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Higher prostaglandin E-major urinary metabolite levels in male versus female students and a small decrease during the menstrual cycle

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Background: Prostaglandin E-major urinary metabolite (PGE-MUM) may be a suitable biomarker to monitor the activity of inflammatory diseases such as ulcerative colitis and interstitial pneumonitis.
Objective: Levels of PGE-MUM were analyzed in male and female students as well as the influence of sex hormones.
Methods: PGE-MUM and sex hormones were measured from void urine and blood samples, respectively, using radioimmunoassay and chemiluminescent enzyme immunoassay kits, respectively, and compared in male and female students. In females, differences in PGE-MUM levels during the menstrual cycle was also analyzed.
Results: Male students showed significantly higher ($P < 0.0001$) PGE-MUM levels than female students. In addition, PGE-MUM levels were significantly lower during the menstrual phase compared to late secretory phase, but the difference was small. In female students, PGE-MUM values were significantly higher in the evening than in the morning.
Conclusions: Prostaglandin E-major urinary metabolite levels were higher in male than female students, with the menstrual cycle showing a slight influence.

Key words: prostaglandin E-major urinary metabolite, sex difference, menstrual cycle, sex hormone

Introduction

Prostaglandin E2 (PGE2) is one of the key molecules of a local inflammatory process and is produced by cyclooxygenase 2 (COX2) activation in active inflammation. Because it is metabolized in a rapid process,[1] it is difficult to measure PGE2 directly in an inflammatory lesion. Prostaglandin E2 is metabolized to 15-keto-PGE2 by 15-hydroxyprostaglandin dehydrogenase (15-PGDH). After conversion, β-oxidation, and ω-oxidation, it is finally excreted as 7α-hydroxy-5,11-diketotetranor-prosta-1,16-dioic acid, i.e., prostaglandin E-major urinary metabolite (PGE-MUM) in urine.[2] Recently, we have developed methods to measure PGE-MUM, such as a radioimmunoassay (RIA)[3] and fully automated chemiluminescent enzyme immunoassay system[4] as well as liquid chromatography/mass spectrometry, which has been used universally.[5] Using these methods, we propose that PGE-MUM is useful as a possible inflammatory biomarker to monitor the activity of inflammatory diseases, particularly ulcerative colitis (UC),[6-9] and interstitial pneumonitis[10] by non-invasive sampling of patients.

Our recent large-scale analysis of healthy people after

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general health checkups revealed that the basal level of PGE-MUM according to an RIA was significantly higher in males than females. It, however, did not differ between genders after menopause since the PGE-MUM level decreased in elderly men and increased in elderly women.[2] This suggests that sex hormones might influence the PGE-MUM level. The number of samples from young adults were not large enough to meaningfully compare the PGE-MUM level between genders. Furthermore, current smokers showed significantly increased levels of PGE-MUM, indicating that smoking induced weak but definite inflammation in the lung.[2] PGE-MUM values among female and male university students were analyzed, and compared with respect to data on the menstrual cycle and serum sex hormones, and any daily variations noted.

Materials and Methods

Samples and data collection

Void urine and blood samples were obtained from all students of Kiryu University and Kiryu University Junior College in the morning (9:00) on the day of annual general health checkups (n = 357 females, mean age ± SD, 20.8 ± 2.8 years; 79 males, 20.7 ± 1.8). The university has schools of nursing, nutrition, and a midwifery program, and the junior college has schools of life science and art. Accordingly, female students were predominant in the present study. Void urine samples were obtained in the afternoon (12:00) and evening (17:00) to observe the difference in PGE-MUM levels in 1 day (139 females, 41 males). Blood samples were obtained from first- and third-year students (e.g., freshmen and juniors). The change in PGE-MUM level during the menstrual cycle was analyzed by referring to documented data obtained from health checkups. Students who could not provide a definite date for their last menstruation were excluded in this analysis. Samples from female students (n = 135) were divided into 5 groups according to the first day of menstruation: (G1, menstruation 1st－5th day; G2, postmenstruation 6th－12th day; G3, 13th－16th day; G4, 17th－25th day; G5, 26th－28th day) (Tables 1 and 2). Smokers (university students) and anyone who took laxatives, anti-allergic drugs, steroid hormones, non-steroidal anti-inflammatory drugs, prostaglandin-related drugs, contraceptives, or steroid hormones were excluded.

Laboratory analyses

After a routine urinary test (protein, sugar, and urobilinogen), PGE-MUM levels were examined using the remaining urine taken from the students. After centrifugation at 1,000 × g for 10 minutes, the supernatants of all urine samples were stored at -60℃ for the analysis of PGE-MUM. After the regular analyses of blood and blood chemistry for health checkups, total estradiol, total progesterone, and total testosterone were examined using the remaining serum. All serum samples were stored -60℃ until the sex hormone analysis.

All urine samples (50 μl) were kept at room temperature for 30 minutes after the addition of 100 μl of 1N NaOH and neutralized with 100 μl of 1N HCl.

Table 1. PGE-MUM samples from female students for each menstrual phase

	Males	Females	Phase	Day	n
Number	79	135	G1 Menstrual phase	1－5	12
			G2 Proliferative phase	6－12	42
			G3 Ovulatory phase	13－16	27
			G4 Secretory phase	17－25	45
			G5 Late secretory phase	26－28	9

Table 2. Serum sex hormone samples available for analysis from male and female students

	Males	Females	Phase	Day	n
Number	39	84	G1 Menstrual phase	1－5	8
			G2 Proliferative phase	6－12	21
			G3 Ovulatory phase	13－16	16
			G4 Secretory phase	17－25	34
			G5 Late secretory phase	26－28	5

PGE-MUM was then assayed using an RIA kit (Institute of Isotopes, Budapest, Hungary) as previously described.[2] PGE-MUM values (ng/ml) were corrected according to the concentration of urinary creatinine (mg/dl) and expressed as PGE-MUM (μg/g creatinine) because the concentration depends on the urinary volume. Serum total estradiol, total progesterone, and total testosterone from students were measured using chemiluminescent enzyme immunoassay kits (Fujirebio, Tokyo) as described previously (SRL, Tokyo).[11]

Data from general health checkups in students included height, body weight, body mass index, high and low blood pressures, urinary protein, urinary sugar, urinary urobilinogen, blood white blood cells, blood red blood cells, hemoglobin, hematocrit, platelet, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, and serum γ glutamyl transferase.

Statistical analyses

Statview software (Abacus Concepts, Berkley, CA, USA) and SPSS version 13.0J (SPSS Japan, Tokyo) were used for statistical analyses. Comparisons between both groups were performed using the Mann-Whitney U test. Because activity scores were not normally distributed, Spearman correlation coefficients were calculated for PGE-MUM and values of other analyzing markers to evaluate relationships. Pearson correlation coefficients calculated from log-transformed data did not differ substantially and are therefore not shown. P values < 0.05 were considered to represent statistical significance.

Ethics

Urine and blood samples, and laboratory data and general health check data from the Wellness Center of Kiryu University and Kiryu University Junior College, were used with the informed consent of study participants. The study was explained verbally to the participants along with printed handouts, after which, they all gave written consent. This study was approved by our Ethics Committee (healthy students: #2,801 in 2016; # 2,901 in 2017;#2,906 in 2018). This study has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. Kazuyuki Umeda, of Fujirebio, measured the PGE-MUM values while blind to the participants' data.

Results

Difference in PGE-MUM levels in healthy male and female students

The PGE-MUM levels (mean 25.7 μg/g Cr) in young males were significantly higher (P < 0.0001) than those in young females (11.4, Figure 1). Differences of PGE-

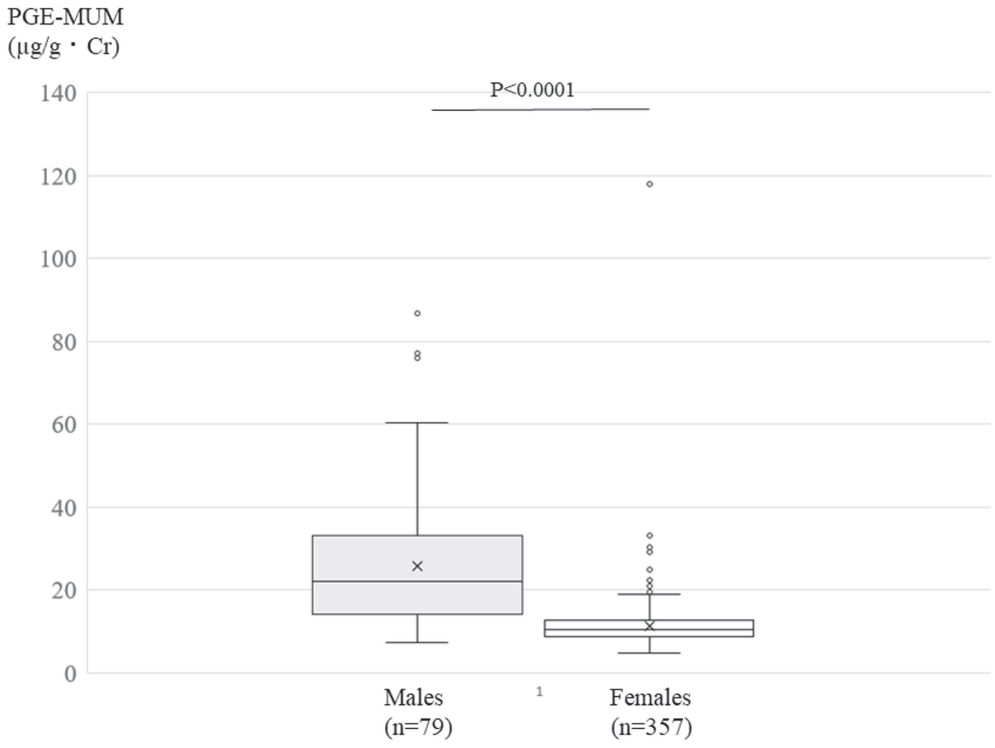

Figure 1. Difference in PGE-MUM levels between male and female students. Void urine samples were obtained at 9:00. PGE-MUM, prostaglandin E-major urinary metabolite

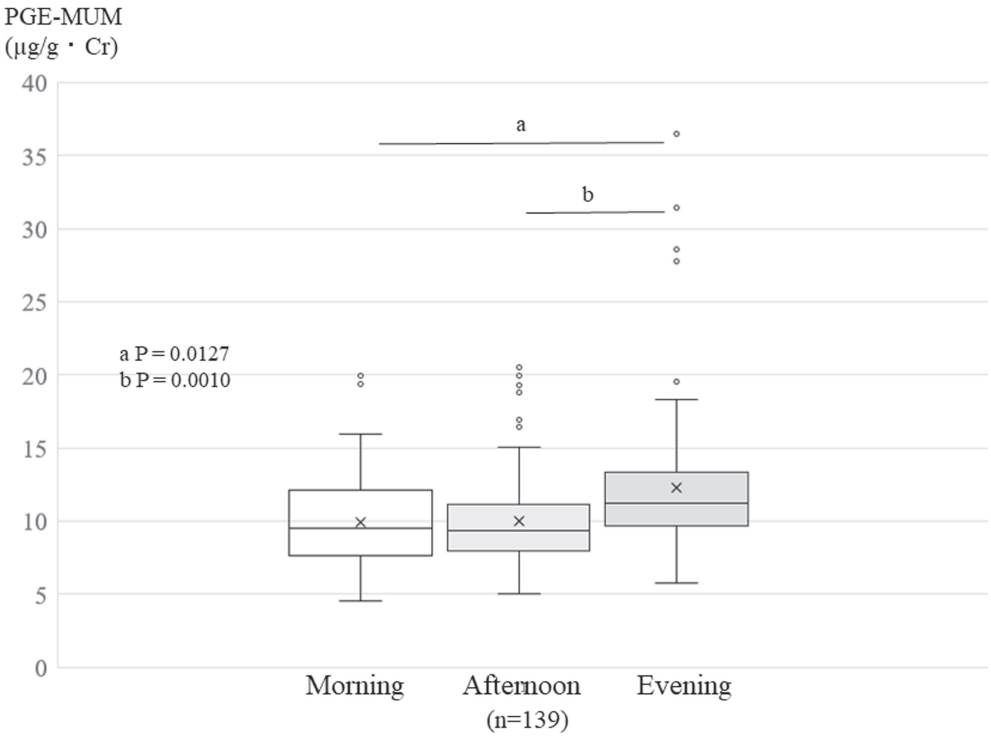

Figure 2A. Difference in PGE-MUM levels over a day in female students. Void urine samples were obtained at 9:00, 12:00, and 17:00. The difference between the 3-time groups was significant ($P < 0.0001$) (Kruskal-Wallis test).

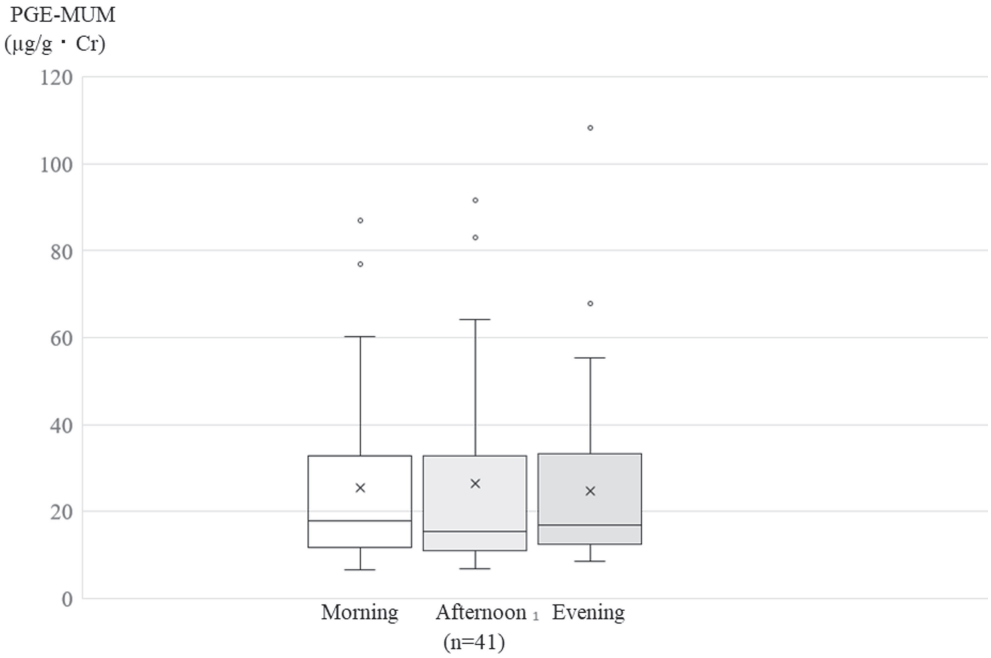

Figure 2B. Difference in PGE-MUM levels over a day in male students. Void urine samples were obtained at 9:00, 12:00, and 17:00. Significant differences were not found between the 3-time groups.

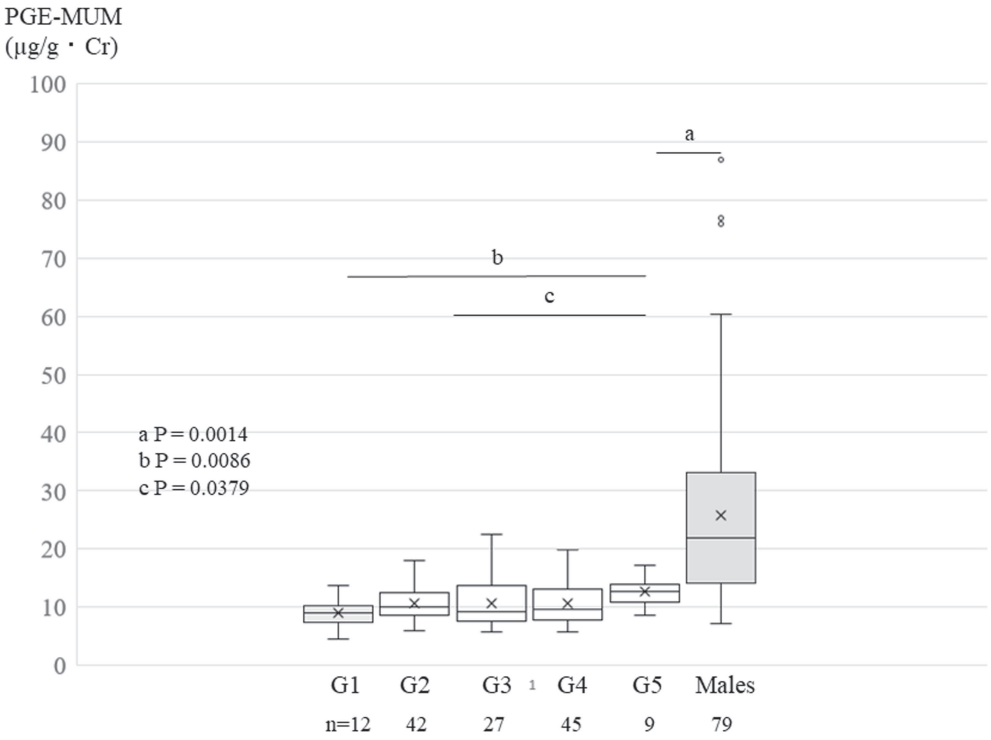

Figure 3A. Difference in PGE-MUM levels during the menstrual cycles of female students (n = 135). Group 1 (G1), Menstruation 1st－5th day; Group 2 (G2), 6th－12th day; Group 3 (G3), 13th－16th day; Group 4 (G4), 17th－25th day; Group 5 (G5), 26th－28th day; Group 6 (G6), male students (n = 79).

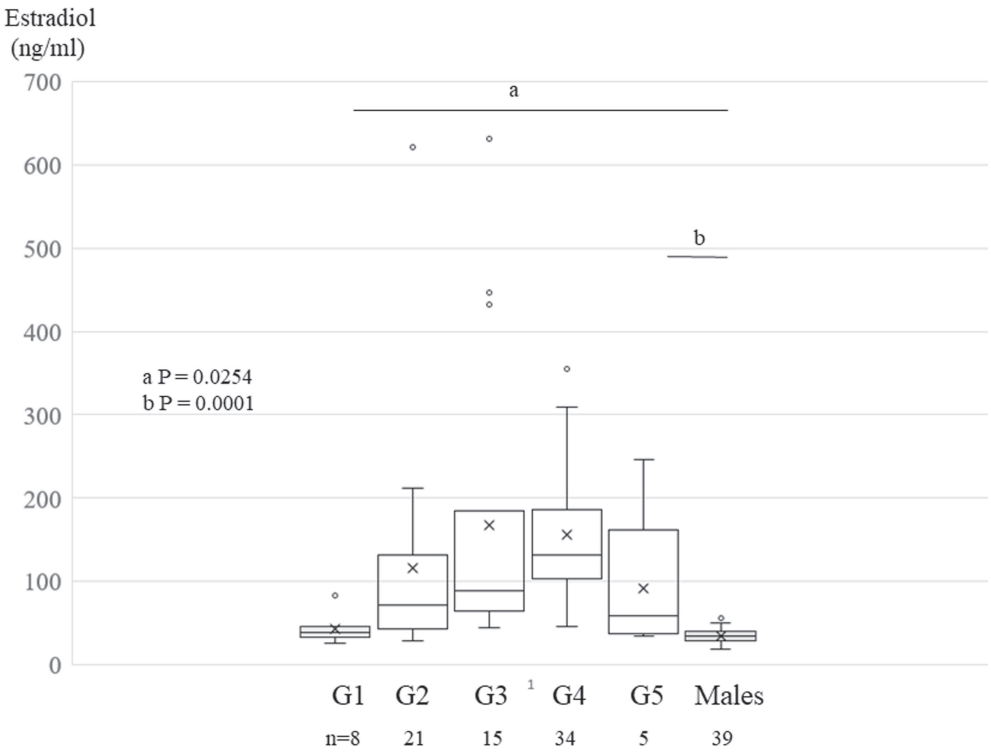

Figure 3B. Difference in serum estradiol levels during the menstrual cycles of female students.

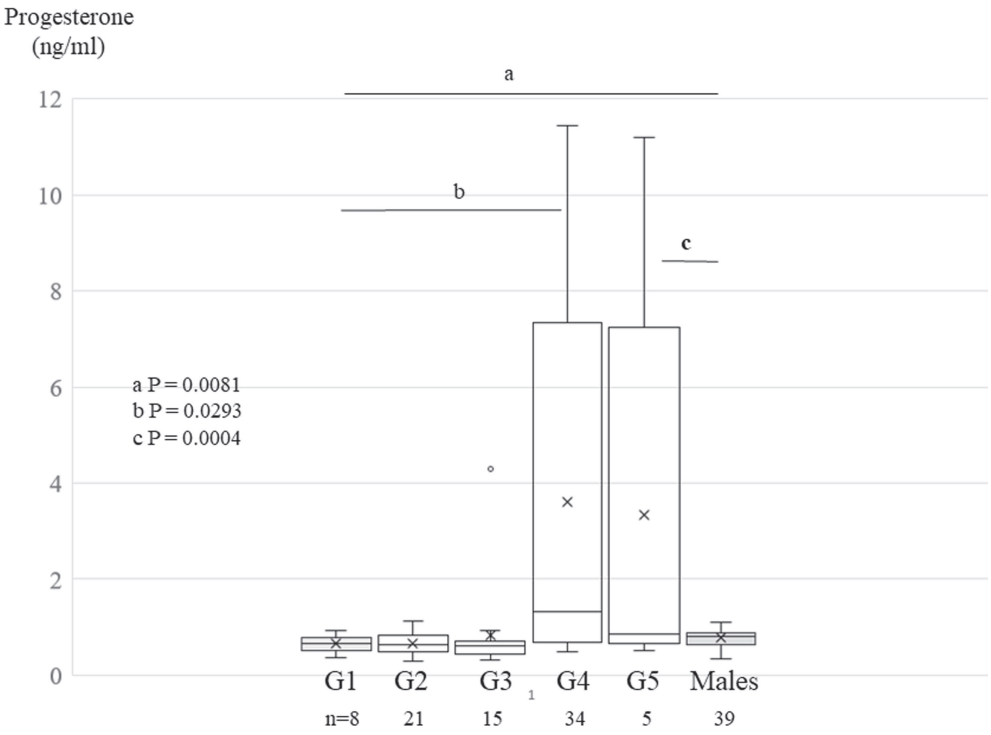

Figure 3C. Difference in serum progesterone levels during the menstrual cycles of female students.

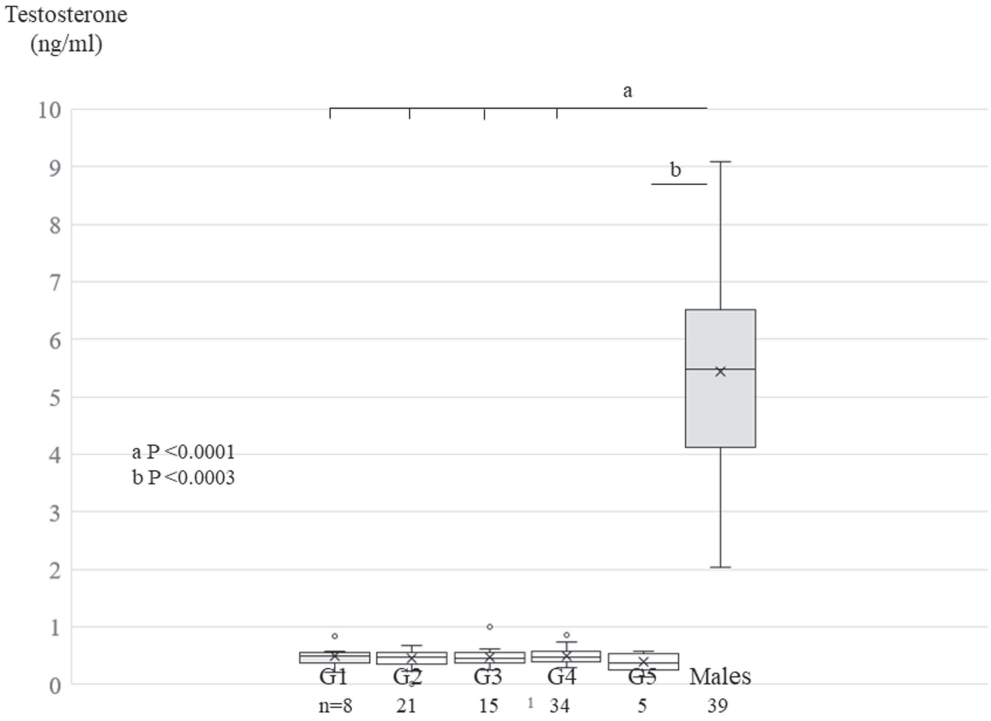

Figure 3D. Difference in serum testosterone levels during the menstrual cycles of female students.

MUM levels in individuals were larger in males than those in females because the standard deviation was significantly larger in males (15.9) compared with that in females (6.8).

The PGE-MUM levels in females were significantly higher in the evening than those in the morning and afternoon, respectively ($P < 0.0001$, Figure 2A), although the difference was small. There were no significant differences in PGE-MUM levels in males between the morning, afternoon, and evening (Figure 2B).

The correlation of the PGE-MUM levels with blood and serum chemistry markers, and serum sex hormones was tested in each gender group. For both females and males, the PGE-MUM levels did not significantly correlate with any markers (data not shown).

Difference in PGE-MUM levels during the menstrual cycle in students

Regarding the relationship of PGE-MUM with female menstruation, the PGE-MUM levels in females were significantly higher ($P = 0.0086$) in group 5 (late secretory phase, 26th－28th day after the start of menstruation) than those in other groups, particularly in group 1 (Menstrual phase, 1st－5th day). I.e., the PGE-MUM level was the lowest in group 1 (Figure 3A). Simultaneously obtained serum showed relatively correct levels of sex hormones during the menstrual phase in females (Figure 3B, 3C). The serum of males showed significantly higher values of total testosterone than did that of females (Figure 3D). Total estradiol and progesterone levels in males were low and almost the same as those of females in group 1 (menstrual phase) (Figure 3B, 3C). The PGE-MUM levels were significantly higher in males than those in females in any phase of the menstrual cycle, even those of group 5 (Figure 3A).

Discussion

Our previous studies revealed that PGE-MUM levels significantly correlated with UC activity as follows. Colonoscopic activity: Mayo score 0 (normal), 3 (moderately active), and 4 (severely active), PGE-MUM 15.9 (mean) ± 6.4 (SD), 48.3 ± 12.3, and 83.1 ± 30.8, respectively); histologic activity: Matts' grading score 1 (normal), 4 (moderately active), and 5 (severely active), PGE-MUM 12.8 ± 4.3, 29.9 ± 9.0, and 76.9 ± 31.0, respectively).[7] Furthermore, PGE-MUM differed significantly in colonoscopic remission vs. non-colonoscopic remission, and histologic remission vs. non-histologic remission.[9] Recently, it has been proposed that mucosal healing (colonoscopic or histologic remission) is more important than clinical remission in clinical care of UC.[7] Children with UC also showed the same tendency of PGE-MUM levels as adult patients, correlating with UC activity.[8] Thus, PGE-MUM seems to be useful for monitoring UC activity, particularly in remission and mildly active phases. Therefore, it is expected that PGE-MUM by the fully automated chemiluminescent enzyme immunoassay system[4] will be adopted as a health care service provided by the national health insurance from the Japanese Ministry of Health, Labor and Welfare. In addition, UC, PGE-MUM levels were significantly increased in patients with chronic fibrosing interstitial pneumonitis, particularly approximately 30 (mean level) in the group of fibrosing score 5－10 on high resolution computed tomography findings.[10] Accordingly, the present study may supply the basic data of young healthy people for the clinical application of PGE-MUM.

The present study revealed and confirmed using large cohorts that the PGE-MUM level was significantly higher in male than in female students, following the results of our previous study.[2] The PGE-MUM level in young females was significantly higher in the evening than in the morning or afternoon, but the difference was not great. The PGE-MUM level may have accumulated due to day activities or hormonal effects. To date, this has been difficult to elucidate. Regarding the PGE-MUM level in males, no significant differences were observed between 3 time points within a day. The PGE-MUM level was significantly higher in young males than in the females, during any stage of their menstrual cycle. In females, the PGE-MUM level was lowest during the menstrual phase. The PGE-MUM level was significantly higher in the late secretory phase, together with an increased level of serum estradiol and progesterone, however the difference was small between the menstrual and late secretory phases. This suggests that sex hormones might have some influence on PGE-MUM,[12-14] but their effects are thought to be small in adolescents.

Correlation analysis performed separately in males or females revealed that other markers did not show a significant correlation with the PGE-MUM level. Accordingly, it is difficult to explain what factors caused the gender difference in the PGE-MUM level in adolescents as observed in the present study. Although PGE-MUM levels in adolescent males were higher than in adolescent females in the present study, a stepwise decrease of PGE-MUM with age was shown in males in the former study. There were no differences of PGE-MUM levels between males and females older than 61

years of age.[2] Regarding the tendency with age, the higher physical activity in young males than that in young females might have some influence on PGE-MUM levels. And regarding other factors, some unknown differences of production and degradation activities of prostaglandin E2 between males and females might cause the difference of PGE-MUM levels. This remains to be clarified.

In addition to our former study, the present study also revealed that individual differences in PGE-MUM levels in males were relatively large due to an unknown mechanism. Although we could not find any significant inflammation during health checks, it may be that the participants with relatively high PGE-MUM levels might have had insidious active inflammation somewhere in their body. With regard to prostaglandin metabolism, PGE-MUM values are related to the activity of 15-hydroxyprostaglandin dehydrogenase (15-PGDH), which degrades PGE2 in local tissues and blood. A mutation of the *15-PGDH* gene has been reported in cases of pachydermoperiostosis or primary hypertrophic osteoarthropathy with high PGE-MUM values.[15] In comparison, multiple, chronic nonspecific ulcers of the small intestine in children and pachydermoperiostosis are caused by a mutation of the prostaglandin transporter encoding gene, solute carrier organic anion transporter family member 2A1 (*SLCO2A1*), a protein that transports prostaglandin into the cytoplasm.[16-19] Furthermore, dysregulation of the PGE2 pathway by genetic variability in the *COX2*, *15-PDH*, and *SLCO2A1* genes may involve tumorigenesis in the stomach and colorectum.[20-23] The reasons for high PGE-MUM levels in healthy people remain unknown.

A significantly higher PGE-MUM level in healthy young males compared with females was revealed, as found previously, despite relatively large individual differences. The effect of sex hormones, and the difference found in the daily PGE-MUM levels, appear to be small, although these may still have some influence.

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Conflicts of Interest: Kazuyuki Umeda, of Fujirebio Inc., the company that has the commercial rights to PGE-MUM tests, measured the PGE-MUM levels blind to any of the participants' data. Isao Okayasu received a research grant for this study from Fujirebio. None of the other authors declare any conflicts of interest.

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