In Japan, patients whose AHI is more than 20 can use CPAP under the health insurance system. In this study, 20 patients were permitted to use the CPAP device (Table 4). Twelve of the 20 patients had severe OSA.

After 2 days of CPAP treatment, morning urinary L-PGDS concentrations were significantly decreased compared with baseline values (Figure 4-A), In contrast, the plasma L-PGDS level, the RH-PAT index (Figure 4-B), and the other biomarkers were not significantly changed (Table 4). In the 12 patients with severe OSA, morning urinary L-PGDS concentrations were significantly decreased compared with baseline values (591.2 at baseline vs. 317.8 ng/mg・Creatinine after 2 days of CPAP, $p = 0.02$). Those decreased L-PGDS concentrations reached the levels present in control subjects ($p = 0.65$).

Circadian variations in urinary L-PGDS concentrations

Circadian variations in urinary L-PGDS concentrations are shown in Figure 5-A. In 25 patients, a multiple analysis of variance with repeated measures revealed a significant time-dependent fluctuation ($p = 0.0002$), with the highest levels at 14:00 and lowest levels at 6:00. Compared to the 6:00 values, urinary L-PGDS concentrations significantly increased at 14:00 ($p < 0.0001$) and 18:00 ($p = 0.02$). Regarding the associations between the AHI and urinary L-PGDS concentrations at each time point, the 6:00 values were only significantly positively correlated with AHI ($r = 0.566$, $p = 0.004$). In the patients with AHI ≥ 30, 6:00

values for urinary L-PGDS were significantly increased compared with patients with AHI < 30 (AHI < 30 (n=13) 227 vs. AHI ≥ 30 (n=12) 780.3, p = 0.003) (Figure 5-B).

DISCUSSION

The major findings of our study are that morning urinary L-PGDS concentrations were positively correlated with the severity of OSA as indicated by AHI, 3% ODI, and the arousal index after adjustment for several confounding factors, and that urinary L-PGDS was significantly elevated in patients with severe OSA in comparison with control subjects and those with moderate OSA. Multivariate modeling of L-PGDS determinants revealed that HDL-C and the AHI, 3% ODI, or the arousal index were significantly and independently associated with the morning urinary L-PGDS (Table 3). However, of interest is that urinary L-PGDS levels at 14:00 were the same in non-to-moderate and severe OSA patients. Our results also showed that morning urinary L-PGDS concentrations were decreased to control values after only 2 days of CPAP treatment although the RH-PAT did not change. These results suggest that the urinary L-PGDS concentration might be a moderately useful marker to identify patients with severe OSA.

In the current study, subjects with severe OSA had elevated urinary L-PGDS values and decreased endothelial function determined by the RH-PAT index whereas the relationship between urinary L-PGDS and the RH-PAT index was not significant. Therefore, the

combined measurement of endothelial function and L-PGDS may be a useful tool for finding and managing patients with severe OSA.

Prostaglandin systems, including PGD_2, are very important in health maintenance and disease prevention, but they are either chemically or metabolically unstable. Therefore, substitutions such as L-PGDS are used as parameters for identifying the functions and effects of prostaglandin systems. Previous studies demonstrated that L-PGDS levels in serum, cerebrospinal fluid, or urine were elevated in patients with cardiovascular, neurological, and renal diseases [21, 23, 29, 30]. The elevation in L-PGDS concentration occurs at an early stage of CVD, and the concentrations of serum or urinary L-PGDS were shown to increase when diseases such as coronary heart disease or type 2 DM worsen [21-23], although the activity of L-PGDS is not well known. OSA induces multi-organ damages such as hypersomnolence and CVD. Therefore, it is supposed that L-PGDS could have significant associations with the pathophysiology and severity of OSA. In addition, a urinary biomarker is attractive because urine is easily obtained and the results of urine testing are easy to follow. Although our present data might not be definitive because of the small sample size, the information gained from this study appears to be of interest and the topic warrants further study.

It was said that serum L-PGDS levels increased with age and were higher in men than in women [19] while urinary L-PGDS was also shown to be higher in males than in females and

was weakly correlated with age in female subjects [22]. In this study, control subjects were slightly younger but not with significance, and those in the moderate and severe OSA groups were almost the same age (Table 1). In addition, morning urinary L-PGDS values were still correlated with the severity of OSA after adjustment for BMI, age, and gender. Therefore, BMI, gender, and age could not be considered to have significant effects on our results. Although L-PGDS concentrations were shown to be increased in essential hypertension [21], morning urinary L-PGDS values were still correlated with the severity of OSA after adjustment for blood pressure. The BMI in individuals with severe OSA was slightly but not significantly higher than in the other groups. It was reported that the BMI [21] was not associated with elevations in blood or urinary L-PGDS. In addition to the fact that morning urinary L-PGDS levels were significantly higher after adjustment for BMI, morning urinary L-PGDS concentrations were also significantly decreased after 2 days of CPAP, which is a specific treatment for OSA, although other factors remained the same. Furthermore, from the viewpoint of circadian measurements, morning urine L-PGDS levels were higher in patients with severe OSA than in the other subjects while values at 14:00 were the same. These findings indicate that L-PGDS is related to OSA independently, especially to severe OSA.

Several causes for elevated L-PGDS concentrations in OSA have been considered, especially in severe OSA. Firstly, intermittent hypoxia induces COX 2 expression and activity, which in turn accelerate arachnoid acid metabolism to PGH_2 and PGE_2 [31, 32]. Han et al.

noted that the expression levels of L-PGDS mRNA and protein in the heart of C57BL/6 mice were significantly increased after 14 days of hypoxia (10% O_2) compared with mice kept under normoxia [33]. OSA is characterized by repeated episodes of oxygen desaturation; however, in OSA there is also sustained hypoxemia, which is expressed by SpO_2 < 90%, %TST. Thus, both sustained and intermittent hypoxemia could possibly increase urinary L-PGDS. Secondly, hypoxia causes pulmonary vasoconstriction and increases pulmonary artery pressure [34]. It has been reported that fluid shear stress increases the expression of L-PGDS in vascular endothelial cells [35, 36] and that urinary PGD_2 metabolites were increased in primary pulmonary hypertension [37]. Semenza reported that intermittent hypoxemia induced systemic hypertension and that sustained hypoxemia induced pulmonary hypertension [38]. Both systemic and pulmonary hypertension induced by OSA might increase L-PGDS values [21, 37].

It is said that sleep fragmentation and arousals caused by sleep apnoea induce an elevation of sympathetic activation [39]. If the severity of OSA might be associated with urinary or plasma L-PGDS concentrations, we thought that it would be important to determine whether there is a significant relationship between sympathetic activation and urinary or plasma L-PGDS. Therefore, we wanted to measure urinary catecholamine concentrations as indicators of sympathetic activity. However, it was difficult to obtain urine in which L-PGDS and catecholamine levels could be measured at the same time. Therefore, even though we

knew that plasma catecholamine levels were unstable and easily variable, the usefulness of which was difficult to understand, we measured plasma catecholamine levels. In the present study, urinary L-PGDS concentrations were also correlated with plasma adrenalin and noradrenalin. Therefore, sympathetic overactivity in OSA [40, 41] might also induce increases in urinary L-PGDS. The relationship between urinary catecholamine and L-PGDS should be studied in the future. We also found that HDL-C was a significant determinant of morning urinary L-PGDS. Miwa et al. reported that L-PGDS played a role in lipid transport [42]. Therefore, HDL-C might be a significant factor along with the arousal index or the AHI in determining the urinary L-PGDS levels.

The present study is the first to demonstrate the circadian variations in urinary L-PGDS concentrations in OSA. Urinary LPGDS values in both severe and non-to-moderate OSA were highest and at the same levels at 14:00, with the lowest values at 6:00 in both groups. However, only 6:00 urinary L-PGDS values significantly correlated with the AHI and were significantly higher in those with severe OSA compared with subjects with AHI < 30. In the current study, we showed that morning urinary L-PGDS concentrations were positively correlated with the AHI, 3% ODI, and arousal index. Through the circadian change in L-PGDS, we propose that L-PGDS might be a stress marker that increases during daytime and decreases during sleep, whereas the decrease in L-PGDS levels in OSA, especially severe OSA, during sleep is attenuated because intermittent or sustained hypoxia, sleep

fragmentation, and arousals caused by OSA lead to increased stress, including oxidative stress and sympathetic activation during sleep [39]. Additionally, occurrences of CVDs peak from morning to noon, while OSA patients have an increased risk of myocardial infarction between 0:00 and 6:00 compared with non OSA patients [43]. These circadian rhythms mimic the pattern of urinary L-PGDS in this study (Figure 4-A).

In the current study, we did not detect a significant relation between plasma L-PGDS and the AHI, whereas morning urinary L-PGDS levels were significantly positively correlated with the AHI. Although the reason for this discrepancy is unclear, Hirawa et al. reported that urinary protein excretion in the early stage of DM was correlated with urinary L-PGDS excretion, but not with plasma L-PGDS levels [44]. In addition, serum L-PGDS levels were not shown to be associated with the AHI [25]. The influence of OSA, such as intermittent hypoxemia, might have a significant effect on the renal system, which induced the differences in values between plasma and urinary L-PGDS. The differences in L-PGDS levels between plasma and urine should be studied in animal models. Furthermore, we did not collect peripheral blood samples at 22:00 in the current study. The night plasma L-PGDS concentrations or the differences between morning and night plasma L-PGDS may contribute to elucidating the relation between plasma L-PGDS and OSA.

This study had some limitations. Firstly, the sample size was small. However, the differences in the urinary L-PGDS values between control, moderate, and severe OSA

patients were large and therefore the results could be considered significant and definitive. Secondly, it is unclear whether the effect of CPAP on the PGD_2 system would persist over the long term. A long-term prospective study is needed to clarify this issue. Thirdly, we could not conduct a comparison between CPAP users and sham CPAP users. A future study that makes comparisons between CPAP users and sham CPAP users is warranted. Fourthly, we used spot urine samples for measurement of L-PGDS. There is a possibility that several factors such as reabsorption at tubules and physical activity influenced the urinary L-PGDS concentrations. However, use of overnight spot urine for measurement of L-PGDS has been validated because of the correlation between L-PGDS values of overnight urine and 24-hour collected urine [44]. Therefore, we believe overnight spot urine sampling is sufficient to evaluate the role of L-PGDS in OSA.

In conclusion, based on our results, in addition to circadian data, urinary L-PGDS might be a moderately useful marker for severe OSA. From this preliminary data, urine L-PGDS measurement may be a simple and cost-effective method to screen for and manage severe OSA. This method should be tested in unselected samples in the future because it is often difficult, costly, and time consuming to find patients with OSA while the number of OSA patients who should be treated is large.

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Tables

Table 1. Patients' characteristics, PSG data, and laboratory data according to severity of obstructive sleep apnoea (OSA)

	Control (n=16)	Moderate OSA (n=25)	Severe OSA (n=23)	P value
Age	47.5 (21 to 76)	55 (31 to 74)	55 (27 to 78)	0.12
Male	12 (75.0)	21 (84.0)	21 (91.3)	0.39
BMI	26.0 (20.4 to 35.8)	24.5 (20.2 to 34.8)	26.6 (21.6 to 39.9)	0.10
Waist circumference (cm)	87 (76 to 118)	90 (78 to 113)	94 (82 to 120)	0.13
Morning systolic BP (mmHg)	114 (95 to 136)	118 (99 to 139)	127 (96 to 150)[a,b]	0.003
Morning diastolic BP (mmHg)	70 (58 to 91)	74 (57 to 96)	80 (69 to 97)[a,b]	0.0004
Ex-smoker	9 (56.3)	12(48.0)	17 (73.9)	0.18
ESS	14 (1 to 24)	12 (2 to 19)	12 (4 to 20)	0.13
Comorbidity				
Hypertension	3 (18.8)	10 (40.0)	10 (47.8)	0.25
Dyslipidemia	9 (56.3)	17 (68.0)	15(65.2)	0.74
Diabetes mellitus	2 (12.5)	2 (8.0)	2 (8.7)	0.88
PSG data				
TST, min	408.5 (256.5 to 510)	389.0 (205.5 to 515.5)	378.0 (240.5 to 499)	0.56
Sleep efficiency, %	81.1 (63.3 to 94.4)	76.6 (42.9 to 94.2)	72.9 (50.4 to 96.4)	0.50
Arousal index, events/h	17.8 (9.5 to 26.7)	22.2 (10.8 to 46.5)	44.3 (12.6 to 61.2)[a,b]	< 0.0001
AHI, events/h	7.3 (1.2 to 14.8)	22.7 (15.2 to 29.8)[a]	47.2 (31.9 to 85.4)[a,b]	< 0.0001
3% ODI, events/h	5.3 (0.5 to 14.1)	17.4 (10.1 to 27.2)[a]	48.0 (26.9 to 86.4)[a,b]	< 0.0001
Min SpO_2, %	90.5 (81 to 97)	81.5 (73 to 90)[a]	75.0 (61 to 86)[a,b]	< 0.0001
SpO_2 < 90%, %TST	0 (0 to 5.0)	2.5 (0 to 8.1)	11.9 (1.2 to	< 0.0001

			87.7)[a,b]	
RH-PAT index	1.91 (1.30 to 2.87)	2.00 (1.32 to 3.91)	1.65 (1.42 to 3.23)[b]	0.02
Blood				
Creatinine (mg/dl)	0.8 (0.4 to 1.0)	0.8 (0.6 to 1.1)	0.8 (0.6 to 1.1)	0.49
TC (mg/dl)	204 (125 to 241)	197 (130 to 255)	199 (141 to 299)	0.58
HDL-C (mg/dl)	51 (35 to 86)	53 (40 to 93)	49 (30 to 82)	0.30
TG (mg/dl)	103 (54 to 245)	100 (44 to 334)	132 (70 to 286)	0.21
CRP (mg/dl)	0.1 (0.0 to 0.2)	0.1 (0.0 to 0.2)	0.1 (0.0 to 0.5)[a,b]	0.004
Glucose (mg/dl)	87 (81 to 114)	95 (75 to 146)	96 (85 to 121)	0.07
Adrenaline (pg/ml)	14 (5 to 31)	16 (5 to 30)	16 (5 to 45)	0.65
Noradrenaline (pg/ml)	241 (67 to 465)	252 (91 to 398)	261 (82 to 569)	0.52
L-PGDS (ng/ml)	422.0 (291.7 to 588.6)	469.3 (270.3 to 657.0)	491.4 (343.3 to 726.0)	0.09
Urine				
Morning L-PGDS (ng/mg・Cre)	262.1 (21.3 to 1178.6)	371.7 (92.3 to 2378.2)	784.7 (124.4 to 3274.1)[a,b]	0.0009
Night L-PGDS (ng/mg・Cre)	745.8 (30.5 to 1754.0)	659.5 (78.9 to 2937.9)	958.3 (221.0 to 5621.8)	0.19

Data are median (range) or number (%)

[a] $p < 0.0167$ versus control, [b] $p < 0.0167$ versus moderate OSA

Abbreviations: PSG, polysomnography; BMI, body mass index; BP, blood pressure; ESS, Epworth Sleepiness Scale; TST, total sleep time; AHI, apnoea-hypopnoea index; ODI, oxygen desaturation index; Min SpO_2, minimum percutaneous oxygen saturation; RH-PAT, reactive hyperemia peripheral arterial tone; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; CRP, C reactive protein; L-PGDS, lipocalin-type prostaglandin D synthase.

Table 2. Associations of L-PGDS level and RH-PAT index with patients' characteristics, PSG data, and biomarkers

	Morning urinary L-PGDS (ng/mg · Cre)		Plasma L-PGDS (ng/ml)		RH-PAT index	
	r	P value	r	P value	r	P value
Age (years)	0.164	0.20	0.362	0.003	0.074	0.56
Gender (Male)	-0.013	0.92	0.048	0.71	-0.160	0.21
BMI (kg/m^2)	-0.060	0.64	-0.132	0.31	-0.135	0.29
Waist circumference (cm)	-0.062	0.63	-0.076	0.55	-0.081	0.53
Morning systolic BP (mmHg)	0.394	0.001*	0.279	0.03*	-0.150	0.24
Morning diastolic BP (mmHg)	0.323	0.009*	0.274	0.03*	0.001	0.99
ESS	-0.139	0.27	-0.035	0.78	0.075	0.56
Arousal, events/h	0.472	< 0.0001*	0.220	0.08	-0.268	0.03*
AHI, events/h	0.426	0.0005*	0.180	0.16	-0.241	0.06
3% ODI, events/h	0.384	0.002*	0.173	0.18	-0.244	0.054
Mini SpO_2, %	-0.112	0.38	0.099	0.44	0.046	0.72
SpO_2 < 90%, %TST	0.128	0.31	0.017	0.90	-0.190	0.14
RH-PAT index	-0.068	0.59	0.030	0.82	-	-
Creatinine (mg/dl)	0.052	0.68	0.256	0.04	-0.067	0.60
TC (mg/dl)	0.189	0.14	0.233	0.07	-0.003	0.98
HDL-C (mg/dl)	0.214	0.09	0.045	0.73	0.079	0.54
TG (mg/dl)	-0.067	0.60	0.170	0.18	0.134	0.30
CRP (mg/dl)	0.071	0.58	0.083	0.52	-0.132	0.30
Glucose (mg/dl)	0.158	0.22	-0.161	0.21	-0.143	0.27
Adrenaline (pg/ml)	0.310	0.02*	0.220	0.10	0.018	0.89
Noradrenaline (pg/ml)	0.329	0.008*	0.149	0.25	0.128	0.32
Plasma L-PGDS (ng/ml)	0.228	0.07	-	-	0.030	0.82
Morning urinary L-PGDS (ng/mg · Cre)	-	-	0.228	0.07	-0.068	0.59
Night urinary	0.868	< 0.0001*	0.090	0.51	0.050	0.71