severe OSA were defined by RDI < 5, 5 to < 15, 15 to < 30, and ≥ 30, respectively. The modified Japanese version of the Epworth Sleepiness Scale (ESS) was used to assess subjective sleepiness. A separate sleep diary was also completed during the survey period.

Results are expressed as the mean plus or minus SD or the number of subjects. We compared the differences in characteristics between the subjects with DL and those without DL. Following testing for normality and equality of variance, continuous variables were compared by unpaired *t* tests, the Welch test, or the Mann-Whitney *U* test, and categorical variables were compared by χ^2 tests. It has been reported that the prevalence of DL and that of severe OSA were approximately 50%[34] and 7%,[35] respectively, among adult men in the general population and that the rate of DL among subjects with severe OSA was 70%.[5] Based on these data, the sample size was set to achieve 80% power at a 5% significance level, and it was determined that there should be a sample size of 40 patients with severe OSA. Subjects were also categorized into four groups by weekly, mean sleep duration as follows: sleep duration < 5 h, 5 to < 6 h, 6 to < 7 h, and ≥ 7 h; two subjects slept longer than 8 h. The significance of an intergroup difference was determined by an analysis of variance, Welch test, or Wilcoxon rank-sum test for the continuous variables following testing for normality and equality of variance. When a significant difference was observed, the Tukey-Kramer test or Steel-Dwass test was performed. For categorical variables, the significance of an intergroup difference was determined by χ^2 tests.

To test our hypothesis that the serum lipid profile was associated with OSA and sleep duration, we performed univariate and multivariate analyses. First, relationships between each serum lipid level and the other variables were analyzed by Pearson correlation coefficients or Spearman rank correlation coefficients following testing for normality and equality of variance. Next, to identify those variables that could best predict each serum lipid level, stepwise multiple regression analyses were performed and coefficients of determination were calculated. The variables entered in these analyses were those yielding a *P* value < .10 by univariate analysis, and when two independent variables had very strong collinearity ($\gamma > 0.70$), one was selected. Cutoff values set in the stepwise analysis were *P* = .05 for both the forward and backward processes. Interactions between any two sets of the variables remaining in the stepwise models were assessed by centered cross terms. *P* values < .05 were considered statistically significant. All analyses were performed using JMP, version 9.0.0 (SAS Institute, Inc).

Results

Characteristics of study subjects with and without DL are shown in Table 1. There were only four subjects whose LDL-C could not be calculated because of TG ≥ 400 mg/dL. Of the 275 subjects, 148 (53.8%) had DL. Besides values for the lipid profile and anthropometric parameters related to obesity, percent sleep time of SpO_2 <90% (%T < 90), systolic BP, diastolic BP, FBG, prevalence of severe OSA, and rate of current smoking were significantly greater, mean SpO_2 during sleep and sleep duration were significantly lower, and RDI tended to be greater in the subjects with DL than in those without DL. Thirteen subjects with DL (8.8%)

were being treated with lipid-lowering drugs. While they had significantly higher TG levels than the others (mean ± SD, 215.1 ± 126.7 mg/dL vs 118.2 ± 75.9 mg/dL; $P < .001$), TC, HDL-C, and LDL-C levels did not differ. Characteristics of the four groups categorized by sleep duration are presented in Table 2. TC level was greater in the subjects who slept 5-< 6 h than in those who slept ≥ 7 h. LDL-C levels tended to differ among the categories. All subjects with moderate to severe OSA slept < 7 h, and 7 (44%) of the 16 patients with severe OSA slept < 5 h. On the other hand, 14 of the 19 subjects (73.7%) who slept ≥ 7 h did not have OSA (Table 2).

Table 1 —Characteristics of Study Subjects With and Without Dyslipidemia

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Table 2 —Characteristics of Study Subjects Categorized by Sleep Duration

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The associations between serum lipid levels and the other variables examined are summarized in Table 3. TG level was positively and significantly correlated with BMI, neck circumference, waist/hip ratio, RDI, %T < 90, FBG, diastolic BP, and alcohol intake, negatively with mean SpO_2 during sleep and lowest SpO_2 during sleep, and tended to be correlated with current smoking. TC level was positively and significantly correlated with age, BMI, neck circumference, waist/hip ratio, RDI, FBS, systolic BP, and diastolic BP, negatively with sleep duration, and tended to be correlated with the lowest SpO_2 during sleep and a history of cardiovascular disease (CVD). HDL-C was negatively correlated with BMI, neck circumference, waist/hip ratio, and current smoking, positively with mean SpO_2 during sleep and with alcohol intake, and tended to be correlated with %T < 90. LDL-C was positively correlated with age, BMI, neck circumference, and waist/hip ratio, negatively with sleep duration and alcohol intake, and tended to be correlated with RDI and FBS.

Table 3 —Correlation Coefficients With Serum Lipid Levels

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With the exception of neck circumference and diastolic BP, these variables were entered into stepwise multiple regression analysis for predicting each serum lipid level for strong collinearity with BMI or systolic BP. These analyses identified BMI ($\beta = 0.20$, $P = .002$), RDI ($\beta = 0.14$, $P = .024$), and alcohol intake ($\beta = -0.20$, $P < .001$) as independent explanatory variables for TG level; age ($\beta = 0.15$, $P = .019$), waist/hip ratio ($\beta = 0.15$, $P = .019$), and sleep duration ($\beta = -0.13$, $P = .030$) for TC level; and BMI ($\beta = -0.36$, $P < .001$), alcohol intake ($\beta = 0.13$, $P = .026$), and current smoking ($\beta = -0.22$, $P < .001$) for HDL-C level (Tables 4-8). Stepwise analysis gave no multivariate model that showed statistical significance for LDL-C, but sleep duration ($\beta = -0.12$, $P = .052$) tended to correlate with LDL-C level (Table 9). The results of analysis using neck circumference instead of BMI are shown in Tables 5 and 8. Almost none of the results changed when systolic BP was changed to diastolic BP. There was no significant interaction between any of two sets of the variables in each stepwise model. Even excluding the 13 subjects who used lipid-lowering drugs, the results of stepwise regression analysis did not change except that current smoking became correlated with TG level.

Table 4 —Stepwise Linear Regression Model to Predict Serum Triglyceride Levels: Model 1

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Table 5 —Stepwise Linear Regression Model to Predict Serum Triglyceride Levels: Model 2

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Table 6 —Stepwise Linear Regression Model to Predict Serum Total Cholesterol Levels

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Table 7 —Stepwise Linear Regression Model to Predict Serum HDL Cholesterol Levels: Model 1

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Table 8 —Stepwise Linear Regression Model to Predict Serum HDL Cholesterol Levels: Model 2

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Table 9 —Stepwise Linear Regression Model to Predict Serum LDL Cholesterol Levels

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Discussion

This was a cross-sectional epidemiologic survey that used portable home-monitoring devices for assessment of sleep duration and OSA, and, to our knowledge, is the first study to evaluate the relationship among the lipid profile, OSA, and sleep duration simultaneously. This study of middle-aged men demonstrated that TG level was correlated with RDI ($P = .02$), BMI ($P < .01$), and alcohol intake ($P < .01$), and that TC level was correlated with sleep duration ($P = .03$), age ($P = .02$), and waist/hip ratio ($P = .02$).

DL is one of the major risk factors for CVD. Among lipid levels clinically measured, LDL-C has been shown to be the strongest risk factor for CVD and TG is also believed to advance atherosclerotic change.[36-38] Recently, two randomized, placebo-controlled studies reported improvements in the serum lipid profile of patients with OSA by CPAP therapy. Phillips et al[39] reported that 8 weeks of CPAP therapy for severe OSA decreased postprandial TG and TC levels, but that fasting TG levels did not change significantly with such treatment. However, Sharma et al[40] reported improvements in fasting TG, TC, and LDL-C levels after 3 months of CPAP therapy for OSA of moderate or greater severity. Therefore, it is still controversial how the lipid profile is affected by CPAP therapy in patients with OSA. On the other hand, severe OSA was related to short sleep duration (Table 2). It has been reported that sleep duration lengthened after adoption of CPAP therapy for OSA.[41] Therefore, the improvements in TC and/or LDL-C levels by CPAP therapy might be partly caused by extension of sleep duration. Indeed, daily use of CPAP for > 5 h in the subanalysis in the Sharma et al study induced improvements in fasting TG, LDL-C, and TC levels[40]; however, these values did not change significantly with 4.4 h of CPAP treatment per day in the report by Phillips et al.[39]

Besides obesity, increases in serum free-fatty-acid (FFA) levels by sympathetic nervous tone,[42] activation of TG biosynthesis in liver through a pathway from hypoxia-inducible factor-1,[6,7,14] and decreases in lipoprotein lipase activity[43] have been advocated as possible mechanisms for the elevation of serum TG levels in OSA patients. Barceló et al[44] reported that patients with OSA had higher serum FFA levels than control subjects, and that this increase in serum FFAs may result in elevated TG levels. Several studies have reported associations between TG level and OSA, but most were clinically based.[4,9,11,14,15] Newman et al[10] reported that in an epidemiologic survey, a correlation between TG level and apnea-hypopnea index was seen only among female patients. Therefore, our study is the first to report a relationship between TG level and OSA in a general male population.

To our knowledge, seven studies have reported associations between the lipid profile and sleep duration,[21-27] but their results were inconsistent. In addition, six of the seven studies estimated sleep duration only by a questionnaire. Our study population was comparable in age to that of Bjorvan et al[23] and relatively close to that of Choi et al.[21] Considering that the subjects in our study included few long sleepers (≥ 8 h), our findings were consistent with the findings of those reports. Two of the seven studies analyzed data specifically according to sex[24,25] and both reported a relationship between sleep duration and HDL-C levels in a female population. It is known that features of the lipid profile differ according to sex[45]; the influence of sleep duration on lipid metabolism may also differ between men and women. Katano et al[27] studied 4,356 healthy workers (including 800 women) from Japan. They studied the correlation between sleep duration and DL and found that those with the lowest sleep duration (< 6 h/night) had DL, which supports our results.

There have been several reports of close relationships between OSA and metabolic syndrome, including several factors that compose metabolic syndrome.[4,5,8,15] Data showed that 3 months of CPAP therapy lowers BP and partially reverses metabolic abnormalities and metabolic syndrome in patients with OSA.[40] In that subanalysis, subjects with mean adherence to CPAP for ≥ 5 h had superior improvements in metabolic parameters to those with mean adherence to CPAP of < 5 h.

Thus, considering previous reports, OSA[4,9,10,12-15] and sleep duration,[21-27] including the treatment duration for OSA,[40] may have significant effects on metabolic parameters, including blood lipid levels. In addition to relationships with metabolic syndrome,[29] BP,[46]

and blood glucose, we have reported relationships among sleep apnea, sleep duration, and lipidemia in this study. Thus, we propose that sleep duration should be considered for relationships between OSA and metabolic parameters whether these conditions are treated or untreated.

In this study, mean sleep duration in urban subjects in Japan was 6.0 h. It was reported that the average sleep duration by self report was 7.2 h/d among middle-aged Japanese people.[48] Actigraph-measured sleep duration was reported to be shorter than self-reported sleep.[28] In addition, recently it was reported that 30% of employed civilian workers in the United States were short sleepers (sleep duration < 6 h/d).[49] Although it is said that the sleep duration among Japanese is one of the shortest in the world,[50] subjects in Western countries might also have developed sleep patterns similar to those shown in the present study. Therefore, our results might apply to urban subjects in Western countries.

Limitations of this study should be mentioned. First, we cannot know causality or the mechanisms of our findings because this was a cross-sectional study. Results of the present study were simple epidemiologic data. Second, the study population was limited to working-age men from an urban company in Japan and included few long sleepers (≥ 8 h). This bias of the study population could have affected the results and they cannot be generalized to women, the elderly, long sleepers, or other ethnic groups. However, limiting the characteristics of study subjects might have enabled us to more clearly identify the relationships under study because lipid metabolism would likely differ considerably in study subjects with a more heterogeneous background. Third, we could not estimate the lifestyles of the participants, namely, dietary habits, exercise habits, and so forth, which are believed to influence lipid metabolism. It is very difficult to evaluate these factors quantitatively by a questionnaire alone. Subjects of the present study, however, were limited to those employed by one company in the service industry, so we might think that their lifestyles, physical workload, and socioeconomic status were relatively similar compared with subjects in previous epidemiologic cohort studies. In addition, the indices associated with obesity, which are usually believed to strongly reflect a daily energy balance, did not differ according to sleep duration. Fourth, the study population was small, especially regarding the number of patients with severe OSA, because this was an exhaustive survey and the number of company employees that we surveyed was smaller than the calculated sample size. Therefore, future testing should include many more subjects among which there is a sufficient number of patients with severe OSA that would correspond to the estimated sample size. Finally, we did not perform polysomnography. However, the interscorer and night-to-night reliability of the RDI were excellent (interclass correlation coefficients of 0.98 and 0.95, respectively).[30] In addition, it has been reported that the nonattached PM is reliable under the specified conditions in which our study was conducted.[33]

In conclusion, we showed that sleep duration was negatively associated with TC levels and that RDI was positively associated with TG levels in middle-aged men working in an urban Japanese company. For working-age men with DL, CPAP therapy for severe OSA may improve the lipid profile and subsequently reduce risk of cardiovascular events. Further study is needed to determine the association among lipid metabolism, OSA, and sleep duration. This will require surveys of various populations with different backgrounds.

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Author contributions: Dr Chin had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Dr Toyama: contributed to data analysis and interpretation, drafted the manuscript, and served as principal author.

Dr Chin: contributed to the design and supervision of the study.

Dr Chihara: contributed to drafting the manuscript and approved the manuscript.

Ms Takegami: performed the primary statistical analysis and approved the manuscript.

Dr Takahashi: evaluated records of portable devices visually and clinically and approved the manuscript.

Dr Sumi: evaluated records of portable devices visually and clinically and approved the manuscript.

Dr Nakamura: evaluated records of portable devices visually and clinically, and approved the manuscript.

Ms Nakayama-Ashida: contributed to data acquisition and approved the manuscript.

Dr Minami: contributed to data acquisition and approved the manuscript.

Ms Horita: contributed to data acquisition and approved the manuscript.

Dr Oka: contributed to data acquisition and approved the manuscript.

Dr Wakamura: contributed to data acquisition and approved the manuscript.

Dr Fukuhara: performed the primary statistical analysis and approved the manuscript.

Dr Mishima: contributed to the study design, and approved the manuscript.

Dr Kadotani: contributed to the study design and data acquisition and approved the manuscript.

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CVD	cardiovascular disease
DL	dyslipidemia
DM	diabetes mellitus
ESS	Epworth sleepiness scale
FBG	fasting blood glucose
FFA	free fatty acid
HDL-C	serum high-density lipoprotein cholesterol
LDL-C	serum low-density lipoprotein cholesterol
OSA	obstructive sleep apnea
%T < 90	percent sleep time of oxygen saturation as measured by pulse oximetry < 90%
PM	type 3 portable monitor
RDI	respiratory disturbance index
SpO_2	oxygen saturation as measured by pulse oximetry
TC	serum total cholesterol
TG	serum triglyceride

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Association between Plasma Neutrophil Gelatinase Associated Lipocalin Level and Obstructive Sleep Apnea or Nocturnal Intermittent Hypoxia

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Abstract

Background: Both obstructive sleep apnea (OSA) and a novel lipocalin, neutrophil gelatinase associated lipocalin (Ngal), have been reported to be closely linked with cardiovascular disease and loss of kidney function through chronic inflammation. However, the relationship between OSA and Ngal has never been investigated.

Objectives: To evaluate the relationship between Ngal and OSA in clinical practice.

Methods: In 102 patients, polysomnography was performed to diagnose OSA and plasma Ngal levels were measured. The correlations between Ngal levels and OSA severity and other clinical variables were evaluated. Of the 46 patients who began treatment with continuous positive airway pressure (CPAP), Ngal levels were reevaluated after three months of treatment in 25 patients.

Results: The Ngal level correlated significantly with OSA severity as determined by the apnea hypopnea index ($r=0.24$, $p=0.01$) and 4% oxygen desaturation index (ODI) ($r=0.26$, $p=0.01$). Multiple regression analysis showed that the Ngal level was associated with 4%ODI independently of other clinical variables. Compliance was good in 13 of the 25 patients who used CPAP. Although the OSA (4%ODI: 33.1 ± 16.7 to 1.1 ± 1.9/h, $p<0.01$) had significantly improved in those with good compliance, the Ngal levels were not significantly changed (60.5 ± 18.1 before CPAP vs 64.2 ± 13.9 ng/ml after CPAP, $p=0.27$).

Conclusions: Plasma Ngal levels were positively associated with the severity of OSA. However, the contribution rate of OSA to systemic Ngal secretion was small and changes in Ngal levels appeared to be influenced largely by other confounding factors. Therefore, it does not seem reasonable to use the Ngal level as a specific biomarker of OSA in clinical practice.

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Introduction

Neutrophil gelatinase associated lipocalin (Ngal), also known as lipocalin 2, is a 25-kDa secretory glycoprotein that was originally identified in human neutrophils. This protein was originally known as an innate immunity antibacterial factor released by activated neutrophils. [1,2] It has also become known to be produced by renal tubular cells in response to different types of injury. [3] Based on experimental and clinical findings, Ngal is widely considered as an excellent indicator of acute and chronic kidney injury.[3–7] Moreover, because this protein is also released by endothelial cells and failing myocardium, a close relationship between blood Ngal levels and heart failure or cardiovascular diseases has been suggested.[8–10].

Obstructive sleep apnea (OSA) is a highly prevalent disorder, affecting about 4–20% of adults and is characterized by repetitive episodes of partial or complete obstruction of the upper airway during sleep associated with transient oxygen desaturation.[11–13] Accumulating clinical evidence suggests that OSA is an independent risk factor for cardiovascular disease and loss of kidney function through nocturnal hypoxia and chronic inflammation.[14–17] From an in vitro model of OSA, it was suggested that the pro-inflammatory transcription factor, nuclear factor-kappa B (NF-κB), plays an important role in the inflammatory

process of a cell's reaction to intermittent hypoxia/reoxygenation. [18] Meanwhile, it has been reported that several inflammatory stimuli, such as interleukin 1β, stimulate systemic Ngal expression and secretion. NF-κB also has been shown to transactivate Ngal expression, suggesting that Ngal might be involved in inflammatory responses. [19,20].

Therefore, a positive correlation between OSA severity and systemic Ngal secretion through chronic inflammation seems possible. However, this relationship has never been investigated. Thus, we hypothesized that blood Ngal levels are elevated in patients with OSA and that its levels are modified by the treatment of OSA with continuous positive airway pressure (CPAP). In the present study, we measured plasma Ngal levels in patients with OSA and evaluated its utility in clinical practice.

Methods

Subjects

Study patients were consecutively recruited from the Sleep Unit of Kyoto University Hospital between January 2009 and May 2012. All had been referred to our sleep unit under suspicion of OSA with symptoms such as habitual snoring or daytime sleepiness. None had been previously diagnosed with or treated for OSA. Patients with overt renal failure (serum creatinine >1.3 mg/dl) or with any history of cardiovascular diseases, heart failure or arrhythmia were excluded because severe renal and/or heart failure can directly affect plasma Ngal levels. Also excluded were patients with pulmonary diseases, chronic infection, history of cancer or collagen disease. Since a consensus about the relationship between Ngal levels and metabolic syndrome has not yet been formed, we aimed to evaluate the correlations between risk factors for metabolic syndrome and plasma Ngal levels in actual clinical practice. We did not exclude patients with components of metabolic syndrome such as hypertension, diabetes and dyslipidemia even if they were under treatment for these comorbidities.[21–23] This study was approved by Kyoto University Graduate School and Faculty of Medicine Ethics Committee, and written informed consent was obtained from all patients.

Polysomnography and CPAP Implementation

The diagnosis of OSA was confirmed by polysomnography (SomnoStar pro, Cardinal Health, Dublin, OH, USA or Alice 4, Philips Respironics, Inc., Murrysville, PA, USA), which was started at 22:00 and ended at 6:00 the following morning. Surface electrodes were attached using standard techniques to obtain an electrooculogram, electromyogram of the chin and 12-lead electroencephalograph. Sleep stages were defined according to the criteria of Rechtchaffen and Kales. [24] Ventilation was monitored by inductive plethysmography (Respitrace QDC, Viasys Healthcare, Palm Springs, CA, USA). Airflow was monitored by a nasal pressure transducer and supplemented by an oronasal thermal sensor. Arterial oxygen saturation (SpO_2) was monitored continuously with a pulse oximeter.

Apnea was defined as the continuous cessation of airflow for more than 10 seconds and hypopnea was defined as a reduction in airflow of 30% or more lasting for 10 seconds or more accompanied by a decrease in SpO_2 of at least 4%. [25] Apnea-hypopnea index (AHI) values were calculated as the number of episodes of apnea and hypopnea per hour over the total sleep time. 4% oxygen desaturation index (ODI) values were defined as the

Table 1. Baseline characteristics and data on metabolic syndrome and its components in study patients.

	non OSA (n = 15)	mild OSA (n = 37)	moderate OSA (n = 24)	severe OSA (n = 26)	p
Age (y)	48.2±17.2	55.1±13.3	57.3±14.5	58.6±11.5	0.12
Sex (male), n(%)	8 (53.3)	24 (64.9)	18 (75)	18 (69.2)	0.56
Smoking status never/ex/current, n	2/3/10	5/13/19	3/5/16	3/8/15	0.88
Body mass index (kg/m^2)	23.5±3.7	25.1±4.6	24.8±4.1	29.2±7.8[a,b,c]	<0.01
Neck circumference (cm)	36.2±3.1	37.9±3.7	37.8±3.6	39.8±4.0[a]	0.03
Waist circumference (cm)	84.3±11.5	90.1±13.1	89.7±11.0	98.6±14.4[a,b]	0.01
Hip circumference (cm)	90.3±8.8	94.6±10.6	92.1±9.3	101.3±15.3[a,c]	0.01
Waist-to-hip ratio	0.93±0.07	0.95±0.05	0.97±0.04	0.97±0.03	0.04
SBP (mmHg)	119.8±16.1	124.2±16.3	127.3±12.9	127.4±16.0	0.40
DBP (mmHg)	72.7±11.9	76.7±11.5	78.6±11.2	76.2±12.0	0.50
Percentages of patients with metabolic syndrome or components of metabolic syndrome					
Hypertension, n (%)	7 (46.7)	20 (54.1)	15 (62.5)	19 (73.1)	0.16
Hyperglycemia, n (%)	5 (33.3)	7 (18.9)	4 (16.7)	5 (19.2)	0.56
Dyslipidemia, n(%)	6 (40.0)	16 (43.2)	11 (45.8)	13 (50.0)	0.98
Visceral fat accumulation, n(%)	7 (46.7)	22 (59.5)	18 (75.0)	23 (88.5)	0.01
Metabolic syndrome, n(%)	5 (33.3)	13 (35.1)	8 (33.3)	11 (42.3)	0.90
Percentages of patients under treatment for components of metabolic syndrome					
Hypertension, n (%)	2 (13.3)	14 (37.8)	9 (37.5)	12 (46.2)	0.16
Diabetes, n (%)	2 (13.3)	3 (8.1)	2 (8.3)	5 (19.2)	0.56
Dyslipidemia, n(%)	4 (26.7)	8 (21.6)	5 (20.8)	6 (23.1)	0.98

Data are expressed in mean ± SD or n (%).
OSA: obstructive sleep apnea; SBP: systolic blood pressure; DBP: diastolic blood pressure;
[a]$p<0.05$ vs non OSA; [b]$p<0.05$ vs mild OSA; [c]$p<0.05$ vs moderate OSA.
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Table 2. OSA parameters and laboratory profiles.

	non OSA (n = 15)	mild OSA (n = 37)	moderate OSA (n = 24)	severe OSA (n = 26)	p
Parameters of OSA					
Apnea hypopnea index/h	2.2±1.6	9.4±2.6	22.2±5.0[a,b]	50.5±19.8[a,b,c]	<0.01
4%ODI/h	1.8±1.6	8.2±3.3	20.7±6.0[a,b]	49.6±20.2[a,b,c]	<0.01
Minimum SpO_2 (%)	90.3±4.5	84.9±4.3	78.4±8.6[a,b]	71.3±12.2[a,b,c]	<0.01
Arousal index/h	24.3±12.1	22.8±9.6	30.7±13.2	43.4±18.3[a,b,c]	<0.01
Length of time SpO_2<90% (m)	3.2±5.4	11.4±17.4	37.3±52.2	121.1±107.8[a,b,c]	<0.01
Laboratory profiles					
FPG (mg/dl)	107.7±39.9	95.4±20.1	96.4±24.3	102.5±20.2	0.35
HbA1c (%)	5.80±1.22	5.49±0.65	5.45±0.91	5.74±0.86	0.45
Total cholesterol (mg/dl)	185.5±32.3	195.2±35.4	203.0±44.0	201.5±44.0	0.53
LDL cholesterol (mg/dl)	103.2±27.9	116.2±27.8	118.5±36.8	109.5±31.8	0.40
HDL cholesterol (mg/dl)	55.2±12.9	52.8±13.5	53.0±16.1	51.9±13.9	0.91
Triglycerides (mg/dl)	125.1±86.5	119.8±58.0	139.1±79.1	168.0±185.2	0.39
BNP (pg/ml)	14.4±8.7	20.6±24.4	22.4±31.8	21.5±18.7	0.73
Creatinine (mg/dl)	0.72±0.19	0.74±0.15	0.80±0.17	0.78±0.21	0.45
Ngal (ng/ml)	46.9±6.0	48.9±10.9	51.3±15.2	55.4±16.7	0.16

Data are expressed in mean ± SD or n (%).
OSA: obstructive sleep apnea; ODI: oxygen desaturation index; SpO_2: saturation of oxygen; FPG: fasting plasma glucose; LDL: low density lipoprotein; HDL: high density lipoprotein; BNP: brain natriuretic peptide; Ngal: neutrophil gelatinase associated lipocalin.
[a]p<0.05 vs non OSA; [b]p<0.05 vs mild OSA; [c]p<0.05 vs moderate OSA.
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number of desaturations ≥4% per hour of sleep. The length of time SpO_2<90% during sleep was calculated in each patient. Patients with central sleep apnea were excluded. OSA severity was defined by the AHI as follows: non OSA (AHI<5), mild OSA (5≤AHI<15), moderate OSA (15≤AHI<30) and severe OSA (30≤AHI).

Patients with an AHI ≥15 were candidates for nasal CPAP. Those who agreed with CPAP implementation underwent a second polysomnography with CPAP titration. We implemented CPAP with the auto adjusting positive airway pressure (PAP) function for all patients. Based on the second sleep study, minimum and maximum PAP were determined to abolish all respiratory events, arousal and desaturation events.

Follow-Up

At the three-month follow-up, we urged the patients to undergo a third sleep study to confirm whether an adjustment of the CPAP setting was necessary. To investigate the effect of CPAP treatment on plasma Ngal levels, at the third sleep study blood samples were collected in the same way as at the first sleep study. We also checked use time of the CPAP machine by reading the time counter on the CPAP machines. Similar to prior studies, we defined 'good compliance' as the use of CPAP for >4 h per night on >70% of nights and categorized the patients into two groups, those with 'good compliance' or 'poor compliance'. [26] We analyzed the data separately for each group and compared clinical variables before and after CPAP treatment.

Blood Sampling and Measurement of Plasma Ngal Level

Blood samples were drawn at 7:00 in the morning after the subjects had fasted beginning at 20:00 the previous night. Blood samples were centrifuged immediately at 3,000 rpm at 4°C for 10 min. The separated samples were stored −80°C until assay. Plasma Ngal concentrations were determined by an ELISA kit provided by Bioporto Diagnostics, Gentofte, Denmark. Intra- and inter-assay coefficients of variation for Ngal were 1.2–4.0% and 2.2–11.2%, respectively.

Definition of Metabolic Syndrome

In classifying patients based on the components of metabolic syndrome, we utilized Japanese criteria. [27] Waist circumference (WC) was measured at the level of the navel with the patient standing, and visceral fat accumulation was determined to be positive at WC ≥85 cm for men and ≥90 cm for women. A diagnosis of metabolic syndrome required the subject to have visceral fat accumulation and 2 or 3 of the following: (a) dyslipidemia (triglycerides ≥150 mg/dL and/or high-density lipoprotein cholesterol level <40 mg/dL, or specific treatment for these lipid abnormalities); (b) hypertension (systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥85 mmHg, or treatment of previously diagnosed hypertension); and (c) hyperglycemia (fasting plasma glucose ≥110 mg/dL or specific treatment for diabetes mellitus). Anthropometric parameters and blood pressure were measured immediately after polysomnography recording ended.

Statistical Analysis

In the analysis of data, we classified the patients depending on the severity of OSA and compared their clinical backgrounds. We also compared plasma Ngal levels between patients with and without each component of metabolic syndrome to investigate the relationships between plasma Ngal levels and metabolic syndrome. Data were expressed as means ± standard deviation. The significance of intergroup differences based on the severity of OSA was determined by an analysis of variance. When a significant difference was found, we used the Tukey's honestly

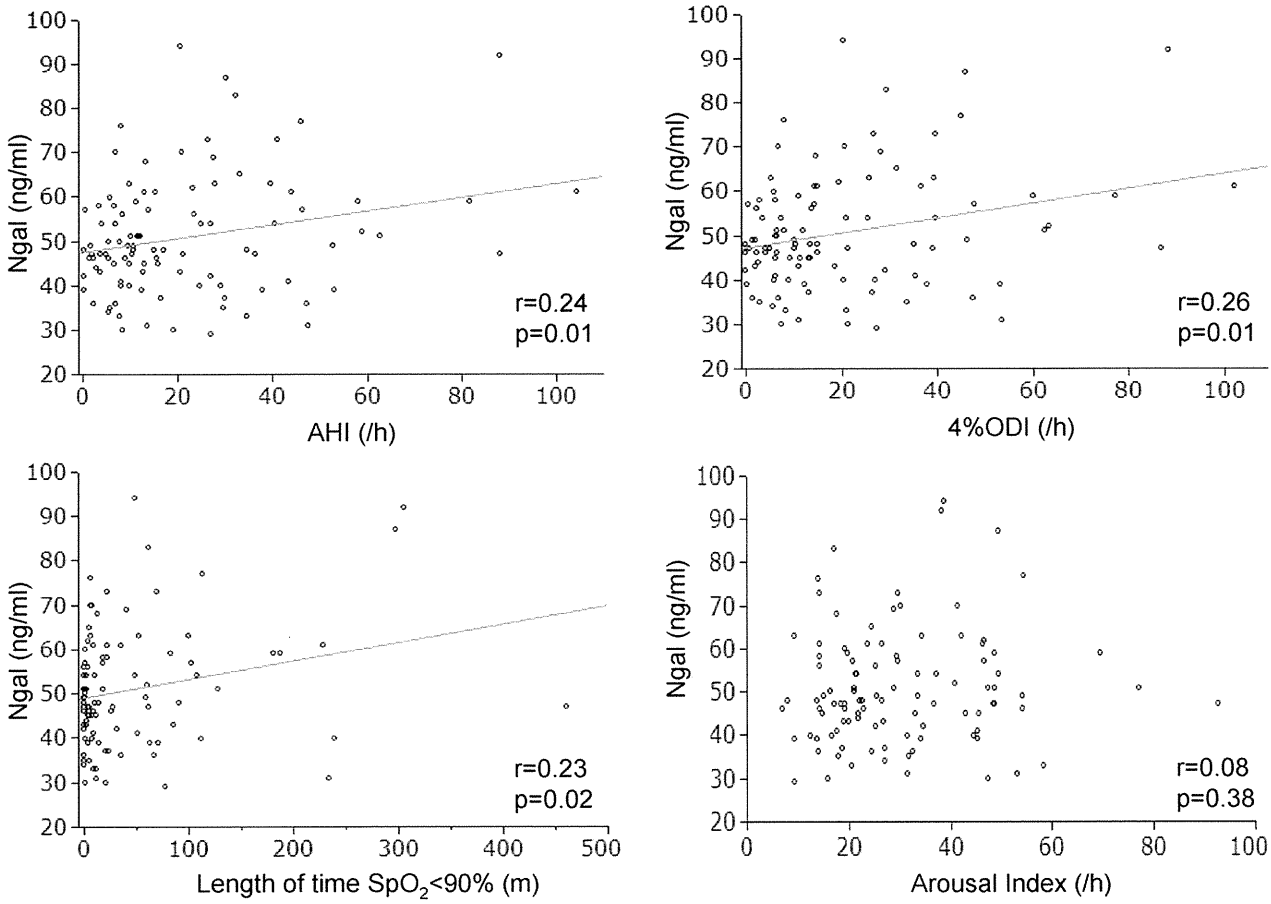

Figure 1. Simple correlations between plasma neutrophil gelatinase associated lipocalin (Ngal) levels and parameters of obstructive sleep apnea. AHI: apnea hypopnea index; ODI: oxygen desaturation index; SpO_2: saturation of oxygen.
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significant difference procedure to identify where the difference was significant. A chi-square test and the Mann-Whitney U test were used to compare categorical and continuous variables, respectively. We used Pearson's coefficient tests to evaluate the relationship between the plasma Ngal level and other continuous variables. Based on the results of this analysis, multiple regression analyses were performed to clarify the contribution rate of OSA and other comorbidities to systemic Ngal secretion. Wilcoxon signed rank test was used to compare clinical variables before and after CPAP treatment. Two-tailed p-values <0.05 were considered statistically significant. All statistical analyses were performed using JMP 7.0.2 statistical software (SAS Institute Inc., Cary, NC, USA).

Results

Baseline Characteristics of Study Patients

A total of 102 patients were studied, and their baseline characteristics are shown in table 1.Those with severe OSA were characterized by a significantly higher body mass index (BMI) than in the other three groups. The percentages of patients who fulfilled the criterion for visceral fat accumulation increased as the severity of OSA increased. Other anthropometric parameters with significant differences among the groups are also shown in table 1. With the exception of the parameters for OSA, there were no significant differences among the four groups in other clinical background factors. (Tables 1 and 2).

Plasma Ngal Levels in Patients at Diagnosis and follow up

Table 2 shows baseline plasma Ngal levels in the four groups, with no statistically significant differences found among them. However, simple linear regression analysis showed significant correlations of the plasma Ngal level with the following parameters of OSA: AHI ($r=0.24$, $p=0.01$), 4%ODI ($r=0.26$, $p=0.01$) and time of $SpO_2<90\%$ ($r=0.23$, $p=0.02$). (Figure 1) The plasma Ngal level was also correlated with values for serum low density lipoprotein (LDL) cholesterol ($r=-0.31$, $p<0.01$), triglycerides ($r=0.24$, $p=0.01$) and creatinine ($r=0.34$, $p<0.01$). On the other hand, none of anthropometric parameters and parameters associated with diabetes such as fasting plasma glucose and HbA1c levels showed significant correlations with Ngal levels.(Table 3) Furthermore, in the present cohort, significant differences were not found in plasma Ngal levels between patients with and without each of the components of metabolic syndrome. (Table 4).

For the multiple regression analysis, we chose 4%ODI as the representative variable for OSA severity as it had the best correlation with the Ngal level among OSA parameters in the simple correlation analysis. The analysis demonstrated that 4%ODI was associated with the Ngal level independently of creatinine and LDL-cholesterol levels. The contribution rate of 4% ODI to the Ngal level was 6.2% (Table 5).

Table 3. Simple correlations between plasma neutrophil gelatinase associated (Ngal) levels and clinical variables.

	r	p
Age (y)	0.04	0.62
Body mass index (kg/m^2)	0.13	0.17
Neck circumference (cm)	0.00	0.84
Waist circumference (cm)	0.00	0.91
Hip circumference (cm)	0.00	0.99
Waist-to-hip ratio	0.03	0.76
SBP (mmHg)	0.00	0.96
DBP (mmHg)	−0.13	0.17
FPG(mg/dl)	−0.08	0.39
HbA1c (%)	−0.06	0.52
Total cholesterol (mg/dl)	0.12	0.21
LDL-cholesterol (mg/dl)	−0.31	<0.01
HDL-cholesterol (mg/dl)	−0.18	0.06
Triglycerides (mg/dl)	0.24	0.01
BNP (pg/ml)	0.00	0.74
Creatinine (mg/dl)	0.34	<0.01

r: correlation coefficient; SBP: systolic blood pressure; DBP: diastolic blood pressure; FPG: fasting plasma glucose; LDL: low density lipoprotein; HDL: high density lipoprotein; BNP: brain natriuretic peptide.
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CPAP was implemented for 46 of the 50 patients with moderate or severe OSA. Of the 46 patients, 27 agreed to a follow-up sleep study. Just before the reevaluation, cardiac medicine was prescribed for one patient and an upper airway infection was found in another patient. These two patients were excluded from the analysis, and the remaining 25 patients were reevaluated. Thirteen were categorized into the good compliance group and the other 12 patients into the poor compliance group. Those in the good compliance group were significantly older than patients in the poor compliance group. The determined maximum and minimum PAP did not differ between the two groups.

After CPAP implementation, OSA was significantly improved in both groups. In the good compliance group, despite improvements in OSA, no significant change was noted in plasma Ngal levels from values before CPAP use. Furthermore, in the poor compliance group, Ngal levels were significantly elevated after CPAP implementation. There were not significant differences in the other confounding factors before and after CPAP treatment (Table 6).

Discussion

In this cross sectional evaluation, although significant differences in plasma Ngal levels were not found among groups classified according to the severity of OSA, parameters of OSA, such as 4%ODI and AHI per se, correlated with plasma Ngal levels in regression analysis. This suggests that OSA contributes, although weakly, to elevated plasma Ngal levels through nocturnal hypoxia. Because it has been reported that hypoxia induces an elevation in plasma Ngal levels in an experimental animal model, it is possible that OSA induces Ngal elevation through nocturnal intermittent hypoxia. [28] To the best of our knowledge, this is the first report to evaluate the relationship between the Ngal protein level and OSA severity in clinical practice.

Table 4. Plasma Ngal levels in patients with and without each component of metabolic syndrome.

	Plasma Ngal levels (ng/ml)		
	Comorbidity(+)	Comorbidity(−)	p
Hypertension	52.7±1.7	48.0±2.1	0.17
	(n=61)	(n=41)	
Hyperglycemia	48.4±14.5	51.4±13.0	0.27
	(n=21)	(n=81)	
Dyslipidemia	51.9±14.7	49.9±12.2	0.49
	(n=46)	(n=56)	
Visceral fat accumulation	50.9±14.1	50.5±11.6	0.92
	(n=70)	(n=32)	
Metabolic syndrome	51.8±16.7	50.2±11.1	0.93
	(n=37)	(n=65)	

Data are expressed in mean ± SD.
Ngal: neutrophil gelatinase associated lipocalin.
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The relationship between another protein in the lipocalin family and OSA has been investigated. Makino et al and Nena et al, respectively, investigated the relationship between the plasma level of retinol binding protein 4 (RBP-4), which also belongs to the lipocalin protein family, and OSA. [29,30] However, neither study found a correlation between RBP-4 levels and apnea-related indices. Although both Ngal and RBP-4 belong to the lipocalin family and share a common tertiary structure, these two proteins appear to have different patterns of regulation in response to inflammatory mediators. [21,23].

Our results also demonstrated a significant inverse correlation between Ngal and LDL cholesterol levels. Wallenius et al also reported such an inverse correlation in their epidemiological study. [21] However, in other studies, correlations between these two variables were not found. [23,31,32] Although this inverse correlation is possible, the results seem to vary depending on the clinical characteristics of the examined cohorts. Furthermore, the mechanisms of this correlation remain utterly unknown.

The relationship between Ngal and metabolic syndrome is quite controversial. Whereas Wang et al and Yan et al reported a close

Table 5. Multiple regression analyses using plasma neutrophil gelatinase associated lipocalin (Ngal) level as a dependent variable.

	p	β	r	R^2(%)
Body mass index (kg/m^2)	0.39	–		
4%ODI/h	<0.01	0.24	0.26	6.2
LDL-cholesterol (mg/dl)	<0.01	−0.29	−0.31	9.0
HDL-cholesterol (mg/dl)	0.71	–		
Triglycerides (mg/dl)	0.31	–		
Creatinine (mg/dl)	<0.01	0.28	0.34	9.5
Cumulative R^2				24.7

β: standard regression coefficient; r: correlation efficient; R^2: contribution rate; ODI: oxygen desaturation index; LDL: low density lipoprotein; HDL: high density lipoprotein.
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Table 6. Changes in clinical variables from baseline to after CPAP implementation.

	CPAP good compliance (n = 13)			CPAP poor compliance (n = 12)			
	before CPAP	after CPAP	p*	before CPAP	after CPAP	p*	p#
Ngal (ng/ml)	60.5±18.1	64.2±13.9	0.27	52.8±16.8	63.1±14.2	<0.01	-
4%ODI (/h)	33.1±16.7	1.1±1.9	<0.01	41.5±22.5	1.5±2.3	<0.01	-
Creatinine (mg/dl)	0.85±0.20	0.88±0.19	0.13	0.77±0.21	0.79±0.20	0.25	-
LDL cholesterol (mg/dL)	107.5±32.7	103.8±30.8	0.70	115.2±28.2	121.0±28.0	0.58	-
Body mass index (kg/m^2)	23.9±2.0	23.9±2.2	0.85	28.2±6.6	28.6±6.6	0.14	
Age (y)	67.5±8.3		-	54.5±12.2		-	0.01
Days with CPAP use >4 h (%)	85.8±9.6		-	43.3±20.7		-	<0.01
Maximum PAP (cmH_2O)	9.9±2.8		-	10.8±2.5		-	0.62
Minimum PAP (cmH_2O)	4.5±0.9		-	4.7±0.8		-	0.35

Data are expressed in mean±SD.

CPAP: continuous positive airway pressure; Ngal: neutrophil gelatinase associated lipocalin; ODI: oxygen desaturation index; LDL: low density lipoprotein; PAP: positive airway pressure; p*: p value for comparison with values before and after CPAP treatment; p# : p value for comparison between CPAP good compliance and CPAP poor compliance groups.

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association between Ngal and obesity or insulin resistance, Wallenius et al found no correlation between these risk factors. [21,22,31] Our results did not show any significant correlation between Ngal levels and obesity or diabetic indices. Also, in the present cohort there were no significant differences in Ngal levels between patients with and without metabolic syndrome. These results also seem to depend on the clinical characteristics of the cohorts. Specifically, we did not exclude patients under treatment for metabolic syndrome to investigate the utility of the plasma Ngal level in actual clinical practice. Because it was reported that the pharmaceutical treatment of diabetes and dyslipidemia can change plasma Ngal levels, treatment of metabolic syndrome in our cohort possibly affected the results. [31,33] Furthermore, in most of these studies, renal function was not taken into account as an explanatory variable of the Ngal level. Risk factors for metabolic syndrome induce latent renal function impairment and even a subtle change in renal function is known to affect blood and urinary Ngal levels. [34] Giaginis et al reported that plasma Ngal levels were higher in patients with than without hypertension and they speculated that the association between elevated Ngal levels and hypertension is secondary to the confounding effect of renal impairment. [35] In our study, even though we did not include patients with overt renal failure, the plasma Ngal levels correlated significantly with serum creatinine levels. Therefore, in evaluating the direct link between metabolic syndrome and Ngal levels, statistical correction for renal function seem to be necessary. In fact, Liu et al reported that the significant correlation between Ngal and insulin resistance detected in their study cohort disappeared after adjustment for serum creatinine values. [32].

Contrary to our expectation, plasma Ngal levels did not change even with the appropriate use of CPAP that effected an improvement in OSA. We could not confirm a direct causality between Ngal levels and OSA. Although we based the three-month treatment observation period on experiences in previous studies, we might need an extended period to find more remarkable changes in Ngal levels in the present cohort. [23,31].

Ngal levels were elevated after CPAP implementation in patients with poor compliance with CPAP. Because we did not include an actual control group that did not use CPAP, we could not judge whether the change in Ngal levels was caused by incomplete CPAP use or other reasons. Although we took every conceivable confounding factor into account, other determinants that we were not aware of might have influenced the results. In the cross sectional studies, the contribution rate of 4%ODI to Ngal levels was not large (6.2%). Therefore, it seems quite possible that other determinants negated the influence of the improvement in OSA. The presence of certain components of metabolic syndrome and their treatment in the present cohort might be among these determinants.

We recognize several limitations in the present study. First, the sample size was small, so it is not reasonable to extrapolate our data to the general population. In addition, the results of this study might have been influenced by the small sample size. Second, as we previously noted, we did not exclude patients with comorbidities such as hypertension and diabetes even if they were under treatment. It is possible that these comorbidities and their treatment affected the results. Third, as mentioned above, we did not have a planned control group without CPAP use. Therefore, we could not judge precisely whether changes in Ngal levels after CPAP implementation were caused by CPAP use or other reasons. Lastly, we did not measure C reactive protein (CRP) levels. Because inflammation has an influence on Ngal, results of measurement of high sensitive CRP would have been a good reflection of the inflammation status of patients and would have been helpful to achieve a more comprehensive understanding of the relationship between OSA and Ngal.

In summary, the present study provides the first clinical evidence demonstrating that plasma Ngal levels were positively but weakly associated with the severity of OSA. Plasma Ngal levels did not change after improvement in OSA, so we could not testify to the causality between Ngal levels and OSA severity. Because Ngal levels were just weakly correlated with the severity of OSA, changes in those levels appear to be influenced largely by other confounding factors. Thus, it would be difficult to use the Ngal level as a specific biomarker representing nocturnal hypoxia in OSA. The links between Ngal levels and metabolic syndrome remain controversial, and unrecognized determinants of plasma Ngal levels are likely to be present. Further studies are warranted to more comprehensively understand the regulation of Ngal in relation to OSA and metabolic syndrome.