

Field study

The impact of night-shift work on platelet function in healthy medical staff

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Abstract: Objectives: Rotating shift work has been reported to increase the risk of cardiovascular diseases. Vascular endothelial dysfunction and platelet activation are among the leading causes of thrombus formation in patients with myocardial infarction or stroke. Endothelial function has been shown to be impaired immediately after night-shift work; however, it is not known whether platelets are also activated. The aim of this study was to investigate the acute impact of night-shift work on platelet function. **Methods:** This observational study included 11 healthy medical staff members (seven women, median age 32 years). We examined each subject's platelet aggregation rates and the serum concentrations of eicosanoid mediators after night-shift work and on day-shift work without preceding night-shift work (baseline). **Results:** Platelet aggregation did not differ from baseline levels after night-shift work. However, serum cyclooxygenase (COX)-metabolized eicosanoid mediators, particularly thromboxane (Tx) B₂ (a stable metabolite of TxA₂ and the most important marker of platelet activation), were significantly higher after the night-shift than at baseline (median 65.3 vs 180.4 ng/ml). **Conclusions:** Although platelet aggregation did not increase, there was an increase in serum COX-metabolized eicosanoid mediators such as TxB₂ in healthy medical staff after night-shift work. This platelet hypersensitivity may be one of the mechanisms underlying the significant association between night-shift work and adverse cardiovascular

outcomes.
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Key words: Cyclooxygenase, Eicosanoids, Platelet aggregation, Thromboxane B₂

Introduction

Various physiological functions of the human body exhibit circadian rhythms, including the autonomic nervous and endocrine systems^{1,2}. Rotating shift work, which disrupts normal circadian rhythms, has recently been reported to be associated with an increased risk of cardiovascular and cerebrovascular diseases³⁻⁵. Platelet activation arises from various causes, including vascular endothelial dysfunction, and is one of the most important causes of thrombus formation in the vessel walls of patients with atherosclerotic diseases such as myocardial infarction or stroke. Previous investigations have demonstrated circadian variation in vascular endothelial function⁶⁻⁸, and have shown that night-shift work disturbs circadian rhythms and impairs brachial endothelial function and coronary microcirculation⁹⁻¹¹, suggesting that these factors may play a role in the mechanisms underlying cardiovascular and cerebrovascular events associated with rotating shift work. Similarly, it has been suggested that there may be circadian variation in platelet function^{12,13}. However, the acute impact of night-shift work on platelet

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function remains unclear. To determine whether platelet function was greater after night-shift work than at the beginning of day-shift work, we examined the platelet aggregation rate using both the conventional light transmission method and a highly sensitive light scattering method. We also measured the serum concentration of eicosanoid mediators (bioactive lipids derived from metabolites of 20-carbon arachidonic acid [AA]), which play important roles in platelet activation.

Subjects and Methods

This observational study compared the platelet aggregation rate, the serum concentration of eicosanoid mediators, hemodynamic data, and laboratory variables after night-shift work with those on day-shift work in healthy medical staff at the University of Tokyo Hospital, Tokyo, Japan. Written informed consent was obtained from all subjects, and the study protocol was approved by the institutional review board at the Graduate School of Medicine and Faculty of Medicine, University of Tokyo, complying with the principles of the World Medical Association's Declaration of Helsinki (IRB number 10767).

Recruitment and subjects

The subjects were recruited between July and December 2015, mainly through advertisements on notice boards of the Department of Clinical Laboratory, University of Tokyo Hospital. The inclusion criteria were as follows: (i) men and women aged >20 years, (ii) medical staff working at the University of Tokyo Hospital who were engaged in rotating shift work, (iii) never smoked, (iv) felt subjectively well, and (v) provided written informed consent. The following exclusion criteria were applied: (i) pregnancy or lactation; (ii) known history of (or currently receiving drugs for) cardiovascular disease, diabetes mellitus, hypertension, or a hematologic disease; (iii) history of any other severe disease, such as cancer or inflammation; or (iv) use of nonsteroidal anti-inflammatory drugs or antiallergic drugs within the 10 days prior to blood collection.

The required sample size was calculated using the free sample size-calculating software G*Power version 3.1.9.2 (University of Kiel, Germany). To achieve a power of 80%, statistical significance at the 0.05 level, and an effect size of 0.8, the required sample size was determined to be 15.

Study protocol

To assess the platelet aggregation rate and serum concentration of eicosanoid mediators, fasting venous blood was collected from each subject at the following times: (1) on day-shift work without previous night-shift work (baseline), and (2) after night-shift work (post-shift). Using paired data for each subject decreased the influence of

variability between subjects and increased the likelihood of detecting differences between the baseline and post-shift conditions. The subjects were asked to refrain from drinking any beverages containing caffeine or glucose and eating any food after midnight on the test day. Blood was collected between 8:00 and 9:00 A.M. to minimize changes in platelet activation associated with circadian variations. The blood samples were carefully drawn from an antecubital vein using a 21-G butterfly needle. A 9-ml sample was collected in a vacuum tube containing 1 ml of 3.2% sodium citrate for platelet aggregation studies, and a 6-ml sample was collected in a vacuum tube containing thrombin and silica for serum eicosanoid analysis. Additional blood samples were also obtained to assess the complete blood count, prothrombin time, activated partial thromboplastin time, and the concentrations of fibrinogen, glucose, hemoglobin A1c, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglycerides. Blood pressure and heart rate were measured immediately before each blood sample collection. The subjects were also asked about how long they slept the previous night and their shift work history.

Measurement of platelet aggregation rate

The citrated samples were centrifuged at $100 \times g$ for 15 min at 23°C to obtain platelet-rich plasma. The remaining samples were centrifuged at $1700 \times g$ for an additional 10 min at 23°C to isolate platelet-poor plasma. The interval between blood collection and aggregation assay was standardized to 1 h. Platelet aggregation rate was measured in the platelet-rich plasma at 37°C using a PA-200 aggregometer (Kowa, Tokyo, Japan). This simultaneously assessed platelet aggregation by two different methods: the light transmission method¹⁴⁾, in which the magnitude of platelet aggregation is expressed as the percentage increase in the light transmission of platelet-rich plasma relative to that of platelet-poor plasma (%LT); and the light scattering method, which measures scattering from small, medium, and large platelet aggregates^{15,16)}. The platelet aggregation rate was assessed in the absence of any agonists as spontaneous platelet aggregation (SPA); or in the presence of adenosine 5'-diphosphate (ADP; MC Medical Co., Tokyo, Japan), collagen (Takeda Austria GmbH, Linz, Austria), or epinephrine (Arkray Factory Co., Shiga, Japan) for 11 min. Final concentrations of each agonist were $0.5 \mu\text{M}$ for ADP, $0.1 \mu\text{g/ml}$ for collagen, and 0.1 and $0.25 \mu\text{M}$ for epinephrine. We measured the maximum light intensity scattered by the small-sized aggregates (SMAX), the maximum of the sum of light intensities scattered by all three aggregate sizes (TMAX) over an 11-min period, the area under the light intensity curve of the small-sized aggregates (SAUC), and the sum of the areas under the light intensity curves for all three aggregate sizes (TAUC).

Measurement of eicosanoid mediators in serum

An internal standard method was used to measure the serum concentrations of eicosanoid mediators. A mixture containing each of the following 12 internal standards at 0.2 µg/ml was prepared: tetranor-prostaglandin (PG) E metabolite-d6 (tetranor-PGEM-d6), PGE₂-d4, PGD₂-d4, PGF_{2α}-d4, 6-keto-PGF_{1α}-d4, thromboxane (Tx) B₂-d4, leukotriene (LT) C₄-d5, LTB₄-d4, 15 (S)-hydroxyeicosatetraenoic acid (HETE)-d8, 5 (S)-HETE-d8, and 12 (S)-HETE-d8, as well as 4.0 µg/ml of AA-d8. Then 200 µl of each serum sample was diluted with 1 ml of methanol, and 10 µl of the internal standard mixture was added. The samples were mixed well, centrifuged (20,000 × g, 10 min, 4°C), and the supernatants were diluted with 3 ml of 0.03% formic acid in water. Solid-phase extraction of eicosanoids was performed using Oasis HLB 1 ml (10 mg) extraction cartridges (Waters) set on a vacuum manifold. The cartridges were preconditioned using 150 µl of methanol followed by 1 ml of 0.03% formic acid in water. The samples were loaded into the columns, and then serially washed with 1 ml of 0.03% formic acid in water, 15% methanol in 0.03% formic acid/water, and 3 ml of petroleum ether. The samples (analytes) were then eluted with 200 µl of methanol. The eluent was evaporated by vacuum evaporator (EC-95C3T, Sakuma, Tokyo, Japan) and reconstituted in 20 µl of methanol. The samples were analyzed by liquid chromatography coupled to a triple quadrupole mass spectrometer, LCMS-8050, equipped with an electrospray ion source (Shimadzu, Kyoto, Japan), into which 5 µl of the samples was injected for analysis. Chromatographic separation was achieved on a reversed-phase column (Kinetex C8, 2.1 × 150 mm, 2.6 µm; Phenomenex, Torrance, CA) at a flow rate of 0.4 ml/min. For mobile phases A and B, 0.1% formic acid in water and acetonitrile were used, respectively. The sample cooler and column oven temperatures were set at 5°C and 40°C, respectively. Methanol was used as the sample solvent. The mass spectrometer was operated in selected reaction monitoring mode. Nitrogen gas was used for nebulizer and drying gas, and argon gas was used for collision-induced dissociation. All compounds were quantified using internal standard methods. LabSolutions Ver. 5.80 and Insight (Shimadzu) software packages were used for data processing, which included peak detection, peak area integration, generation of a calibration curve, and evaluation of linearity and accuracy. The results of the automatic peak integration were manually inspected and corrected if necessary.

Statistical analysis

The distribution of each variable was tested for normality using the Shapiro-Wilk test. As previously reported, the platelet aggregation rate assessed by the light scattering method exhibited a skewed distribution¹⁶. The log-transformed values, which followed a normal distribution,

were therefore used for statistical analysis. Values are expressed as median and interquartile range (IQR). Post-shift and baseline hemodynamic and laboratory data, platelet aggregation rates, and serum concentrations of eicosanoids in each subject were compared using paired *t*-tests or the Wilcoxon signed-rank test when the differences were not normally distributed. Correlations between the increase in serum TxB₂ concentration after night-shift work and clinical and laboratory factors were determined by univariate linear regression analysis, or by Spearman's rank correlation when the data were not normally distributed. Independent *t*-tests were used to analyze the effect of gender on the increase in serum TxB₂ concentrations after night-shift work. All hypothesis tests were two-tailed. Differences were considered statistically significant at *p*-value < 0.05. Data were analyzed with IBM SPSS statistics version 22.0 (IBM Corp., Armonk, NY, USA).

Results

Fifteen subjects were selected among the 21 volunteers who expressed interest in participating. All completed the study; however, following the analysis four of the subjects reported that they had taken nonsteroidal anti-inflammatory drugs or antiallergic drugs within the 10 days preceding the study. We therefore excluded them from the analysis. Thus, the final study subjects comprised 11 medical staff (four men and seven women), consisting of 10 medical technologists and 1 physician. None was taking any medications. The median (IQR) age and body mass index was 32 years (29-42 years) and 21.4 kg/m² (19.7-21.8 kg/m²). The median period they had worked night shifts was 8.3 years (IQR, 6-16 years). The median hours slept during night-shift work was significantly less than that at baseline (Table 1). There was no significant difference in any hemodynamic or laboratory variable between baseline and after night-shift work (Table 1 and 2).

Platelet aggregation rates

We initially considered platelet aggregation after stimulation by stirring without agonists (i.e., SPA). SPA is considered to have occurred if SMAX is ≥2.5×10⁴ mV × count¹⁷. We examined the primary aggregation process, as shown by the SMAX and SAUC values, and the overall platelet aggregation rate shown by TMAX, TAUC, and %LT, and compared these parameters between baseline and after night-shift work. At baseline, SPA was negative in all subjects, apart from one with hystero-myoma (data not shown). SMAX and SAUC did not differ between baseline and after night-shift work (Supplementary Table 1). Similarly, there was no significant difference in TMAX, TAUC, or %LT between the two time points (Table 3 and Supplementary Table 1). There was

Table 1. Sleeping hours, hemodynamics, and laboratory data at baseline and after night-shift work ($n = 11$)

Parameter	Baseline median (IQR)	After the shift median (IQR)	Post-shift—baseline mean (95% CI)	<i>p</i> -value
Sleep duration, hour	6.0 (5.0-7.0)	3.0 (4.0-7.0)	3.0 (2.0 to 4.0)	<.001
SBP, mmHg	113.0 (100.0-121.0)	112.0 (101.0-128.0)	0.2 (−7.0 to 7.0)	.954
DBP, mmHg	75.0 (65.0-81.0)	74.0 (69.0-77.0)	0.6 (−5.2 to 6.5)	.814
HR, beats/min	73.0 (64.0-82.0)	65.0 (62.0-72.0)	6.3 (−0.2 to 12.7)	.055
Fasting glucose, mg/dl	90.0 (84.0-96.0)	92.0 (91.0-94.0)	3.0 (−5.0 to 7.0)	.246 [†]
Total cholesterol, mg/dl	188.0 (163.0-235.0)	203.0 (167.0-214.0)	1.0 (−21.0 to 18.0)	.722 [†]
LDL cholesterol, mg/dl	109.0 (99.0-130.0)	118.0 (85.0-136.0)	3.1 (−12.1 to 18.3)	.660
HDL cholesterol, mg/dl	70.3 (60.8-94.6)	79.3 (60.9-93.8)	−1.8 (−5.6 to 2.1)	.336
Triglycerides, mg/dl	57.0 (52.0-86.0)	54.0 (50.0-63.0)	5.3 (−7.5 to 18.1)	.380

SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; LDL, low-density lipoprotein; HDL, high-density lipoprotein; IQR, interquartile range; post-shift—baseline, difference in parameter values between baseline and after night-shift work; CI, confidence interval. [†]Wilcoxon signed-rank test; all other variables were compared using paired *t*-tests.

Table 2. Complete blood count and coagulation tests at baseline and after night-shift work ($n = 11$)

Parameter	Baseline median (IQR)	After the shift median (IQR)	Post-shift—baseline mean (95% CI)	<i>p</i> -value
Leukocyte count, $\times 10^9/L$	5.0 (4.6-5.7)	4.9 (4.6-5.9)	−0.1 (−0.6 to 0.5)	.762
Hematocrit, %	42.6 (38.7-44.3)	40.2 (37.7-42.4)	1.0 (−0.2 to 2.1)	.101
Reticulocyte, %	1.21 (1.04-1.40)	1.14 (1.12-1.40)	0.0 (−0.1 to 0.1)	.927
Platelet count, $\times 10^9/L$	220.0 (203.0-238.0)	225.0 (208.0-232.0)	0.0 (−1.1 to 1.0)	.970
PDW, fL	11.4 (10.7-12.6)	11.8 (10.2-12.6)	0.0 (−0.4 to 0.5)	.854
MPV, fL	10.3 (10.0-10.5)	10.2 (9.5-10.7)	0.0 (−0.2 to 0.3)	.688
IPF, %	4.2 (2.5-4.6)	4.3 (2.6-4.9)	−0.2 (−0.5 to 0.2)	.359
PT, %	106.0 (101.0-115.0)	102.0 (99.0-108.0)	4.1 (−1.1 to 9.3)	.111
aPTT, s	32.9 (31.2-36.3)	33.4 (31.9-36.9)	−0.5 (−1.4 to 0.4)	.225
Fibrinogen, mg/dl	253.0 (204.0-311.0)	242.0 (202.0-308.0)	8.6 (−18.4 to 35.7)	.493

PDW, platelet distribution width; MPV, mean platelet volume; IPF, immature platelet fraction; PT, prothrombin time; aPTT, activated partial thromboplastin time; IQR, interquartile range; post-shift—baseline, difference in parameter values between baseline and after night-shift work; CI, confidence interval. Baseline and after shift median values were compared using a paired *t*-test.

Table 3. Comparison of platelet aggregation rates estimated by the light transmission method between baseline and after night-shift work ($n = 11$)

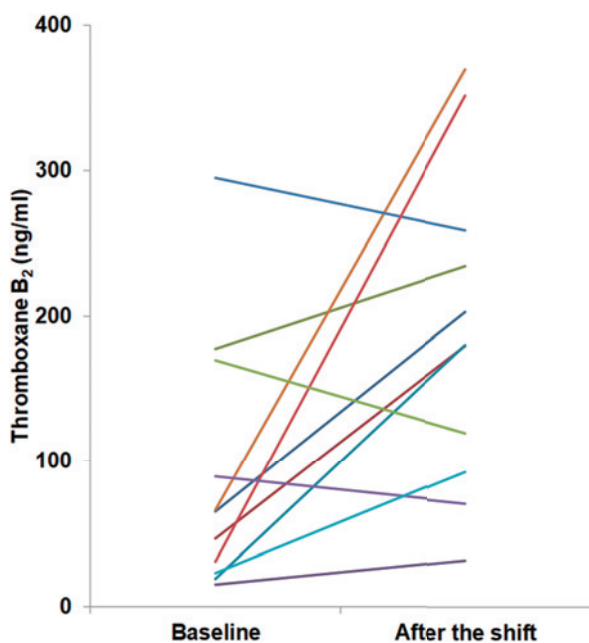
Agonist	Baseline %LT median (IQR)	Post-shift %LT median (IQR)	%LT (post-shift—baseline) mean (95% CI)	<i>p</i> -value
None (SPA)	1 (1-2)	1 (1-1)	0.09 (−0.5 to 0.7)	.756
ADP (0.5 μ M)	17 (7-20)	13 (10-16)	−1 (−13 to 4)	.397 [†]
Collagen (0.1 μ g/ml)	7 (6-16)	7 (5-25)	−1 (−8 to 35)	.553 [†]
Epinephrine (0.1 μ M)	6 (5-22)	7 (6-10)	0 (−56 to 3)	.766 [†]
Epinephrine (0.25 μ M)	10 (6-73)	13 (8-42)	−1 (−60 to 24)	1.000 [†]

SPA, spontaneous platelet aggregation; ADP, adenosine 5'-diphosphate; %LT, percentage increase in the light transmission of platelet-rich plasma relative to that of platelet-poor plasma; IQR, interquartile range; post-shift—baseline, difference in parameter values between baseline and after night-shift work; CI, confidence interval. [†]Wilcoxon signed-rank test. SPA was assessed using a paired *t*-test.

Table 4. Comparison of serum concentrations of major eicosanoid mediators between baseline and after night-shift work ($n = 11$)

Parameter	Baseline median (IQR)	After the shift median (IQR)	Post-shift—baseline mean (95% CI)	<i>p</i> -value
TxB ₂ , ng/ml	65.3 (23.1-169.4)	180.4 (93.5-258.9)	99.7 (14.4 to 184.9)	.026
12-HHT, ng/ml	44.4 (17.2-107.7)	116.7 (67.1-156.9)	57.3 (1.2 to 113.5)	.046
12-HETE, ng/ml	24.0 (11.9-46.5)	32.3 (20.9-80.5)	28.2 (-3.5 to 60.1)	.075
15-HETE, ng/ml	1.8 (1.0-5.3)	4.9 (3.5-8.4)	2.8 (0.1 to 5.4)	.043
11-HETE, ng/ml	2.6 (0.9-5.9)	6.9 (3.8-9.6)	3.6 (0.6 to 6.6)	.025

TxB₂, thromboxane B₂; 12-HHT, 12-hydroxyheptadecatrienoic acid; HETE, hydroxyeicosatetraenoic acid; IQR, interquartile range; post-shift—baseline, difference in parameter values between baseline and after night-shift work; CI, confidence interval. Baseline and after shift median values were compared using a paired *t*-test.

**Fig. 1.** Serum thromboxane B₂ concentrations for each subject at baseline and after night-shift work ($n = 11$)

also no difference in any of these values between baseline and after night-shift work when the samples were stimulated with ADP, collagen, or epinephrine (Table 3 and Supplementary Table 1). This suggests that night-shift work did not affect platelet aggregation rates.

Serum eicosanoid mediators

Serum TxB₂, which is dependent on cyclooxygenase (COX) activity, has been shown to be a useful platelet activation marker¹⁸. In this study, we performed multiplex quantitative analysis of serum eicosanoids, including COX metabolites, using liquid chromatography-tandem mass spectrometry (LC-MS/MS)¹⁹. The results for the major eicosanoid mediators with concentrations greater than 5 ng/ml are shown in Table 4; and the results for all

of the tested mediators are shown in Supplementary Table 2. As expected, we detected relatively high baseline concentrations of the COX-metabolized eicosanoid mediators TxB₂ and 12-HHT in the serum samples. Additionally, we found a high baseline concentration of the 12-lipoxygenase (LOX)-metabolized eicosanoid mediator 12-HETE, which is consistent with 12-LOX expression in platelets.

Following night shift work, serum concentrations of TxB₂ and 12-HHT were significantly elevated over baseline values (Fig. 1 and Table 4). This result contrasted with the lack of change in platelet aggregation reported above. Serum concentrations of other COX-metabolized eicosanoid mediators, such as PGE₂, PGF_{2 α} , and PGD₂ were also significantly higher after night-shift work than at baseline (Supplementary Table 2).

There were no significant relationships between clinical and laboratory parameters and the increase in serum TxB₂ after night-shift work compared with baseline levels (Table 5). Moreover, no significant difference in the increase in serum TxB₂ concentrations after a night shift was observed between males and females (men vs. women, median (IQR) 115.8 (-19.7-281.1) vs. 56.5 (-18.2-137.5) ng/ml, $p = .632$). Other COX-metabolized eicosanoid mediators, 15-HETE and 11-HETE, were also found in serum (Table 4). Importantly, the concentrations of these mediators increased after night-shift work, as was the case with TxB₂ and 12-HHT.

Discussion

To our knowledge, this is the first report to show an acute impact of night-shift work on serum concentrations of eicosanoid mediators. Our study showed no increase in platelet aggregation rate after night-shift work, but found that the concentrations of COX-metabolized eicosanoid mediators in serum increased, primarily due to platelet activation elicited during blood coagulation. This change may partially explain the pathophysiology underlying the

Table 5. Linear regression analysis of the association between clinical and laboratory factors and the increase in serum thromboxane B₂ level after night-shift work (*n* = 11)

Parameter	<i>r</i>	<i>p</i> -value
Age	-.234	.488 [†]
Duration of night-shift work	-.380	.248
BMI	-.350	.291
HbA1c	.318	.341
Sleep duration during shift	.483	.133
Decrease in sleep duration during shift compared to baseline	.053	.877
SBP at baseline	-.485	.131
DBP at baseline	-.558	.074
HR at baseline	.183	.591
Fasting glucose at baseline	.138	.685 [†]
LDL cholesterol at baseline	.105	.760
HDL cholesterol at baseline	.403	.218
Triglycerides at baseline	.082	.811 [†]
Leukocyte count at baseline	-.142	.677
Hematocrit at baseline	.237	.482
Platelet count at baseline	-.286	.394
PT at baseline	.369	.264
aPTT at baseline	.040	.907
Fibrinogen at baseline	-.211	.534

BMI, body mass index; HbA1c, hemoglobin A1c; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; LDL, low-density lipoprotein; HDL, high-density lipoprotein; PT, prothrombin time; aPTT, activated partial thromboplastin time. [†]Spearman's rank correlation; all other samples were assessed using univariate linear regression analysis.

increased cardiovascular risk observed in shift workers.

Many epidemiological studies have sought to elucidate this pathophysiology^{20,22}. Previous investigations^{9-11,23} have shown that impairment of vascular endothelial function is a major contributor to this increased risk. A properly functioning endothelium exhibits antiplatelet activity that prevents platelet aggregation²⁴, whereas an impaired endothelium tends to cause pathologic thrombosis that can lead to myocardial infarction and stroke²⁵. Flow-mediated dilation of the brachial artery, a marker of endothelial function, has been reported to be decreased after night-shift work^{10,23}. However, it has not been established whether platelet activity increases after night-shift work. In this study, we demonstrated that night-shift work did indeed have an impact on platelet function.

First, we examined the effect of night-shift work on the rates of SPA and platelet aggregation induced by a low-dose agonist. SPA and small aggregate formation in low concentrations of ADP or epinephrine have been reported to be elevated in patients with arterial or venous throm-

botic diseases such as anterior ischemic optic neuropathy, retinal vein occlusion²⁶, or acute coronary syndrome^{27,28} due to platelet hypersensitivity resulting from platelet stimulation. Thus, rates of SPA and platelet aggregation induced by a low-dose agonist can be used to document platelet sensitivity to platelet stimuli *ex vivo*. In this study, these rates did not differ between baseline and after night-shift work. Contrary to expectations, these results indicated that platelet sensitivity was not increased after night-shift work when the platelet aggregation rate was used as an index. However, the subjects of this study were healthy, and it remains unclear whether the platelet aggregation rate would be further increased after night-shift work in patients with pathological conditions that impair endothelial function and increase baseline platelet aggregation rate, such as diabetes mellitus²⁹ or hypertension³⁰.

Second, we examined serum concentrations of eicosanoid mediators to evaluate platelet hypersensitivity resulting from the COX pathway. In activated platelets, AA is released from membrane phospholipids and oxygenated by COX-1 to produce pro-aggregatory eicosanoids (mainly TxA₂). TxA₂ is rapidly hydrolyzed to the biologically inactive TxB₂. Serum TxB₂ is mainly produced in the blood collection tube by platelets activated by thrombin, a product of the coagulation cascade. Therefore the concentration of serum TxB₂ is thought to reflect the ability of platelets to synthesize TxA₂ and *ex-vivo* responsiveness to thrombin³¹. In this study, serum concentrations of TxB₂ and other COX-metabolized eicosanoid mediators were higher after night-shift work than at baseline, indicating that platelet sensitivity assessed using these indicators was enhanced after night-shift work. In contrast, no increase in the rate of platelet aggregation induced by a low-dose agonist was observed after night-shift work. In other words, there was a discrepancy between the synthesis of TxB₂ by platelets activated by thrombin during blood coagulation, and the rate of platelet aggregation induced by a low-dose agonist. In this context, it should be noted that TxB₂ biosynthesis and platelet aggregation were observed under different experimental conditions. We examined TxB₂ biosynthesis in whole blood, containing white and red blood cells in addition to platelets, as well as thrombin produced during the coagulation process. Conversely, we examined platelet aggregation in isolated platelets and also in the presence of low-dose ADP, collagen, or epinephrine. The activation threshold for the former pathway may be lower than for the latter pathway. Furthermore, there are TxA₂-dependent and TxA₂-independent pathways associated with platelet aggregatory reactivity. Indeed, a discrepancy has been reported between *ex vivo* platelet aggregation and TxB₂ production in aggregated platelets¹⁸, supported by reports that serum TxB₂ and agonist-induced platelet aggregation did not correlate with each other in patients treated with aspi-

rin^{18,32,33}).

Nevertheless, this study revealed that platelet sensitivity assessed by serum TxB₂ concentration increased after night-shift work. The measurement of TxB₂ concentrations can be performed using commercial kits, and sampling serum specimens is not difficult in daily medical practice. Therefore, the serum level of TxB₂ may be a useful marker of platelet activation in clinical settings.

What are the determinants of platelet hypersensitivity? Amir et al.¹⁰ investigated the influence of night-shift work on brachial artery endothelial function and demonstrated that the decrease in function was independently related to the number of sleeping hours during night-shift work and the length of shift work history. In the present study, we found that neither these factors nor other clinical and laboratory factors such as age, blood pressure, fasting glucose, and LDL cholesterol were associated with an increase in serum TxB₂ concentrations after night-shift work (Table 5). Serum TxB₂ levels decreased after night-shift work in three of the four subjects with the highest baseline levels of serum TxB₂ (Fig. 1). Therefore, the unadulterated effects of night-shift work on serum TxB₂ levels may not have been examined in subjects whose baseline serum TxB₂ levels were already enhanced due to factors that were not investigated in this study. Night-shift work also induces fatigue through psychological or physical stressors. To examine the effect of night-shift work-induced fatigue on brachial artery endothelial function, previous studies had utilized either the self-reported shift difficulty score¹⁰ or plasma levels of epinephrine and norepinephrine¹¹. Nevertheless, further investigations are required to assess the influence of these parameters on platelet function after night-shift work. Night-shift work has also been known to promote not only sleep restriction and fatigue but also disturbances in normal physiological circadian rhythms. Heart rate has been reported to exhibit circadian variation and to decrease during sleep, and then peak during the early morning³⁴. Amir et al.¹⁰ reported that heart rates tended to be lower after night-shift work, suggesting that circadian misalignment had been induced. However, it is not clear whether circadian misalignment caused platelet hypersensitivity in that study. Further study is needed to simultaneously investigate platelet function alongside indicators such as body temperature that can be used monitor disturbances in the circadian rhythm.

In this study, we applied multiplex quantitative analysis using LC-MS/MS to serum eicosanoid mediators, allowing us to assay not only COX metabolites but also a broad range of eicosanoid mediators. Unexpectedly, in addition to TxB₂ serum 15-HETE and 11-HETE were found to increase significantly after night-shift work. Because the serum samples were prepared by coagulation of whole blood, platelets were not necessarily the source of these mediators^{35,36}, and their presence remains to be ex-

plained. However, given a recent report that plasma levels of 15-HETE and 11-HETE were significantly increased in acute coronary syndrome³⁷, these mediators may make novel prothrombotic markers.

This study had several limitations. In accordance with previous studies, agonists were applied at 0.5 μM, 0.1 μg/ml, and 0.1 and 0.25 μM for ADP, collagen, and epinephrine, respectively^{27,28,38}. However, agonist-induced platelet aggregation rates varied among the subjects. To examine whether platelet aggregation was truly activated by night-shift work, further studies that examine platelet aggregation using at least two concentrations of each agonist are required. Additionally, we determined that at least 15 subjects were required to examine the effect of shift work; unfortunately, four of our subjects were later excluded from the study. Moreover, among the major eicosanoid mediators, serum 12-HETE concentrations did not significantly differ after night-shift work in this study. Therefore, further studies with a larger population are needed to confirm the results of the present study. An investigation is also needed to determine whether platelet sensitivity is enhanced not only after night-shift work but also after day-shift work. In this study, we compared platelet function at the beginning of day-shift work and after night-shift work, and measured platelet function in all subjects between 8:00 and 9:00 A.M. to avoid the influence of circadian variation on platelet function. However, platelet aggregation is reported to be decreased in the evening after day-shift work is ended, compared with morning levels^{12,13}. A subject's platelet function may differ between the beginning of a day-shift work day, after day-shift work, and after night-shift work because of circadian variation, but it is important to establish whether stress from work enhances platelet sensitivity. This study focused on the short-term effect of night-shift work on platelet function; further investigation is needed to clarify when platelet function returns to baseline level. Several studies have reported conflicting results regarding the effects of sex differences or menstrual cycles on platelet function³⁹. Consequently, further studies are needed to examine the effect of these factors on platelet function after night-shift work. Furthermore, whether platelet activation also increases after night-shift work in patients with increased baseline platelet function, such as those with diabetes mellitus or hypertension, should also be clarified. The results of such studies would help in establishing appropriate intervals between night shifts according to the comorbidities of staff members.

In conclusion, this study revealed that serum concentrations of COX-metabolized eicosanoid mediators, but not platelet aggregation parameters, increased after night-shift work compared with day-shift work in the same subject. Increased platelet hypersensitivity monitored by serum COX-metabolized eicosanoid mediators rather than platelet aggregation may be related to the mechanisms

that underlie the association between night-shift work and adverse cardiovascular outcomes. Because serum samples can be easily obtained in clinical settings, serum eicosanoid mediators may represent promising prothrombotic markers.

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