discussed with regard to this rare experience. Firstly, when the patient was transferred to our hospital, PVOD was clinically highly suspected; however, treatment for the other types of PAH is entirely different from that for PVOD. Because the obstructive vasculopathy in PVOD is predominantly located on the venous aspect of the pulmonary circulation, treatment with PAH-specific vasodilator therapies may cause augmentation of the pulmonary arteriolar blood flow against the fixed resistance of occluded pulmonary venules and veins. Therefore, we performed open lung biopsy before LDLLT, although PVOD was most likely from a clinical point of view.

Secondly, as cadaveric donors for pediatric patients are not practically expected in Japan, a single LDLLT from her mother was the last and only practical option. If the patient's condition is stable, physicians could consider medical therapy to wait for the patient to grow. However, in the current case, the patient was intubated again and under deep sedation to prevent attacks due to pulmonary edema and pulmonary hypertension. According to the recent guidelines for the treatment of pulmonary hypertension [1], there is no established medical therapy for PVOD. Most importantly, vasodilators and especially prostanoids must be used with great caution because of the high risk of pulmonary edema. Epoprostenol can make the patient's condition better and could be a bridge to lung transplantation [1], but there is a report showing epoprostenol-induced pulmonary edema in a patient with PVOD [3].

Thirdly, we have accepted size mismatches between recipients and donor lobar grafts only if the predicted FVC of the graft is at least more than 45% of the expected recipient FVC. In the present case, the predicted FVC of the graft was 78.9% of the expected recipient FVC. Furthermore, to assess the use of oversized grafts, we also have used three-dimensional CT volumetry [2, 4, 5]. In single LDLLT, size matching is crucial because the use of oversized grafts can cause high airway resistance, atelectasis, and hemodynamic instability at the time of chest closure. However, because of our experiences [2, 6], size discrepancy would probably be acceptable if the graft is less than 200% bigger than the right chest cavity of the recipient. In the current case, the graft was calculated to be 173% bigger than the right chest cavity of the recipient and was thus adjustable in size for the recipient's chest. However, delayed chest closure was required after LDLLT. Conversely, the evaluated volume of the right lobe of the patient's father was 1,495 mL, indicating that the graft would be 243% bigger than the right chest cavity of the recipient. This calculation confirmed that the father's graft was too large for the recipient. Furthermore, the recipient's growth should be taken into consideration when performing LDLLT for a child. At 10 months after LDLLT, the grafted lung was still compressed to less than 50% of its original size, indicating that the grafted lung has a potential ability to adjust to some extent to the recipient's growth. In the present case, we did not want to downsize the adult lobe because of the future growth of the recipient. Despite no available data on how much compression can be tolerated by the donor lungs in the recipient's thorax [4], the present case revealed that the lungs could function when compressed to approximately 50% of their original size. Long-term durability of the lung function as the patient grows remains a major unresolved issue, and life-long follow-up should be mandatory in such cases of single LDLLT.

Fourthly, various methods of downsizing donor lungs can be applied in lung transplantation [7]. In LDLLT, if a moderate amount of differences need to be adjusted, superior segment of the lower lobe can be performed easily from the anatomic point of view [4]. By contrast, nonanatomic peripheral resection of the anterior donor lung might also be considered more favorably when we consider the adaptation of the downsized lung to the shape of the recipient's thorax. Furthermore, in this setting of lung transplantation, cadaveric lung transplantation using the upper lobe only or the lower lobe only from an adult cadaveric donor might provide an even better size match, resulting in no requirement of living donation.

Lastly, only 1 case report documented that no attacks of pulmonary edema occurred after single lung transplantation for PVOD [8]; however, it is necessary to perform a close follow-up with regard to how the graft and native lung behave.

References

1. Task Force for Diagnosis and Treatment of Pulmonary Hypertension of European Society of Cardiology (ESC); European Respiratory Society (ERS); International Society of Heart and Lung Transplantation (ISHLT). Guidelines for the diagnosis and treatment of pulmonary hypertension. Eur Heart J 2009;30:2493–537.
2. Chen F, Matsukawa S, Ishii H, et al. Delayed chest closure assessed by transesophageal echocardiogram in single-lobe lung transplantation. Ann Thorac Surg 2011;92:2254–7.
3. Palmer SM, Robinson LJ, Wang A, Gossage JR, Bashore T, Tapson VF. Massive pulmonary edema and death after prostacyclin infusion in a patient with pulmonary veno-occlusive disease. Chest 1998;113:237–40.
4. Chen F, Fujinaga T, Shoji T, et al. Perioperative assessment of oversized lobar graft downsizing in living-donor lobar lung transplantation using three-dimensional computed tomographic volumetry. Transpl Int 2010;23:e41–4.
5. Chen F, Kubo T, Shoji T, Fujinaga T, Bando T, Date H. Comparison of pulmonary function test and computed tomography volumetry in living lung donors. J Heart Lung Transplant 2011;30:572–5.
6. Sonobe M, Bando T, Kusuki S, et al. Living-donor single-lobe lung transplantation and simultaneous contralateral pneumonectomy in a child. J Heart Lung Transplant 2011;30:471–4.
7. Aigner C, Mazhar S, Jaksch P, et al. Lobar transplantation, split lung transplantation and peripheral segmental resection—reliable procedures for downsizing donor lungs. Eur J Cardiothorac Surg 2004;25:179–83.
8. Cassart M, Gevenois PA, Kramer M, et al. Pulmonary veno-occlusive disease: CT findings before and after single-lung transplantation. AJR Am J Roentgenol 1993;160:759–60.

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Brief Communication

Adaptation Over a Wide Range of Donor Graft Lung Size Discrepancies in Living-Donor Lobar Lung Transplantation

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Living-donor lobar lung transplantation (LDLLT), unlike deceased donor lung transplantation, often involves a wide range of size discrepancies between donors and recipients. The aim of this study was to evaluate the function of donor lung grafts in the recipient thorax in 14 cases of bilateral LDLLT involving 28 successfully transplanted lower-lobe grafts. Pulmonary function tests and three-dimensional computed tomography (3D-CT) volumetry were performed perioperatively. According to 3D-CT size matching, donor graft volumes ranged from 40% to 161% of the hemilateral thoracic volumes of the recipients. Graft forced vital capacity (FVC) values increased over time, reaching 102 ± 39% of preoperatively estimated values at 12 months postoperatively. Graft volumes also increased over time, reaching 120 ± 38% of the original values at 12 months postoperatively. Undersized donor grafts expanded more after LDLLT than oversized donor grafts, producing greater FVC values than those estimated preoperatively, whereas oversized donor grafts became inflated to their original size and maintained FVC values that approached the preoperative estimates. Thus, donor grafts were found to overinflate or underinflate to the extent that they could preserve their native function in the new recipient's environment.

Key words: Alveolar dilation, computed tomography volumetry, forced vital capacity, graft lungs, living-donor lobar lung transplantation, lung volume

Abbreviations: DLCO, diffusion capacity for carbon monoxide; FEV1, forced expiratory volume in 1 s; FEV1%, FEV1/FVC; FVC, forced vital capacity; LDLLT, living-donor lobar lung transplantation; PF, pulmonary fibrosis; PFT, pulmonary function test; VA, alveolar volume; 3D-CT, three-dimensional computed tomography.

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Introduction

Living-donor lobar lung transplantation (LDLLT) has been performed successfully in order to save the lives of rapidly deteriorating critically ill patients (1–3). In deceased donor lung transplantation, an appropriately size-matched recipient can be intentionally selected for a specific donor. However, in LDLLT, ideal size matching between donor and recipient is usually difficult because of the limited population of potential donors. As a result, in LDLLT, there is a wide range of size discrepancies between donor and recipient. If the donor's lungs are small, native lung-sparing surgical procedures may be necessary, whereas if the donor's lungs are too large, volume reduction of the donor lungs is required (4). Size matching between donor and recipient is particularly important for safe transplantation with better outcomes in LDLLT. Forced vital capacity (FVC) has been used to facilitate size matching in LDLLT, and we have been using a segment-based estimation formula of graft FVC that we have previously proposed as a size matching method in LDLLT (2,5).

Three-dimensional computed tomography (3D-CT) volumetry provides informative data for size matching in liver transplantation, and quantitative CT has also been used for predicting postoperative lung function in patients with lung cancer (6–8). 3D-CT volumetric data are true volume data and can also be used for size matching in LDLLT (9–11). We have previously reported that certain pulmonary function test (PFT) results are well correlated with 3D-CT volumetric data in healthy subjects (12).

Although several reports have described trends of lung function after LDLLT (13), there are as yet no reports investigating these trends in terms of both size and function of the donor lung grafts after LDLLT. Thus, we decided to investigate the behavior of the donor lung grafts in the new recipient environment after LDLLT based on PFTs and 3D-CT volumetry. We also investigated trends of postoperative graft volume and function according to preoperative size matching.

Table 1: Donor selection criteria for living donor lobar lung transplantation

Relatives within the third degree or a spouse
20 ≤ age ≤ 60 years
ABO identical or compatible
No significant medical history or active medical problems
No recent viral infection
No abnormalities on the electrocardiograph and echocardiogram
No significant pulmonary pathology on computed tomography on donor side
Arterial oxygen tension ≥ 80 Torr
Forced vital capacity, forced expiratory volume in 1 second ≥ 85% of predicted
No previous thoracic operation on the side to be donated
Nonsmokers (If current smokers, cessation of smoking is required at the time of the offer for donation and continuous cessation is required after donor lobectomy)
Absence of coercion
Satisfactory psychosocial evaluation

Patients and Methods

Between August 2008 and September 2011, 18 patients underwent bilateral LDLLT at Kyoto University Hospital. Four of these patients were excluded from this study: two patients underwent native lung-sparing bilateral LDLLT; one died of severe aspiration pneumonia 3 months after LDLLT; and one suffered from malnutrition due to severe postoperative gastroparesis, developed multiple thoracic vertebral fractures, and had severe kyphosis within 1 year. Thus, we analyzed LDLLT results in 14 patients who underwent successful transplantation of 28 lower-lobe lung grafts procured from living donors. All 14 recipients were discharged within 3 months after surgery without any physical limitations. Donor selection criteria are outlined in Table 1. All 28 donors met the donor criteria for LDLLT (2). No potential donors for the 14 LDLLTs were rejected solely because of PFT results or 3D-CT volumetric data. All 28 donors remained alive and well 1 year after donor lobectomies, without any limitations in daily activities. The included recipients have also survived well without any significant complications, including chronic lung allograft dysfunction. The preoperative evaluation included 3D-CT volumetry and PFTs performed using a Minato System 21 (Minato Medical Science Co Ltd., Osaka, Japan) to measure FVC, forced expiratory volume in 1 s (FEV1), diffusion capacity for carbon monoxide (DLCO) and DLCO/alveolar volume (VA).

For size accommodation in LDLLT, we have previously proposed a graft FVC calculation method based on the number of segments in the graft (2,5,11). Given that the right lower lobe consists of 5 segments, the left lower lobe of 4, and the whole lung of 19, we estimated the graft FVC using the following equation: Graft FVC = measured FVC of the right donor × 5/19 + measured FVC of the left donor × 4/19. When the graft FVC was >45% of the predicted FVC of the recipient calculated according to height, age and sex, we accepted the size disparity regardless of the recipient's diagnosis.

The 3D-CT volumetry is a newly developed method for size matching in LDLLT (9–12) that allows us to calculate the whole lung volume and also allows us to calculate the volume of a particular part of the lung, e.g. a single lobe. CT images for this study were obtained during a single respiratory pause at the end of maximum inspiratory effort using a multidetector CT scanner (Aquilion 64; Toshiba Medical Systems, Tochigi, Japan). Contiguous 0.5-mm slices, reconstructed using a standard lung reconstruction algorithm (FC 51), were used for volumetric analysis and the entire CT image could be exported to a workstation (AZE Virtual Place Lexus; AZE Co., Ltd., Tokyo, Japan) for 3D-CT volumetry. Using automated segmentation, the volumes of each lung and the lower lobes were calculated automatically and whole lungs and grafts were shown as 3D-volume rendering images.

Inpatient and outpatient medical records, PFT results, 6-min walk test results, chest CT films, 3D-CT volumetric data and pulmonary ventilation scintigraphy for all donors and recipients were reviewed. All cases were retrospectively analyzed, particularly for PFT results and 3D-CT volumetric data recorded before LDLLT and at 3, 6 and 12 months after LDLLT. As previously described, preoperative donor graft FVC and FEV1 were calculated by the number of segments in the graft (2,5), whereas donor graft FVC and FEV1 after LDLLT were assessed by the percentages of ventilation counts in pulmonary ventilation scintigraphy. In addition, FEV1% was defined as FEV1/FVC. DLCO and DLCO/VA were calculated by the same method.

PFT and 3D-CT volumetry data from the 28 donor grafts before and after LDLLT were reviewed. For analysis of the trends of postoperative graft volume and function according to preoperative size matching, the 28 donor grafts were divided into two groups, undersized (US) and oversized (OS), based on 3D-CT size matching between donor graft volumes and the corresponding recipient hemithorax volumes. There were 15 grafts in the US group (donor graft volume < recipient hemithorax volume) and 13 grafts in the OS group (donor graft volume ≥ recipient hemithorax volume). This study protocol (E-1531) was approved by the Institutional Review Board of Kyoto University Hospital.

Statistical analysis

Statistical analysis was performed using the StatView (version 4.5) software package (Abacus Concepts, Berkeley, CA, USA). All values are presented as the mean ± the standard deviation of the mean. We calculated the correlation coefficients between the given parameters. We also used a Student's unpaired t-test and repeated-measures analysis of variance (ANOVA) to explore differences between groups. Differences were considered significant when $p < 0.05$.

Results

Recipient and donor characteristics

As shown in Table 2, the study included 14 female and 14 male healthy donors aged 39.6 ± 13.3 years and 9 female and 5 male recipients aged 39.6 ± 17.6 years.

Preoperative size matching and postoperative course

According to 3D-CT volumetry, the donor graft lung volumes were 95.6 ± 35.8% of the hemilateral thoracic volumes of the recipients (range 39.8–160.8%). LDLLT was successfully performed without any severe complications in all included cases. All of the recipients were well and at home without oxygen supplementation by 3 months postoperatively. During a follow-up period of 1 year after LDLLT, all but two patients in this study were free of significant pulmonary complications. Two patients developed bronchiolitis obliterans, but neither of them required oxygen supplementation. There were no recipients who grew taller during the study period.

Perioperative trends of donor graft function and volume

Trends of donor graft function and volume are shown in Table 3. Because of technical issues, DLCO was not measured in patients with FVC values of <1 L.

Table 2: Preoperative and early postoperative characteristics of the US and OS groups

Variables	US (n = 15)	OS (n = 13)	Sum (n = 28)
Age (years)	39.1 ± 13.8	40.3 ± 13.3	39.6 ± 13.3
Gender (male: female)	6:9	8:5	14:14
Height (cm)	164.0 ± 8.2	165.9 ± 9.0	164.9 ± 8.5
Weight (kg)	60.0 ± 11.1	63.4 ± 10.2	61.6 ± 10.7
%FVC (% of predicted value)	110.9 ± 11.2	114.3 ± 12.6	112.4 ± 11.7
%FEV1 (% of predicted value)	100.1 ± 10.4	104.7 ± 9.0	102.3 ± 9.9
Donor graft side (right:left)	6:9	8:5	14:14
Donor graft FVC (mL)	858 ± 266	975 ± 299	912 ± 283
Donor graft FEV1 (mL)	702 ± 229	803 ± 219	749 ± 226
Donor graft DLCO	5.68 ± 1.37	6.45 ± 2.03	6.04 ± 1.72
Donor graft DLCO/VA	5.62 ± 0.93	5.81 ± 1.00	5.71 ± 0.95
Donor graft volume (mL)	1134 ± 362	1268 ± 325	1197 ± 346
Recipient hemithorax volume (mL)	1752 ± 624	1038 ± 328	1420 ± 617
Recipient original disease			
Bronchiolitis obliterans	7	3	10
Interstitial pneumonia	4	8	12
Others	4	2	6
FVC-based size matching (%)	66.1 ± 20.2	78.4 ± 31.7	71.8 ± 26.4
3D-CT volumetric size matching (%)	68.8 ± 18.9	126.4 ± 23.2	95.6 ± 35.8
ICU stay (days)	12.9 ± 7.1	14.2 ± 7.8	13.5 ± 7.4
Period of mechanical ventilation (days)	13.3 ± 15.7	13.8 ± 14.4	13.5 ± 14.9

DLCO, diffusion capacity for carbon monoxide; FEV1, forced expiratory volume in 1 s; FEV1%, FEV1/FVC; ICU, intensive care unit; FVC, forced vital capacity; OS, oversized donor graft group; US, undersized donor graft group; VA, alveolar volume; 3D-CT, three-dimensional computed tomography.

Table 3: Trends of donor graft function and volume

	Preoperatively estimated value	Post-LDLLT values		
		3 months	6 months	12 months
Donor graft FVC (mL) (ratio to preoperatively estimated value,%)	912 ± 283	745 ± 278 (86.8 ± 38.0)	829 ± 331 (94.7 ± 40.4)	884 ± 310 (102.2 ± 39.0)
Donor graft FEV1% (%)	82.5 ± 7.0	90.3 ± 9.2	88.1 ± 10.7	88.2 ± 12.0
Donor graft volume (mL) (ratio to preoperatively estimated value,%)	1197 ± 346	1249 ± 360 (108.4 ± 30.9)	1360 ± 366 (118.4 ± 33.1)	1377 ± 427 (119.8 ± 38.4)
Donor graft DLCO (ratio to preoperatively estimated value,%)	6.04 ± 1.72	5.67 ± 1.95 (92.7 ± 28.5)	5.71 ± 2.02 (95.6 ± 26.3)	5.43 ± 2.17 (90.8 ± 24.6)
Donor graft DLCO/VA (relative to preoperatively estimated value,%)	5.71 ± 0.95	4.73 ± 1.32 (77.1 ± 20.5)	4.54 ± 1.09 (77.8 ± 17.7)	4.32 ± 1.03 (75.0 ± 12.3)

DLCO, diffusion capacity for carbon monoxide; FEV1, forced expiratory volume in 1 s; FEV1%, FEV1/FVC; LDLLT, living-donor lobar lung transplantation; FVC, forced vital capacity; VA, alveolar volume.

Donor graft FVC was significantly correlated with donor graft volume preoperatively ([Donor graft FVC = 61 + 0.71 × donor graft volume], $r^2 = 0.76$, $p < 0.0001$) and at 3 months after LDLLT ([Donor graft FVC = 296 + 0.36 × donor graft volume], $r^2 = 0.22$, $p = 0.013$), 6 months after LDLLT ([Donor graft FVC = 48 + 0.57 × donor graft volume], $r^2 = 0.40$, $p = 0.0003$) and 12 months after LDLLT ([Donor graft FVC = 153 + 0.53 × donor graft volume], $r^2 = 0.53$, $p < 0.0001$).

Donor graft volume at 12 months after LDLLT was significantly correlated with recipient hemithorax volume ([Donor graft volume = 705 + 0.47 × recipient hemithorax volume], $r^2 = 0.47$, $p < 0.0001$), but donor graft FVC at 12 months after LDLLT was not correlated with recipient hemithorax volume ($p = 0.31$).

Pulmonary function and volume based on 3D-CT size matching

We evaluated the association between 3D-CT volumetry size matching and donor graft FVC ratios to the preoperatively estimated values at 12 months after LDLLT and found a significant correlation between these parameters ([Donor graft FVC ratio to preoperatively estimated

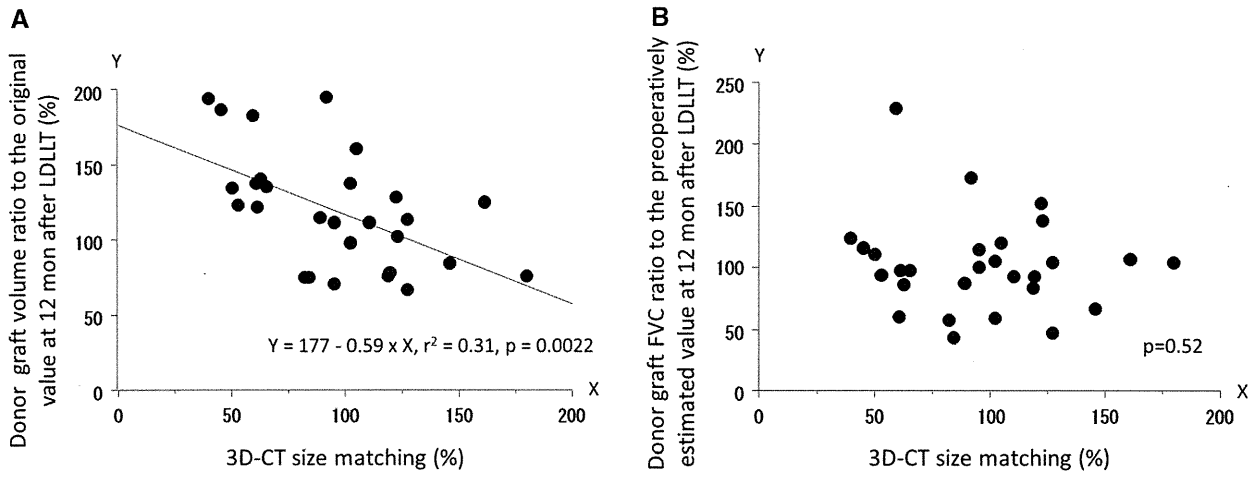

Figure 1: (A) Association between three-dimensional computed tomography (3D-CT) volumetry size matching and donor graft forced vital capacity (FVC) ratios to preoperatively estimated values at 12 months after living donor lobar lung transplantation (LDLLT). We found a significant correlation between these parameters ([Donor graft FVC ratio to preoperatively estimated value = 177 − 0.59 × 3D-CT volumetry size matching], $r^2 = 0.31$, $p = 0.0022$). (B) Association between three-dimensional computed tomography (3D-CT) volumetry size matching and donor graft volume ratios to the original values at 12 months after living donor lobar lung transplantation (LDLLT). There was no significant correlation between these parameters ($p = 0.52$).

value = 177 − 0.59 × 3D-CT volumetry size matching], $r^2 = 0.31$, $p = 0.0022$, Figure 1A). In contrast, when we checked the association between 3D-CT volumetry size matching and donor graft volume ratios to the original values at 12 months after LDLLT, no significant correlation was found ($p = 0.52$, Figure 1B).

The trends of graft function and volume in the US and OS groups are shown in Table 4 and Figure 2. Of note, donor graft volumes in the US group compared to their original values were significantly larger than those in the OS group ($p = 0.045$, Figure 2B), but there were no significant differences between the groups in donor graft FVC values compared to preoperatively estimated values ($p = 0.78$, Figure 2A). Six-min walk distance was dramatically improved by 3 months after LDLLT in both groups, and had reached more than 500 m at 12 months postoperatively.

Discussion

Size matching has been discussed from various points of view (14–17) and it is one of the most important components of safe and successful lung transplantation. Size matching is even more crucial in LDLLT than in deceased donor lung transplantation because of the variety of combinations of donor and recipient sizes. For example, when considering LDLLT for an adult recipient, the volume of graft lungs is usually small because the recipient will receive, at most, the bilateral lower lobes from the donor. On the other hand, in the setting of LDLLT from a parent to a small child, an OS graft might be transplanted into the small chest cavity of the recipient. Either way, size matching is always a great concern, particularly in LDLLT, and in this study, in fact, the 3D-CT size matching showed a wide range of size discrepancies, from 40% to 160%, between donors and recipients.

According to previous studies (2,13), recipient FVC values at 6 months after LDLLT were equal to the estimated graft FVC values of two lobes, and FVC reached 123% of these values at 36 months after transplantation. In this study, although we estimated FVC for each lobar lung graft, the graft FVC values approached the original estimates by approximately 12 months after LDLLT, which was consistent with the findings of the previous studies. More importantly, although US donor grafts expanded significantly compared to OS donor grafts, graft FVC values were irrelevant to preoperative donor size matching. Furthermore, 6-min walk distance dramatically improved after LDLLT in both groups. Thus, despite the larger size discrepancies between donors and recipients associated with LDLLT vs. deceased donor lung transplantation, we found that both OS and US donor grafts functioned very well in the patients selected for our analysis. To be more specific, lung grafts in the US group expanded beyond their original size and up to 133% of their original size by 12 months after LDLLT and the FVC values reached 106% of the preoperatively estimated values without any decrease in FEV1%. In contrast, in the OS group, the lung graft segments expanded to their original size by 6 months after LDLLT regardless of the recipient's thorax volume. The OS grafts could expand to >120% of the recipient's thorax volume, but because of the limitation of the recipient's thorax capacity, the grafts did not expand for more

Table 4: Trends of graft function and volume by group

	Preoperatively estimated value	Post-LDLLT values		
		3 months	6 months	12 months
Donor graft FVC ratio to preoperatively estimated value (%)				
US	100	88.3 ± 44.3	94.8 ± 48.6	106.2 ± 46.2
OS	100	85.0 ± 30.9	94.6 ± 30.2	97.6 ± 30.0
Donor graft FEV1 (%)				
US	81.6 ± 6.7	90.5 ± 9.2	88.8 ± 11.1	89.4 ± 12.3
OS	83.1 ± 8.2	90.1 ± 9.5	87.3 ± 10.7	86.8 ± 11.9
Donor graft volume ratio to preoperatively estimated value (%)				
US	100	120.8 ± 30.4	127.5 ± 35.7	133.1 ± 41.8
OS	100	94.1 ± 25.7	107.9 ± 27.5	104.4 ± 28.1
Donor graft DLCO ratio to preoperatively estimated value (%)				
US	100	104.3 ± 30.2	94.8 ± 17.7	95.4 ± 25.8
OS	100	81.1 ± 22.7	96.3 ± 33.7	85.5 ± 23.2
Donor graft DLCO/VA ratio to preoperatively estimated value (%)				
US	100	76.7 ± 21.4	73.8 ± 18.1	73.6 ± 13.4
OS	100	77.5 ± 20.8	81.7 ± 20.8	76.6 ± 11.3
6-min walk distance (m)				
US	224 ± 78[1]	404 ± 98	483 ± 90	507 ± 72
OS	235 ± 101[1]	444 ± 97	508 ± 100	529 ± 70

DLCO, diffusion capacity for carbon monoxide; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 s; FEV1%, FEV1/FVC; LDLLT, living-donor lobar lung transplantation; OS, oversized donors; US, undersized donors; VA, alveolar volume.
[1]Five patients could not perform 6-min walk test because of severe preoperative respiratory compromise.

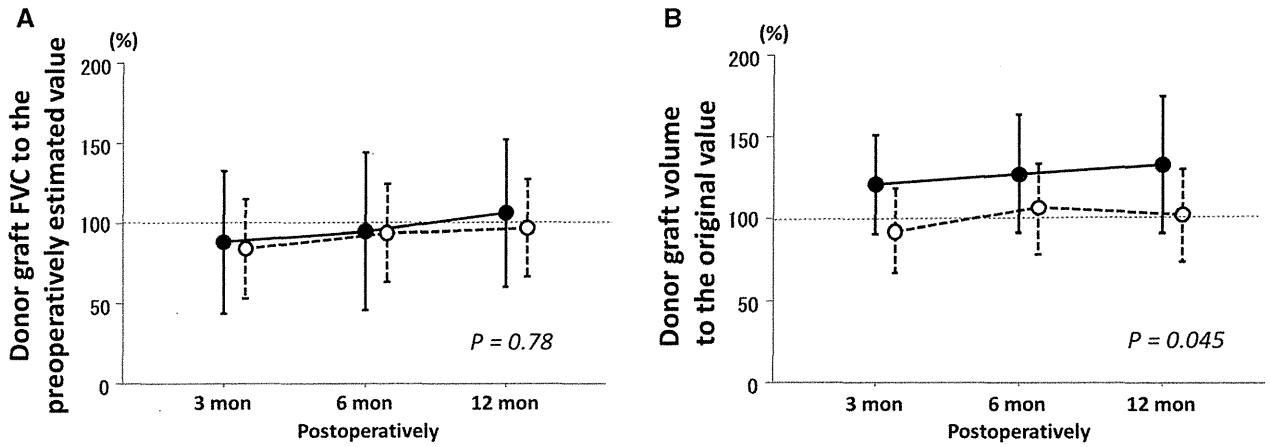

Figure 2: (A) Trend of donor graft forced vital capacity (FVC) in the undersized (US) and oversized (OS) groups. There was no significant difference in donor graft FVC compared to the preoperatively estimated values between the groups. Donor graft FVC values were 106.2 ± 46.2% and 97.6 ± 30.0% of the preoperatively estimated values at 12 months after LDLLT in the US and OS groups, respectively. Open circle: OS group. Closed circle: US group. (B) Trend of donor graft volumes in the US and OS groups. Ratio of donor graft volumes to original values in the US group were significantly larger than in the OS group ($p = 0.045$). Donor graft volumes were 133.1 ± 41.8% and 104.4 ± 28.1% of the original values at 12 months after LDLLT in the US and OS groups, respectively. Open circle: OS group. Closed circle: US group.

than 12 months after LDLLT. In these cases, FVC also continued increasing, approximating preoperatively estimated values by 12 months after LDLLT. Of note, in both groups, the donor lung grafts became larger than the original size but produced lower FVC values in comparison with the rates of volume increase. This might be partly because of the topographic differences between donor grafts and recipient hemithoraces as well as mechanical issues that may result when the lobe is not perfectly opposed to the chest wall (18). Accumulation of a larger number of cases with longer follow-up periods is needed to accurately confirm the extent of FVC and lung volume changes in each lobar lung graft after LDLLT, but from this study, we have determined that greater lung expansion is needed in the

new recipient environment in order for the donor graft to provide the same lung function as it had provided preoperatively for the donor.

Another interesting finding of our study was that DLCO and DLCO/VA values in the US group indicated that the increases of lung volume could be attributed to alveolar dilation. In the US group, theoretically, implanted donor lung grafts could have expanded to the same extent of the recipient's original hemithoracic volume, and the donor graft volumes among the recipients in this study at 12 months after LDLLT reached 133% of the original values. However, DLCO values in these patients at 12 months were almost the same as the preoperatively estimated values. Furthermore, DLCO/VA at 12 months was 74% of the preoperatively estimated value, indicating that alveolar volume expanded by 135% (100/74). These numbers strongly indicated that the increase in lung volume was caused by a passive increase in the size of the pulmonary capillary beds and alveolar dilation (13,19).

In this study, we found that donor graft volume was significantly correlated with recipient hemithorax volume, which suggested the importance of preoperative measurement of the recipient hemithorax volume. This finding also implied that a donor graft will accommodate itself to the size of the recipient hemithorax. In LDLLT, the donor grafts stretched or shrank in comparison to the original size, and there was also significant correlation between donor graft FVC values and lung volumes not only before transplantation but also at all of the appointed measurement times after transplantation. Furthermore, in both OS and US groups, FEV1% was neither increased nor decreased significantly before or after LDLLT. These results demonstrate that, by our donor selection criteria, donor grafts will overinflate or underinflate after LDLLT to an extent that allows them to maintain their original function. Furthermore, in this study, we did not observe any emphysematous changes on routine chest CT scans, and according to the trends of FEV1% in both groups, obstructive patterns of pulmonary dysfunction did not occur. Previous studies have also presumed that significant increase in lung volumes after LDLLT may be caused by alveolar dilation (13,19), and physiological investigation on lung size mismatch and allograft function that was conducted previously with deceased donor lung transplantation (20) indicated that an OS allograft was associated with higher expiratory airflow capacity and less frequent occurrence of bronchiolitis obliterans syndrome, possibly due to the relatively more abundant surfactant in a chronically underinflated lungs. However, further investigation was necessary to understand the true mechanism, particularly in LDLLT with its wider range of size discrepancies.

Limitations of this study included the relatively small sample size, relatively short duration of patient follow-up, and disparate demographics of the transplant recipients. In this study, we selected only patients who had complete preoperative data and 1-year follow-up after LDLLT in order to accurately evaluate the state of the donor graft lungs within at least 1 year of LDLLT. Moreover, because no patients grew taller during this study period, the influence of growth for understanding the current data is excluded. Another important limitation of this study was that recipients in both groups presented with a variety of original diseases. In cases of bronchiolitis obliterans, a recipient's hemithoracic volume might become larger because of the disease itself, whereas in interstitial pneumonia it could become smaller. These disease-specific characteristics would affect the results of this kind of study. We are still accumulating data in order to establish a definite criterion for the safe range of size discrepancies between donor and recipient, and are planning to evaluate patients with the same original disease with longer follow-up times in the near future.

In conclusion, we found that donor grafts overinflated or underinflated to the extent that they could maintain their native function in a new recipient environment. After LDLLT, US donor grafts expanded more than OS donor grafts by alveolar dilatation, producing greater FVC values than preoperative estimates. In contrast, OS donor grafts expanded to their original size, and their FVC values also approached the preoperative estimates.

Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

References

1. Starnes VA, Barr ML, Cohen RG, et al. Living-donor lobar lung transplantation experience: Intermediate results. J Thorac Cardiovasc Surg 1996; 112: 1284–1291.
2. Date H, Aoe M, Nahagiho I, et al. Living-donor lobar lung transplantation for various lung diseases. J Heart Lung Transplant 2003: 126: 476–481.
3. Cohen RG, Barr ML, Schenkel FA, DeMeester TR, Wells WJ, Starnes VA. Living-related donor lobectomy for bilateral lobar transplantation in patients with cystic fibrosis. Ann Thorac Surg 1994; 57: 1423–1428.
4. Chen F, Fujinaga T, Bando T, Date H. Pulmonary function of individual lung lobes after complex living-donor lobar lung transplantation using inspiratory and expiratory three-dimensional computed tomographic volumetry. Interact Cardiovasc Thorac Surg 2012; 15: 1077–1079.
5. Date H, Aoe M, Nagahiro I, et al. How to predict forced vital capacity after living-donor lobar-lung transplantation. J Heart Lung Transplant 2004; 23: 547–551.
6. Hiroshige S, Shimada M, Harada N, et al. Accurate preoperative estimation of liver-graft volumetry using three-dimensional computed tomography. Transplantation 2003; 75: 1561–1564.

7. Ming-Ting W, Jinn-Ming C, Ambrose AA, et al. Use of quantitative CT to predict postoperative lung function in patients with lung cancer. Radiology 1994; 191: 257–262.
8. Ueda K, Tanaka T, Hayashi M, Li TS, Tanaka N, Hamano K. Computed tomography-defined functional lung volume after segmentectomy versus lobectomy. Eur J Cardio-Thorac Surg 2010; 37: 1433–1437.
9. Chen F, Fujinaga T, Shoji T, et al. Perioperative assessment of oversized lobar graft downsizing in living-donor lobar lung transplantation using three-dimensional computed tomographic volumetry Transpl Int 2010; 23: e41–44.
10. Shiraishi T, Hiratsuka M, Munakata M, et al. Living-donor single-lobe lung transplantation for bronchiolitis obliterans in a 4-year-old boy. J Thorac Cardiovasc Surg 2007; 134: 1092–1093.
11. Camargo JJP, Irion KL, Marchiori E, et al. Computed tomography measurement of lung volume in preoperative assessment for living donor lung transplantation: Volume calculation using 3D surface rendering in the determination of size compatibility. Pediatr Transplantation 2009; 13: 429–439.
12. Chen F, Kubo T, Shoji T, Fujinaga T, Bando T, Date H. Comparison of pulmonary function test and computed tomographic volumetry in living lung donors. J Heart Lung Transplant 2011; 30: 572–575.
13. Yamane M, Date H, Okazaki M, Toyooka S, Aoe M, Sano Y. Long-term improvement in pulmonary function after living donor lobar lung transplantation. J Heart Lung Transplant 2007; 26: 687–692.
14. Egan TM, Thompson JT, Detterbeck FC, et al. Effect of size (mis)matching in clinical double-lung transplantation. Transplantation 1995; 59: 707–713.
15. Shigemura N, Bermundez C, Hattler BG, et al. Impact of graft volume reduction for oversized grafts after lung transplantation on outcome in recipients with end-stage restrictive pulmonary diseases. J Heart Lung Transplant 2009; 28: 130–134.
16. Ouwens JP, van der Mark TW, van der Bij W, Geertsma A, de Boer WJ, Koeter GH. Size matching in lung transplantation using predicted total lung capacity. Eur Respir J 2002; 20: 1419–1422.
17. Miyoshi S, Schaefers HJ, Trulock EP, et al. Donor selection for single and double lung transplantation. Chest size matching and other factors influencing posttransplantation vital capacity. Chest 1990; 98: 308–313.
18. Bowdish ME, Pessotto R, Barbers RG, Schenkel FA, Starnes VA, Barr ML. Long-term pulmonary function after living-donor lobar lung transplantationinadults. Ann Thorac Surg 2005; 79: 418–425.
19. Sritippayawan S, Keens TG, Horn MV, et al. Does lung growth occur when mature lobes are transplanted into children? Pediatr Transplant 2002; 6: 500–504.
20. Eberlein M, Permutt S, Chahla MF, et al. Lung size mismatch in bilateral lung transplantation is associated with allograft function and bronchiolitis obliterans syndrome. Chest 2012; 141: 451–460.

A urine biomarker for severe obstructive sleep apnoea patients: lipocalin-type prostaglandin D synthase

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ABSTRACT Lipocalin-type prostaglandin D synthase (L-PGDS), which is responsible for the biosynthesis of prostaglandin D_2, has been reported to have a close connection with cardiovascular disease and sleep regulation. This study aimed to test the hypothesis that the L-PGDS level is a useful marker to identify patients with obstructive sleep apnoea.

64 subjects were enrolled in this prospective study. Urinary concentrations of L-PGDS were measured in the morning. Measurements were made every 4 h in 25 of the 64 patients. Endothelial function was assessed by the reactive hyperaemia peripheral arterial tone index.

Circadian variations in L-PGDS concentrations had a significant time-dependent fluctuation ($p=0.0002$). L-PGDS was higher in the subjects with severe obstructive sleep apnoea (median 784.7 ng per mg of creatinine, $n=23$) than in control subjects (262.1 ng per mg of creatinine, $n=16$; $p=0.004$) and in those with moderate obstructive sleep apnoea (371.7 ng per mg of creatinine, $n=25$; $p=0.0008$). After 2 days of continuous positive airway pressure treatment, L-PGDS concentrations in severe obstructive sleep apnoea subjects ($n=12$) decreased significantly ($p=0.02$) to levels present in control subjects whereas endothelial function did not change significantly. Morning urinary L-PGDS concentrations had significant correlations with the apnoea/hypopnoea index ($R^2=13.9\%$) and serum high-density lipoprotein cholesterol ($R^2=6.2\%$), but not with sleepiness.

Urinary L-PGDS might be a moderately useful marker to identify patients with severe obstructive sleep apnoea.

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Introduction

Obstructive sleep apnoea (OSA) is one of the most important medical conditions identified in the last 50 years and is a major cause of morbidity and mortality throughout the world [1].

Most patients with OSA that should be treated are undiagnosed. One reason is that the diagnostic methods for OSA, such as polysomnography (PSG), are difficult to perform. Therefore, a reasonable biomarker for OSA would be extremely helpful, especially in identifying patients who have OSA with a degree of severity that would put them at risk for cardiovascular disease (CVD).

Prostaglandin (PG)D_2 is formed by the action of PGD synthases on the cyclooxygenase (COX) product PGH_2. PGD_2 is widely distributed in rat and human brain [2]. In peripheral tissues, PGD_2 executes a wide range of functions, including vasodilatation, inhibition of platelet aggregation, glycogenolysis, vasoconstriction, allergic reaction mediation and intraocular pressure reduction [3–8]. In the brain, PGD_2 has been shown to contribute to sleep induction, modulation of body temperature, olfactory function, hormone release, nociception and neuromodulation [9–12]. Thus, because PGD_2 has significant effects on platelet aggregation, vasodilation and vasoconstriction, it has been supposed that PGD_2 is relevant to the occurrence of CVD. Although the prostanoids, including PGD_2, are released from cells immediately after synthesis, it is believed that prostanoids work only locally, near their site of production, because they are either chemically or metabolically unstable [13].

One of the enzymes characterised as a PGD synthase, which catalyses the isomerisation of PGH_2 to PGD_2, is the lipocalin-type prostaglandin D synthase (L-PGDS) [14], and is responsible for the biosynthesis of PGD_2 in the brain and heart (cardiovascular system). L-PGDS is a unique protein with enzyme activity and ligand-binding properties. L-PGDS binds various lipophilic compounds, such as retinoids, bilirubin, biliverdin, gangliosides and amyloid-β peptides, with high affinity, acting as an extracellular transporter of these compounds and serving as an endogenous amyloid-β chaperone to prevent amyloid deposition *in vivo* [15]. The half-life of L-PGDS in canines was reported to be 0.77 h [16].

L-PGDS was confirmed to be secreted into blood and urine [14], and we have established a system to measure its urine, serum or plasma levels by an ELISA system [14, 17–19]. L-PGDS is a very stable enzyme and is highly resistant to heat treatment [14] and protease digestion [20], whereas PGD_2 is an unstable substance, as mentioned earlier [13]. L-PGDS is localised where PGD_2 would have its effect, that is, in the central nervous system, male genital organs and the human heart. In the human heart, L-PGDS is localised in myocardial and atrial endocardial cells, smooth muscle cells in the arteriosclerotic intima, and in the atherosclerotic plaques of severely stenotic coronary arteries. In addition, the chemical properties of L-PGDS are similar to those of serum albumin; however, its molecular weight is much smaller than that of serum albumin (26 000 *versus* 66 000 Da). Thus, L-PGDS more easily passes through glomerular capillary walls of the kidney than serum albumin. Indeed, it has been reported that urinary L-PGDS excretion increased in the microalbuminuric stage in patients with type 2 diabetes mellitus (DM) and in hypertensive patients who were apparently free from overt proteinuria [21, 22]. Although the enzymatic activity of L-PGDS cannot be determined, the amount of serum or urinary L-PGDS increased when conditions such as coronary heart disease, hypertension or type 2 DM worsened [21–23].

As OSA induces multi-organ damage and diseases such as hypertension, DM, renal insufficiency, coronary disease and cerebral-cardiovascular diseases, the degree of severity of OSA in individuals with OSA would have significant associations with morbidity and mortality from these conditions [24]. In addition, serum L-PGDS levels were slightly elevated in individuals with OSA with excessive daytime sleepiness [25]. Thus, we hypothesised that L-PGDS could be a biomarker for OSA because of its close relationships with sleep and CVD. In consideration of this hypothesis, we tested whether plasma or urine L-PGDS would be a powerful biomarker for OSA.

Methods

Study subjects

64 clinically stable adults (age >20 years) with suspected OSA were consecutively enrolled in the present prospective study. This study is registered at www.clinicaltrials.gov with identifier number NCT01096433.

Major exclusion criteria were history of CVD, DM under treatment with hypoglycaemic agents or insulin, use of glucocorticoid or nonsteroidal anti-inflammatory drugs, and being a current smoker. This study was approved by the Ethics Committee of Kyoto University (Kyoto, Japan). All patients gave written informed consent to participate. The other exclusion criteria are shown, in detail, in the online supplementary material.

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Study design

At baseline, the subjects' medical history was recorded and a physical examination was performed. In the medical history, hypertension was defined as a systolic blood pressure $\geqslant$140 mmHg or diastolic blood pressure $\geqslant$90 mmHg, or use of an antihypertensive medication. Dyslipidaemia was defined as serum low-density lipoprotein cholesterol $\geqslant$140 mg·dL^{-1} or high-density lipoprotein cholesterol (HDL-C) <40 mg·dL^{-1}, triglycerides $\geqslant$150 mg·dL^{-1} [26], or use of an antilipidaemic medication.

All patients underwent attended diagnostic overnight PSG. Before the patient slept, urine samples were collected at 22:00 h. In addition, the first urine voided in the morning following the overnight PSG was collected at 06:00 h. Peripheral venous blood samples were collected from 06:00 h to 7:00 h following a 12-h overnight fast and PSG. Morning endothelial dysfunction measured by reactive hyperaemia peripheral arterial tone (RH-PAT) [27] was measured. After the overnight PSG, five blood pressure measurements, each 1 min apart, were taken in the morning after the patient had rested for at least 5 min in the supine position. The average of the latter two readings was calculated.

After 2 days of continuous positive airway pressure (CPAP) treatment, urine and blood samples were collected at the same time as during and after PSG. In all of the patients, adequate CPAP pressure was determined to have overcome obstruction and all flow limitations by a full night's titration.

During the PSG day, we investigated the circadian variations in urinary L-PGDS concentrations in 25 patients who were randomly selected from all 64 subjects at 4-h intervals except for midnight (14:00, 18:00, 22:00, 06:00 and 10:00 h). Urinary sampling was not performed at midnight in order not to disturb physiological sleep.

Polysomnography

PSG was performed according to recommendations in American Academy of Sleep Medicine manual (online supplementary material). Apnoea/hypopnoea index (AHI) values were expressed as the number of episodes of apnoea and hypopnoea per hour over the total sleep time. OSA severity was defined by the AHI as follows: control (AHI <15 events·h^{-1}), moderate OSA (15$\leqslant$AHI<30 events·h^{-1}) and severe OSA (AHI $\geqslant$30 events·h^{-1}).

Measurements of plasma and urinary L-PGDS concentrations

In the present study, plasma samples were centrifuged immediately at 1470 × *g* at 4°C for 15 min and urine samples were pooled (not centrifuged), as described previously [15, 17–19]. The separated samples were stored at -80°C until assay. Concentrations of urinary or plasma L-PGDS were measured by an ELISA using two monoclonal antibodies, Mab-7F5 and Mab-1B7, as described previously (online supplementary material) [15, 17–19].

With this ELISA system, it has been demonstrated that intra- and interassay coefficients of variation in urine samples ranged from 3.2% to 5.8% and 7.6% to 8.3%, respectively. The intra- and interassay coefficients of variation in serum samples were 3.6% and 5.8%, respectively. The ELISA showed no significant interference by a variety of urinary constituents [18]. In addition, it was shown that serum and plasma L-PGDS values in individual subjects were almost the same [15]. All the samples were measured in duplicate and the results were averaged.

Other parameters

Venous blood samples were taken in the fasting state in the morning after one night of PSG and examined for markers of glucose and lipid metabolism and C-reactive protein. As it was difficult to measure urinary catecholamine and L-PGDS levels at the same time, we measured plasma catecholamine levels.

Measurements of the RH-PAT

The RH-PAT is a newly established method to measure endothelial function [27]. Endothelial dysfunction as measured by RH-PAT has been reported in patients with OSA [28]. Morning endothelial function assessed by a finger plethysmographic device (Itamar Medical Ltd., Caesarea, Israel) that allows the isolated detection of pulsatile arterial volume changes [27] was measured after an overnight PSG and after 2 days of CPAP.

Statistical analysis

Data were analysed using JMP 9.0 (SAS Institute, Inc., Cary, NC, USA). Continuous variables were expressed as mean$\pm$SE or median values and ranges because the sample size of each group was small. The associations between patients' characteristics, PSG data, biomarkers (blood and urine) and OSA severity were assessed by the Kruskal–Wallis test. When a significant difference was observed, we used the Bonferroni-corrected t-test to identify where differences were significant. We evaluated the sensitivity and

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specificity of the cut-off value of L-PGDS for predicting severe OSA with the use of receiver operating characteristic curve analysis, estimating the area under the curve (AUC). The relationship between L-PGDS concentrations (urine and plasma), the RH-PAT index and other parameters were analysed by Pearson's correlation coefficient test. Multiple regression analysis was performed to adjust for confounders such as age, sex, body mass index (BMI), and morning systolic and diastolic blood pressure. Next, multiple regression analyses, with a p-value <0.10 required for entry into the models, were performed to identify those variables that could best predict morning urinary L-PGDS.

To investigate changes in L-PGDS concentrations, the RH-PAT index, and other parameters before and after 2 days of CPAP, comparisons of data between those two time-points were tested by a paired t-test. Multiple ANOVA with repeated measures was performed to analyse urinary L-PGDS concentrations across the 24-h period. In all analyses, p-value <0.05 was considered statistically significant.

Results

Clinical characteristics of study subjects, L-PGDS concentration and RH-PAT index according to OSA severity

Patient characteristics, PSG data and laboratory data are shown in table 1. There were significant differences among the groups in morning urinary L-PGDS concentrations ($p=0.0009$) but not in night urinary L-PGDS concentrations ($p=0.19$) and plasma L-PGDS levels ($p=0.09$) (table 1 and fig. 1a). After adjustment for age and BMI, subjects with severe OSA had significantly higher morning urinary L-PGDS values than control subjects ($p=0.007$) and subjects with moderate OSA ($p=0.002$). There were significant differences among the groups in the RH-PAT index (fig. 1b).

The cut-off value for predicting severe OSA with minimal false-negative and false-positive errors was 621.8 $ng \cdot mg^{-1}$ of creatinine (sensitivity 65.2%, specificity, 85.4%). This cut-off value had moderate accuracy for predicting severe OSA (AUC 0.78) (fig. 2).

Relationship between urinary L-PGDS concentrations, RH-PAT index and clinical indices

Morning urinary L-PGDS concentrations were positively correlated with several parameters, including the AHI (table 2 and fig. 3). Morning urinary L-PGDS concentrations were positively correlated with AHI after adjustment for age, sex, BMI and morning systolic and diastolic blood pressure (fig. 3). There was a strongly positive correlation between morning and night urinary L-PGDS concentrations ($p<0.0001$). The Epworth Sleepiness Scale did not correlate significantly with morning urinary L-PGDS (table 2).

After adjustment for age, sex, BMI, and morning systolic and diastolic blood pressure, morning urinary L-PGDS concentrations were still positively correlated with the AHI (β coefficient=0.373; $p=0.006$), 3% oxygen desaturation index (ODI) ($\beta=0.322$; $p=0.02$), arousal index ($\beta=0.370$; $p=0.007$) and plasma noradrenaline ($\beta=0.258$; $p=0.04$). The RH-PAT index was negatively correlated with the AHI ($\beta=-0.305$; $p=0.04$) and arousal index ($\beta=-0.359$; $p=0.01$) after adjustment.

Relationship between plasma L-PGDS concentrations and clinical indices

Plasma L-PGDS levels were positively correlated with several parameters (table 2). Plasma L-PGDS also tended to have a positive correlation with morning urinary L-PGDS ($p=0.07$). After adjustment for age, sex, BMI, and morning systolic and diastolic blood pressure, plasma L-PGDS levels were only positively correlated with serum creatinine levels ($\beta=0.361$; $p=0.03$).

Clinical determinants of morning urinary L-PGDS concentrations

Table 3 shows results of multiple regression analyses to identify those variables (morning systolic and diastolic blood pressure, arousal index, AHI, 3% ODI, serum HDL-C, plasma adrenalin, plasma noradrenalin and plasma L-PGDS) that could predict morning urinary L-PGDS concentrations. Then, one of the variables that had very strong co-linearity ($r>0.70$), such as the arousal index, AHI and 3% ODI, was selected.

In these three models, morning urinary L-PGDS had a significant and independent correlation with the AHI or the 3% ODI, or the arousal index and serum HDL-C (table 3).

Effects of CPAP treatment on urinary L-PGDS concentrations

In Japan, patients whose AHI is $\geqslant 20$ can use CPAP under the health insurance system. In this study, 20 permitted patients were investigated (table 4). 12 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 (fig. 4a). In contrast, the plasma L-PGDS level, the RH-PAT index (fig. 4b) 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

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

	Control	Moderate OSA	Severe OSA	p-value
Subjects	16	25	23	
Age years	47.5 (21–76)	55 (31–74)	55 (27–78)	0.12
Male	12 (75.0)	21 (84.0)	21 (91.3)	0.39
BMI kg·m^{-2}	26.0 (20.4–35.8)	24.5 (20.2–34.8)	26.6 (21.6–39.9)	0.10
Waist circumference cm	87 (76–118)	90 (78–113)	94 (82–120)	0.13
Morning systolic BP mmHg	114 (95–136)	118 (99–139)	127 (96–150)$^{\#,\P}$	0.003
Morning diastolic BP mmHg	70 (58–91)	74 (57–96)	80 (69–97)$^{\#,\P}$	0.0004
Ex-smoker	9 (56.3)	12(48.0)	17 (73.9)	0.18
ESS score	14 (1–24)	12 (2–19)	12 (4–20)	0.13
Comorbidity				
Hypertension	3 (18.8)	10 (40.0)	10 (47.8)	0.25
Dyslipidaemia	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 (256.5–510)	389.0 (205.5–515.5)	378.0 (240.5–499)	0.56
Sleep efficiency %	81.1 (63.3–94.4)	76.6 (42.9–94.2)	72.9 (50.4–96.4)	0.50
Arousal index events·h^{-1}	17.8 (9.5–26.7)	22.2 (10.8–46.5)	44.3 (12.6–61.2)$^{\#,\P}$	<0.0001
AHI events·h^{-1}	7.3 (1.2–14.8)	22.7 (15.2–29.8)$^{\#}$	47.2 (31.9–85.4)$^{\#,\P}$	<0.0001
3% ODI events·h^{-1}	5.3 (0.5–14.1)	17.4 (10.1–27.2)$^{\#}$	48.0 (26.9–86.4)$^{\#,\P}$	<0.0001
Minimum Sp_{O_2} %	90.5 (81–97)	81.5 (73–90)$^{\#}$	75.0 (61–86)$^{\#,\P}$	<0.0001
Sp_{O_2} <90% % TST	0 (0–5.0)	2.5 (0–8.1)	11.9 (1.2–87.7)$^{\#,\P}$	<0.0001
RH-PAT index	1.91 (1.30–2.87)	2.00 (1.32–3.91)	1.65 (1.42–3.23)¶	0.02
Blood				
Creatinine mg·dL^{-1}	0.8 (0.4–1.0)	0.8 (0.6–1.1)	0.8 (0.6–1.1)	0.49
TC mg·dL^{-1}	204 (125–241)	197 (130–255)	199 (141–299)	0.58
HDL-C mg·dL^{-1}	51 (35–86)	53 (40–93)	49 (30–82)	0.30
TG mg·dL^{-1}	103 (54–245)	100 (44–334)	132 (70–286)	0.21
CRP mg·dL^{-1}	0.1 (0.0–0.2)	0.1 (0.0–0.2)	0.1 (0.0–0.5)$^{\#,\P}$	0.004
Glucose mg·dL^{-1}	87 (81–114)	95 (75–146)	96 (85–121)	0.07
Adrenaline pg·mL^{-1}	14 (5–31)	16 (5–30)	16 (5–45)	0.65
Noradrenaline pg·mL^{-1}	241 (67–465)	252 (91–398)	261 (82–569)	0.52
L-PGDS ng·mL^{-1}	422.0 (291.7–588.6)	469.3 (270.3–657.0)	491.4 (343.3–726.0)	0.09
Urine				
Morning L-PGDS ng·mg^{-1} of creatinine	262.1 (21.3–1178.6)	371.7 (92.3–2378.2)	784.7 (124.4–3274.1)$^{\#,\P}$	0.0009
Night L-PGDS ng·mg^{-1} of creatinine	745.8 (30.5–1754.0)	659.5 (78.9–2937.9)	958.3 (221.0–5621.8)	0.19

Data are presented as n, median (range) or n (%), unless otherwise stated. BMI: body mass index; BP: blood pressure; ESS: Epworth Sleepiness Scale; TST: total sleep time; AHI: apnoea/hypopnoea index; ODI: oxygen desaturation index; Sp_{O_2}: arterial oxygen saturation measured by pulse oximetry; RH-PAT: reactive hyperaemia 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. $^{\#}$: p<0.0167 *versus* control; ¶: p<0.0167 *versus* moderate OSA.

at baseline *versus* 317.8 ng·mg^{-1} of 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 5a. In 25 patients, a multiple ANOVA with repeated measures revealed a significant time-dependent fluctuation (p=0.0002), with the highest levels at 14:00 h and lowest levels at 06:00 h. Compared with the 06:00 h values, urinary L-PGDS concentrations significantly increased at 14:00 h (p<0.0001) and 18:00 h (p=0.02). Regarding the associations between the AHI and urinary L-PGDS concentrations at each time-point, the 06:00 h values were only significantly positively correlated with AHI (r=0.566; p=0.004). In the patients with AHI ⩾30, 06:00 h values for urinary L-PGDS were significantly increased compared with patients with AHI <30 (AHI <30 (n=13) 227 ng·mg^{-1} of creatinine *versus* AHI ⩾30 (n=12) 780.3 ng·mg^{-1} of creatinine; p=0.003) (fig. 5b).

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

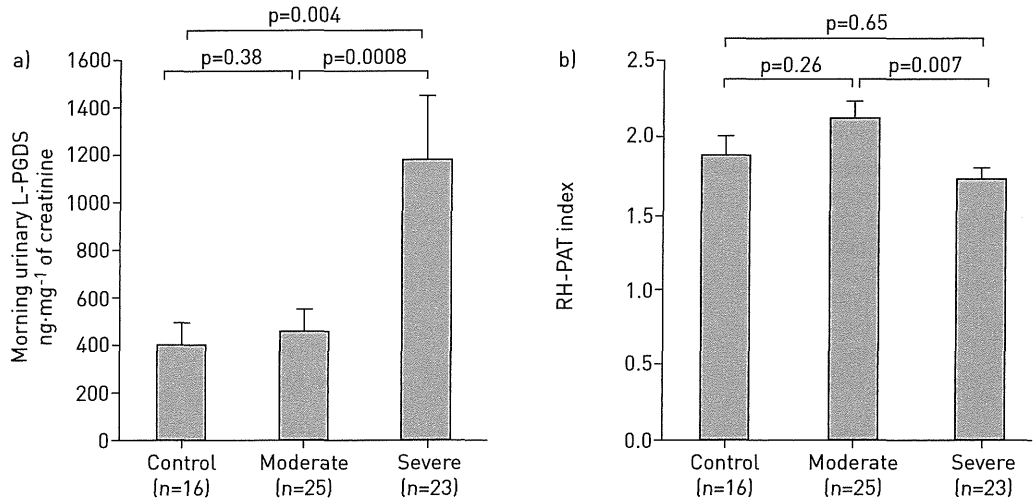

FIGURE 1 Comparison of a) morning urinary lipocalin-type prostaglandin D synthase (L-PGDS) concentrations and b) reactive hyperaemia peripheral arterial tone (RH-PAT) index between controls and moderate and severe obstructive sleep apnoea patients. Data are presented as mean±SE.

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 modelling 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, it is of interest that urinary L-PGDS levels at 14:00 h were the same in the 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 have 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

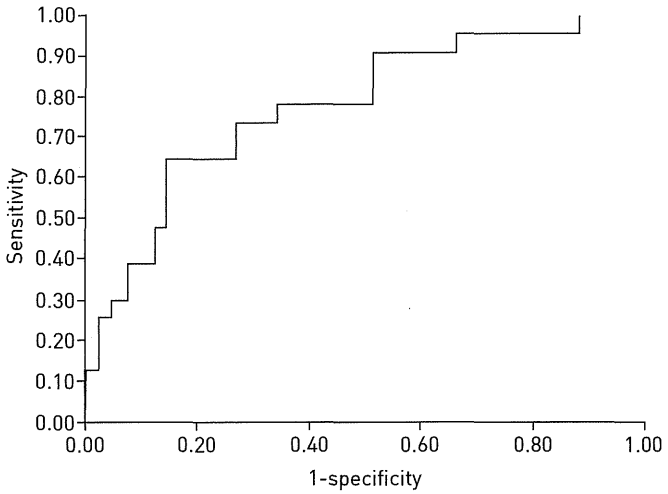

FIGURE 2 Receiver operating characteristic curve analysis to assess the diagnostic validity of morning urinary lipocalin-type prostaglandin D synthase concentrations to detect severe obstructive sleep apnoea.

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TABLE 2 Associations of lipocalin-type prostaglandin D synthase (L-PGDS) level and reactive hyperaemia peripheral arterial tone (RH-PAT) index with patients' characteristics, polysomnography data and biomarkers

	Morning urinary L-PGDS ng·mg^{-1} of creatinine		Plasma L-PGDS ng·mL^{-1}		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
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^{-1}	0.472	<0.0001*	0.220	0.08	-0.268	0.03*
AHI events·h^{-1}	0.426	0.0005*	0.180	0.16	-0.241	0.06
3% ODI events·h^{-1}	0.384	0.002*	0.173	0.18	-0.244	0.054
Minimum Sp_{O_2} %	-0.112	0.38	0.099	0.44	0.046	0.72
Sp_{O_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^{-1}	0.052	0.68	0.256	0.04	-0.067	0.60
TC mg·dL^{-1}	0.189	0.14	0.233	0.07	-0.003	0.98
HDL-C mg·dL^{-1}	0.214	0.09	0.045	0.73	0.079	0.54
TG mg·dL^{-1}	-0.067	0.60	0.170	0.18	0.134	0.30
CRP mg·dL^{-1}	0.071	0.58	0.083	0.52	-0.132	0.30
Glucose mg·dL^{-1}	0.158	0.22	-0.161	0.21	-0.143	0.27
Adrenaline pg·mL^{-1}	0.310	0.02*	0.220	0.10	0.018	0.89
Noradrenaline pg·mL^{-1}	0.329	0.008*	0.149	0.25	0.128	0.32
Plasma L-PGDS ng·mL^{-1}	0.228	0.07			0.030	0.82
Morning urinary L-PGDS ng·mg^{-1} of creatinine			0.228	0.07	-0.068	0.59
Night urinary L-PGDS ng·mg^{-1} of creatinine	0.868	<0.0001*	0.090	0.51	0.050	0.71

BMI: body mass index; BP: blood pressure; ESS: Epworth Sleepiness Scale; AHI: apnoea/hypopnoea index; ODI: oxygen desaturation index; Sp_{O_2}: arterial oxygen saturation measured by pulse oximetry; TST: total sleep time; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglycerides; CRP: C-reactive protein. *: $p<0.05$.

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 damage 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 has been said that serum L-PGDS levels increase with age and are higher in males than in females [19], while urinary L-PGDS was also shown to be higher in males than in females and is weakly correlated with age in female subjects [22]. In that study [22], control subjects were slightly younger, but not with significance, and those in the moderate and severe OSA groups were almost the same age as those in the present study (table 1). In addition, morning urinary L-PGDS values were still correlated with the severity of OSA after adjustment for BMI, age and sex. Therefore, BMI, sex 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

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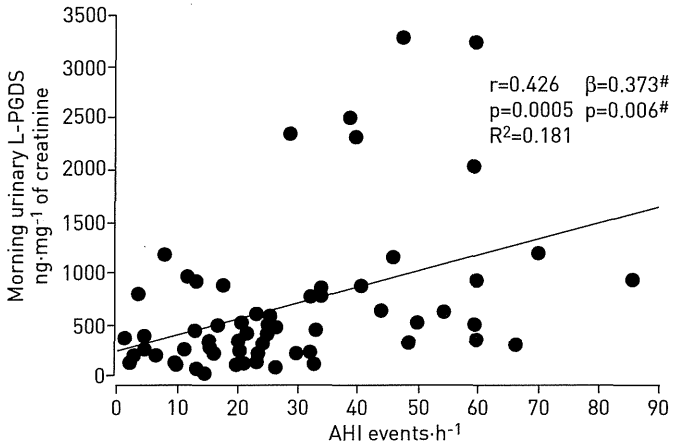

FIGURE 3 Relationship between apnoea/hypopnoea index (AHI) and morning urinary lipocalin-type prostaglandin D synthase (L-PGDS) concentrations. #: values after adjustment for age, sex, body mass index, and morning systolic and diastolic blood pressure.

patients with severe OSA than in the other subjects, while values at 14:00 h were the same. These findings indicate that L-PGDS is related to OSA independently, and is especially related to severe OSA.

Several causes for elevated L-PGDS concentrations in OSA have been considered, especially in severe OSA. First, intermittent hypoxia induces COX2 expression and activity, which in turn accelerate arachnoid acid metabolism to PGH_2 and PGE_2 [31, 32]. HAN *et al.* [33] 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% oxygen) compared with mice kept under normoxia. OSA is characterised by repeated episodes of oxygen desaturation; however, in OSA there is also sustained hypoxaemia, which is expressed by arterial oxygen saturation measured by pulse oximetry <90% (% of total sleep time). Thus, both sustained and intermittent hypoxaemia 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 [38] reported that intermittent hypoxaemia induced systemic hypertension and that sustained hypoxaemia induced pulmonary hypertension. Both systemic and pulmonary hypertension induced by OSA might increase L-PGDS values [21, 37].

It has been shown 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

TABLE 3 Multivariate linear regression analysis for morning urinary lipocalin-type prostaglandin D synthase

	β	r	p-value	R^2 %
Model 1				
AHI events·h^{-1}	0.326	0.426	0.02	13.9
HDL-C mg·dL^{-1}	0.297	0.214	0.02	6.4
Cumulative R^2				20.3
Model 2				
Arousal, events·h^{-1}	0.411	0.472	0.001	19.4
HDL-C mg·dL^{-1}	0.290	0.214	0.01	6.2
Cumulative R^2				25.6
Model 3				
3% ODI events·h^{-1}	0.291	0.384	0.03	11.2
HDL-C mg·dL^{-1}	0.280	0.214	0.02	6.0
Cumulative R^2				17.2

β: standard regression coefficient; r: correlation coefficient; R^2: contribution rate; AHI: apnoea/hypopnoea index; HDL-C: high-density lipoprotein cholesterol; ODI: oxygen desaturation index.

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TABLE 4 Effects of continuous positive airway pressure (CPAP) on the reactive hyperaemia peripheral arterial tone (RH-PAT) index, various parameters and lipocalin-type prostaglandin D synthase (L-PGDS)

	Before CPAP	After 2 days of CPAP	p-value
BMI	25.2 (21.0–39.9)	24.8 (20.8–39.9)	0.11
Morning systolic BP mmHg	126 (102–138)	121 (103–138)	0.15
Morning diastolic BP mmHg	80 (69–94)	79 (63–94)	0.44
Arousal index events·h^{-1}	31.6 (10.8–54.8)	16.8 (8.6–42.8)	0.0005
AHI events·h^{-1}	33.6 (20.3–59.6)	4.4 (0–8.8)	<0.0001
3% ODI events·h^{-1}	27.9 (16.2–61.3)	3.5 (0–7.6)	<0.0001
RH-PAT index	1.75 (1.46–3.91)	1.82 (1.41–2.83)	0.61
Blood			
Creatinine mg·dL^{-1}	0.8 (0.6–1.0)	0.8 (0.6–1.0)	0.41
TC mg·dL^{-1}	189 (130–299)	184 (132–293)	0.78
HDL-C mg·dL^{-1}	53 (41–66)	52 (38–68)	0.13
TG mg·dL^{-1}	95 (44–215)	98 (58–304)	0.30
CRP mg·dL^{-1}	0.1 (0.0–0.5)	0.0 (0.0–0.4)	0.07
Glucose mg·dL^{-1}	96 (75–133)	96 (83–140)	0.23
Adrenaline pg·mL^{-1}	16 (5–45)	16 (5–36)	0.38
Noradrenaline pg·mL^{-1}	257 (82–521)	216 (113–529)	0.31
L-PGDS ng·mL^{-1}	480.4 (323.2–567.4)	466.0 (323.9–599.2)	0.92
Urine			
Morning L-PGDS ng·mg^{-1} of creatinine	591.2 (227–3274.1)	317.8 (130.3–1587.6)	0.007

Data are presented as median (range), unless otherwise stated. BMI: body mass index; BP: blood pressure; AHI: apnoea/hypopnoea index; ODI: oxygen desaturation index; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglycerides; CRP: C-reactive protein.

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 adrenaline and noradrenaline. 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.* [42] reported that

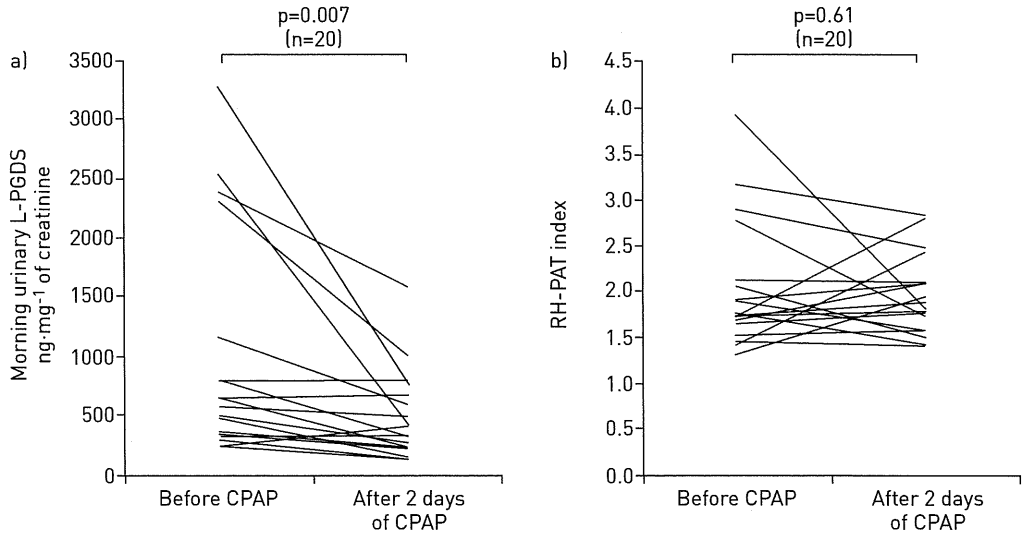

FIGURE 4 Change in a) morning urinary lipocalin-type prostaglandin D synthase (L-PGDS) concentrations and b) reactive hyperaemia peripheral arterial tone (RH-PAT) index before and after 2 days of continuous positive airway pressure (CPAP). Individual data are presented.

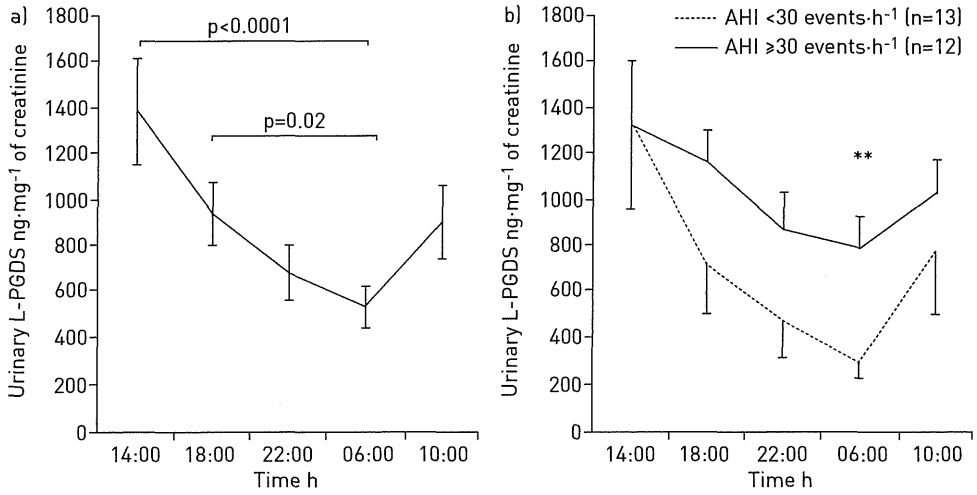

FIGURE 5 a) Urinary lipocalin-type prostaglandin D synthase (L-PGDS) concentrations in 25 study patients at 4-h intervals within a day (except midnight). b) shows the comparison of circadian variations in urinary L-PGDS between patients with apnoea/hypopnoea index (AHI) $\geqslant$30 events·h^{-1} (n=12) and patients with AHI <30 events·h^{-1} (n=13). Data are shown as mean±SE. **: p<0.01 between patients with AHI $\geqslant$30 events·h^{-1} and those with AHI <30 events·h^{-1}.

L-PGDS played a role in lipid transport. 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 L-PGDS values in both severe and non-to-moderate OSA were highest and at the same levels at 14:00 h, with the lowest values at 06:00 h in both groups. However, only 06:00 h urinary L-PGDS values correlated significantly with the AHI and were significantly higher in those with severe OSA compared with subjects with AHI <30 events·h^{-1}. 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 00:00 h and 06:00 h compared with non-OSA patients [43]. These circadian rhythms mimic the pattern of urinary L-PGDS in this study (fig. 4a).

In the current study, we did not detect a significant relationship 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.* [44] 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. In addition, serum L-PGDS levels were not shown to be associated with the AHI [25]. The influence of OSA, such as intermittent hypoxaemia, 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 h 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. First, 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

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values of overnight urine and urine collected over 24 h [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 these 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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References

1 Douglas NJ. Sleep apnea. *In:* Longo DL, Fauci AS, Kasper DL, *et al.*, eds. Harrison's Principle of Internal Medicine. 17th Edn. New York, McGraw-Hill, 2008; pp. 1665–1668.
2 Ogorochi T, Narumiya S, Mizuno N, *et al.* Regional distribution of prostaglandins D_2, E_2, and $F_{2\alpha}$ and related enzymes in postmortem human brain. *J Neurochem* 1984; 43: 71–82.
3 Whittle BJ, Moncada S, Mullane K, *et al.* Platelet and cardiovascular activity of the hydantoin BW245C, a potent prostaglandin analogue. *Prostaglandins* 1983; 25: 205–223.
4 Narumiya S, Toda N. Different responsiveness of prostaglandin D_2-sensitive systems to prostaglandin D_2 and its analogues. *Br J Pharmacol* 1985; 85: 367–375.
5 Casteleijn E, Kuiper J, Van Rooij HC, *et al.* Prostaglandin D_2 mediates the stimulation of glycogenolysis in the liver by phorbol ester. *Biochem J* 1988; 250: 77–80.
6 Darius H, Michael-Hepp J, Thierauch KH, *et al.* Inhibition of human platelets and polymorphonuclear neutrophils by the potent and metabolically stable prostaglandin D_2 analog ZK 118.182. *Eur J Pharmacol* 1994; 258: 207–213.
7 Matsugi T, Kageyama M, Nishimura K, *et al.* Selective prostaglandin D_2 receptor stimulation elicits ocular hypotensive effects in rabbits and cats. *Eur J Pharmacol* 1995; 275: 245–250.
8 Matsuoka T, Hirata M, Tanaka H, *et al.* Prostaglandin D_2 as a mediator of allergic asthma. *Science* 2000; 287: 2013–2017.
9 Eguchi N, Minami T, Shirafuji N, *et al.* Lack of tactile pain (allodynia) in lipocalin-type prostaglandin D synthase-deficient mice. *Proc Natl Acad Sci USA* 1999; 96: 726–730.
10 Urade Y, Hayaishi O. Prostaglandin D_2 and sleep regulation. *Biochim Biophys Acta* 1999; 1436: 606–615.
11 Mizoguchi A, Eguchi N, Kimura K, *et al.* Dominant localization of prostaglandin D receptors on arachnoid trabecular cells in mouse basal forebrain and their involvement in the regulation of non-rapid eye movement sleep. *Proc Natl Acad Sci USA* 2001; 98: 11674–11679.
12 Hayaishi O. Molecular genetic studies on sleep-wake regulation, with special emphasis on the prostaglandin D_2 system. *J Appl Physiol* 2002; 92: 863–868.
13 Matsuoka T, Narumiya S. The roles of prostanoids in infection and sickness behaviors. *J Infect Chemother* 2008; 14: 270–278.
14 Urade Y, Fujimoto N, Hayaishi O. Purification and characterization of rat brain prostaglandin D synthetase. *J Biol Chem* 1985; 260: 12410–12415.
15 Oda H, Eguchi N, Urade Y. Quantitative sandwich enzyme-linked immunosorbent assay for human secretory prostagrandin D synthase (β-trace). *Proc Japan Acad* 1996; 72: 108–111.
16 Li W, Mase M, Inui T, *et al.* Pharmacokinetics of recombinant human lipocalin-type prostaglandin D synthase/beta-trace in canine. *Neurosci Res* 2008; 61: 289–293.
17 Eguchi Y, Eguchi N, Oda H, *et al.* Expression of lipocalin-type prostaglandin D synthase (beta-trace) in human heart and its accumulation in the coronary circulation of angina patients. *Proc Natl Acad Sci USA* 1997; 94: 14689–14694.
18 Oda H, Shiina Y, Seiki K, *et al.* Development and evaluation of a practical ELISA for human urinary lipocalin-type prostaglandin D synthase. *Clin Chem* 2002; 48: 1445–1453.
19 Miwa Y, Oda H, Shiina Y, *et al.* Association of serum lipocalin-type prostaglandin D synthase levels with subclinical atherosclerosis in untreated asymptomatic subjects. *Hypertens Res* 2008; 31: 1931–1939.
20 Urade Y, Ujihara M, Horiguchi Y, *et al.* Mast cells contain spleen-type prostaglandin D synthetase. *J Biol Chem* 1990; 265: 371–375.
21 Hirawa N, Uehara Y, Yamakado M, *et al.* Lipocalin-type prostaglandin d synthase in essential hypertension. *Hypertension* 2002; 39: 449–454.
22 Uehara Y, Makino H, Seiki K, *et al.* Urinary excretions of lipocalin-type prostaglandin D synthase predict renal injury in type-2 diabetes: a cross-sectional and prospective multicentre study. *Nephrol Dial Transplant* 2009; 24: 475–482.
23 Inoue T, Eguchi Y, Matsumoto T, *et al.* Lipocalin-type prostaglandin D synthase is a powerful biomarker for severity of stable coronary artery disease. *Atherosclerosis* 2008; 201: 385–391.
24 Somers VK, White DP, Amin R, *et al.* Sleep apnea and cardiovascular disease: an American Heart Association/American College Of Cardiology Foundation Scientific Statement from the American Heart Association Council

for High Blood Pressure Research Professional Education Committee, Council on Clinical Cardiology, Stroke Council, and Council On Cardiovascular Nursing. In collaboration with the National Heart, Lung, and Blood Institute National Center on Sleep Disorders Research (National Institutes of Health). *Circulation* 2008; 118: 1080–1111.

25 Barceló A, de la Peña M, Barbé F, *et al.* Prostaglandin D synthase (beta trace) levels in sleep apnea patients with and without sleepiness. *Sleep Med* 2007; 8: 509–511.

26 Teramoto T, Sasaki J, Ueshima H, *et al.* Diagnostic criteria for dyslipidemia. Executive summary of Japan Atherosclerosis Society (JAS) guideline for diagnosis and prevention of atherosclerotic cardiovascular diseases for Japanese. *J Atheroscler Thromb* 2007; 14: 155–158.

27 Bonetti PO, Barsness GW, Keelan PC, *et al.* Enhanced external counterpulsation improves endothelial function in patients with symptomatic coronary artery disease. *J Am Coll Cardiol* 2003; 41: 1761–1768.

28 Itzhaki S, Lavie L, Pillar G, *et al.* Endothelial dysfunction in obstructive sleep apnea measured by peripheral arterial tone response in the finger to reactive hyperemia. *Sleep* 2005; 28: 594–600.

29 Melegos DN, Freedman MS, Diamandis EP. Prostaglandin D synthase concentration in cerebrospinal fluid and serum of patients with neurological disorders. *Prostaglandins* 1997; 54: 463–474.

30 Melegos DN, Grass L, Pierratos A, *et al.* Highly elevated levels of prostaglandin D synthase in the serum of patients with renal failure. *Urology* 1999; 53: 32–37.

31 Gozal D, Kheirandish L. Sleepiness and neurodegeneration in sleep-disordered breathing: convergence of signaling cascades. *Am J Respir Crit Care Med* 2005; 171: 1325–1327.

32 Li RC, Row BW, Gozal E, *et al.* Cyclooxygenase 2 and intermittent hypoxia-induced spatial deficits in the rat. *Am J Respir Crit Care Med* 2003; 168: 469–475.

33 Han F, Takeda K, Ishikawa K, *et al.* Induction of lipocalin-type prostaglandin D synthase in mouse heart under hypoxemia. *Biochem Biophys Res Commun* 2009; 385: 449–453.

34 Tilkian AG, Guilleminault C, Schroeder JS, *et al.* Hemodynamics in sleep-induced apnea. Studies during wakefulness and sleep. *Ann Intern Med* 1976; 85: 714–719.

35 Taba Y, Sasaguri T, Miyagi M, *et al.* Fluid shear stress induces lipocalin-type prostaglandin D_2 synthase expression in vascular endothelial cells. *Circ Res 2000; 86:* 967–973.

36 Miyagi M, Miwa Y, Takahashi-Yanaga F, *et al.* Activator protein-1 mediates shear stress-induced prostaglandin D synthase gene expression in vascular endothelial cells. *Arterioscler Thromb Vasc Biol* 2005; 25: 970–975.

37 Robbins IM, Barst RJ, Rubin LJ, *et al.* Increased levels of prostaglandin D_2 suggest macrophage activation in patients with primary pulmonary hypertension. *Chest* 2001; 120: 1639–1644.

38 Semenza GL. Regulation of oxygen homeostasis by hypoxia-inducible factor 1. *Physiology (Bethesda)* 2009; 24: 97–106.

39 Arnardottir ES, Mackiewicz M, Gislason T, *et al.* Molecular signatures of obstructive sleep apnea in adults: a review and perspective. *Sleep* 2009; 32: 447–470.

40 Narkiewicz K, van de Borne PJ, Cooley RL, *et al.* Sympathetic activity in obese subjects with and without obstructive sleep apnea. *Circulation* 1998; 98: 772–776.

41 Ziegler MG, Mills PJ, Loredo JS, *et al.* Effect of continuous positive airway pressure and placebo treatment on sympathetic nervous activity in patients with obstructive sleep apnea. *Chest* 2001; 120: 887–893.

42 Miwa Y, Takiuchi S, Kamide K, *et al.* Identification of gene polymorphism in lipocalin-type prostaglandin D synthase and its association with carotid atherosclerosis in Japanese hypertensive patients. *Biochem Biophys Res Commun* 2004; 322: 428–433.

43 Kuniyoshi FH, Garcia-Touchard A, Gami AS, *et al.* Day-night variation of acute myocardial infarction in obstructive sleep apnea. *J Am Coll Cardiol* 2008; 52: 343–346.

44 Hirawa N, Uehara Y, Ikeda T, *et al.* Urinary prostaglandin D synthase (beta-trace) excretion increases in the early stage of diabetes mellitus. *Nephron* 2001; 87: 321–327.

DOI: 10.1183/09031936.00133512