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Effect of the menstrual cycle on serum diamine oxidase levels in healthy women

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ABSTRACT

Objectives: Serum diamine oxidase (DAO) level is employed as a useful marker of intestinal mucosal integrity. As reported previously, the range of serum DAO levels in women is wider than that in men. We hypothesized that the menstrual cycle may affect DAO levels.

Design and methods: Thirty-six women of Japanese descent were recruited. All participants, aged 20–29 years, were healthy. Food surveys utilized in this study were based on questionnaires validated by dietitians. Complete blood counts, biochemical parameters, female hormones, and serum DAO levels during the follicular and luteal phases were measured in each subject.

Results: Biochemical parameters, except for DAO levels, were comparable between the two phases. However, serum DAO levels during the luteal phase were significantly higher than those during the follicular phase.

Conclusions: Serum DAO levels were influenced by the menstrual cycle. Furthermore, our findings suggest that serum DAO levels should be interpreted cautiously in premenopausal women.

Introduction

The gut plays a critical role in the absorption of nutrients and in immune defense. Starvation, sepsis, surgery, and injury result in mucosal atrophy and increased intestinal permeability, indicating damage to the intestinal barrier. Subsequently, impaired absorption of nutrients, bacterial translocation, and multiple organ failure may be induced [1]. Thus, it is very important to clinically evaluate the gut function of patients. For example, an evaluation of intestinal permeability includes gas chromatography analysis of ethylenediaminetetraacetic acid [2] and sugar molecules [3]. However, these tests are poorly used in the clinical environment because of the complicated procedures involved. Moreover, it is difficult to directly measure small intestinal mucosal integrity due to inaccessibility, unlike the more accessible organs such as the stomach or rectum.

Serum diamine oxidase (DAO; ABP1; EC 1.4.3.6) level has often been employed as a useful marker of intestinal mucosal integrity. DAO belongs to the class of copper-containing amine oxidases that convert primary amines to corresponding aldehydes, hydrogen peroxide, and ammonia [4]. DAO is mainly expressed in the intestinal mucosa, kidney, placenta, thymus, and seminal vesicles [5,6]. Among them, DAO activity is particularly high in the upper portion of the small intestinal villi [7–9]. Since DAO levels in the layers of intestinal mucosal villi indicate the function and structure of the small intestine, serum DAO level can be used as a marker to evaluate intestinal mucosa maturation and integrity [10]. Until date, a decrease in serum DAO levels has been reported in various diseases, including severe burns [11], gut injury, diverse enteropathies, abdominal surgery [12,13], and kidney injury [14,15]. Furthermore, a decrease in serum and mucosal DAO activity during cancer chemotherapy has been reported [16,17].

In contrast, García-Martín et al. reported gender-related differences in serum DAO levels, in which women had a wider range of serum DAO levels than men [18]. In addition, DAO is synthesized by decidual and trophoblast cells, leading to high DAO levels during pregnancy [19–21]. Based on these findings, we hypothesized that the menstrual cycle influence serum DAO levels. Thus, we evaluated the effect of the menstrual cycle on serum DAO levels in healthy women.

Materials and methods

Subjects

Thirty-six women of Japanese descent were recruited among the students and staff at Kobe University Graduate School of Health Sciences and Kobe University Hospital. All participants were aged 20–29 years, were healthy, and had regular menstrual cycles and lacked all exclusion criteria. Exclusion criteria were marriage, pregnancy, vaginal infection, use of antibiotics and hormonal contraceptive pills,

Abbreviation: DAO, diamine oxidase.

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and history of any abnormal findings through medical examination. Responses for food surveys and blood samples were collected from each subject during the follicular and luteal phases, which were determined by self-report of the menstrual cycle.

Food surveys involved individual interviews of subjects by dieticians using self-administered 3-day dietary records and using a food frequency questionnaire validated by national registered dietitians of Japan. The subjects did not have any food and fiber restrictions during the study and were requested to maintain their usual dietary habits and normal physical activity. Dietary information was obtained for 3 consecutive days during the follicular and luteal phases. Energy and nutrient content of each diet was calculated using Healthy Maker Pro 501 R5 software (MushroomSoft Co., Ltd., Okayama, Japan). Fasting blood samples were usually collected by antecubital vein puncture from each subject during the follicular and luteal phases. Blood was collected using 10 mL tubes and centrifuged for 10 min at 1500 ×*g* and 4 °C. Complete blood counts were determined immediately after collection. Biochemical parameters and female hormones were measured immediately after centrifugation. Hematological parameters and biochemical assessments were determined in the hospital laboratory using commercially available test kits. In brief, complete blood counts were measured using the XE2100 hematology analyzer (Sysmex, Kobe, Japan). Biochemical parameters, except for prealbumin, were measured using the TBA200FR NEO Automated Analyzer (Toshiba Medical Systems, Tochigi, Japan). Prealbumin was determined by an immunonephelometry method (BN2; Siemens, Munich, Germany). Estrogen and progesterone were measured using the Modular Analytics analyzer (Hitachi, Ltd, Tokyo, Japan). The study protocol was approved by the Ethics Committee of Kobe University School of Medicine and complied with the Declaration of Helsinki. All participants were included in the study after informed consent.

Measurement of serum DAO levels

Blood samples were centrifuged at 3000 ×*g* for 10 min at 4 °C and stored at −80 °C until serum DAO levels were measured by the colorimetric method of Takagi et al. [22]. In brief, DAO catalyzes the substrate cadaverine and DA-67 (Wako Pure Chemical Industries Co. Ltd, Osaka, Japan) is quantitatively oxidized by peroxidase in proportion to the amount of hydrogen peroxide produced, resulting in the production of methylene blue with an absorption maximum at 668 nm. All samples were run in duplicate; the mean DAO levels are reported along with an inter- and intra-assay CV.

Statistical analysis

Data are expressed as mean ± SD. Paired *t*-tests were used to compare the follicular and luteal phases. All statistical analyses were conducted using JMP version 9 software (SAS Institute, Cary, NC, USA). $p<0.05$ was considered to be statistically significant.

Results

Subject characteristics

Table 1 summarizes the characteristics of the subjects included in the study. All participants were healthy and unmarried women of Japanese descent. Their mean age was 23.1 ± 2.3 years, body height was 158.3 ± 4.8 cm, and body weight was 49.9 ± 4.8 kg. The body mass index of all subjects was within the normal range.

Serum levels of female hormones during the follicular and luteal phases

As shown in Fig. 1, serum estrogen and progesterone levels during the luteal phase (135 ± 113 pg/mL and 5.64 ± 6.91 ng/mL, respectively) were significantly higher than those during the follicular phase (67 ± 53 pg/mL and 0.62 ± 0.75 ng/mL, respectively). These results verify that the follicular and luteal phases were correctly identified.

Table 1
Subject characteristics.

	Mean	Range
Age (years)	23.1 ± 2.3	(20–29)
Height (cm)	158.3 ± 4.8	(148.0–165.0)
Body weight (kg)	49.9 ± 4.8	(42.0–63.0)
Body mass index (kg/m^2)	20.0 ± 2.4	(16.6–28.4)

All participants ($n = 36$) were healthy and unmarried women of Japanese descent. Values are mean ± SD.

Food intake

No differences in energy and nutrient intake were observed during the follicular and luteal phases. Furthermore, the ratios of protein to energy, fat to energy, and carbohydrate to energy were comparable between the follicular and luteal phases (Table 2).

Complete blood counts and biochemical parameters

As shown in Table 3, complete blood counts and serum total protein, albumin, prealbumin, cholinesterase, blood urea nitrogen, and creatinine levels were comparable between the follicular and luteal phases. All parameters were within the normal range.

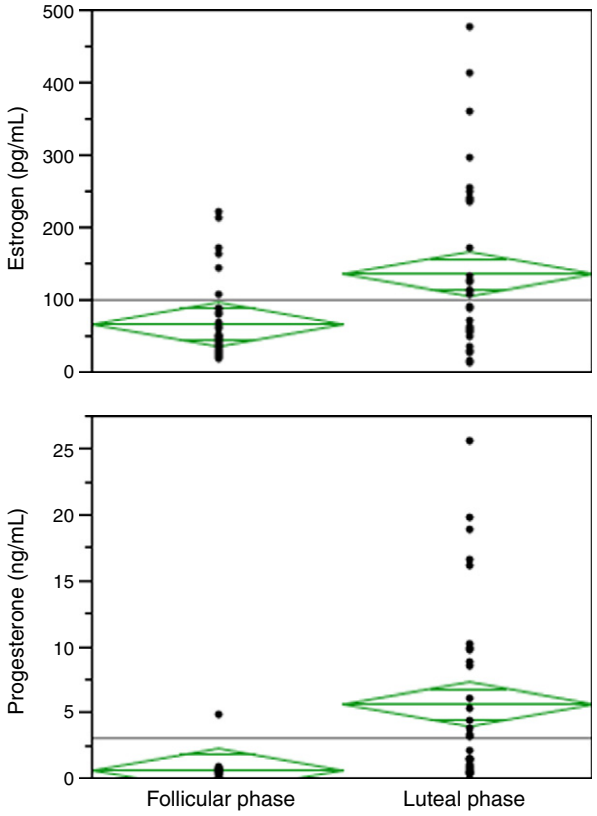

Fig. 1. Serum levels of female hormones during the follicular and luteal phases. Serum levels of estrogen (upper panel) and progesterone (lower panel) during the luteal phase (135 ± 113 pg/mL and 5.64 ± 6.91 ng/mL, respectively) were significantly higher ($p<0.05$) than those during the follicular phase (67 ± 53 pg/mL and 0.62 ± 0.75 ng/mL, respectively).

Table 2
Food intake.

	Menstrual cycle		
	Follicular phase	Luteal phase	*p*
Energy/body weight (kcal/kg)	34±9	34±9	NS
Kcal from proteins (%)	14±2	14±2	NS
Kcal from fats (%)	30±5	31±6	NS
Kcal from carbohydrates (%)	54±6	53±5	NS

Values are mean±SD.
NS, not significant.
p Values obtained from the paired *t*-test.

Serum DAO activity

As shown in Fig. 2 and consistent with previous reports [18], serum DAO levels in healthy women showed a broad range during the follicular and luteal phases, ranging from 2.87 to 8.93 U/l with an average value of 5.92 U/l during the follicular phase and 4.81 to 8.87 U/l with an average value of 6.25 U/l during the luteal phase. In addition, serum DAO levels in each subject during the luteal phase were significantly higher (6.25±0.92 U/l) than those during the follicular phase (5.92±1.20 U/l; $p<0.05$).

Discussion

We confirmed that serum DAO levels in healthy women were significantly higher during the luteal phase than those during the follicular phase. In contrast, food intake, complete blood count, and biochemical parameters such as total protein, albumin, prealbumin, cholinesterase, blood urea nitrogen, and creatinine were similar between the two phases. Taken together, serum DAO levels are closely associated with the menstrual cycle in healthy premenopausal women.

DAO levels are particularly high in the upper portion of the small intestinal villi in humans [7,9]. Therefore, serum DAO level has been used as an index of small intestinal mucosal mass and integrity [23–25]. DAO is localized in the small intestine, kidney, and placenta, with rapid cellular metabolic turnover [22]. Because of this localization, we hypothesized that the menstrual cycle may influence serum DAO levels in healthy premenopausal women. The lining of the uterus thickens during the follicular phase, and the endometrium undergoes changes to prepare for potential implantation of an embryo to establish a pregnancy in the luteal phase. It has been reported that maternal plasma DAO levels increase exponentially during the first 20 weeks of gestation, leading to decreases in circulating maternal histamine levels [19]. DAO levels of ≥500 U/mL have been observed beyond week 20 [20]. After delivery, serum DAO levels decrease to normal non-pregnancy levels within 10–15 days [21]. DAO is located predominately in the cytosol [26] and intercellular spaces [27] of decidual cells. DAO mRNA expression has also been observed in the villous trophoblast and decidual cells [28]. This indicates that DAO is synthesized by decidual and trophoblast cells, leading to high DAO levels during pregnancy.

Table 3
Complete blood count and biochemical parameters.

	Menstrual cycle		
	Follicular phase	Luteal phase	*p*
Red blood cell count (10000/mL)	443±29	447±31	NS
Hemoglobin (g/dl)	12.9±0.8	13.0±0.9	NS
White blood cell count (100/mL)	61±18	67±17	NS
Platelet count (10000/mL)	25±5	25±5	NS
Total protein (g/dl)	7.3±0.4	7.4±0.4	NS
Albumin (g/dl)	4.7±0.3	4.7±0.3	NS
Prealbumin (mg/dl)	26.2±3.8	25.8±4.0	NS
Cholinesterase (U/l)	261±42	266±41	NS
Blood urea nitrogen (mg/dl)	11.4±2.8	11.9±2.7	NS
Creatinine (mg/dl)	0.63±0.07	0.63±0.07	NS

All values are mean±SD.
NS, not significant.
p Values obtained from the paired *t*-test.

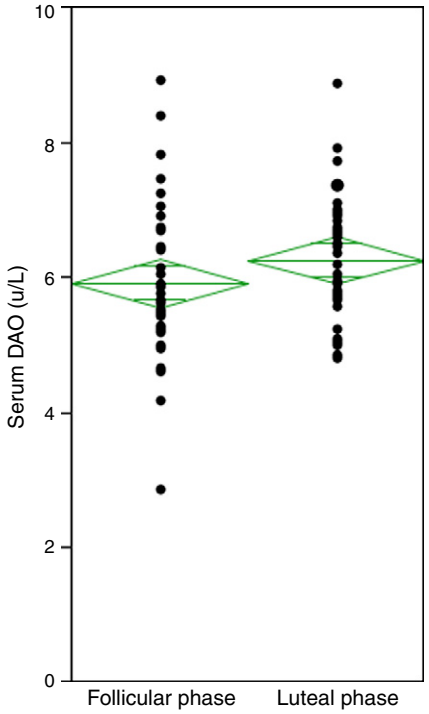

Fig. 2. Serum diamine oxidase (DAO) activity during the follicular and luteal phases. Serum DAO levels in each subject during the luteal phase were significantly higher (6.25±0.92 U/l) than those during the follicular phase (5.92±1.20 U/l; $p<0.05$). Serum DAO activity in healthy women showed a broad range during both phases.

The present study showed that healthy women had a broad range of serum DAO levels during the follicular and luteal phases, ranging from 2.87 to 8.93 U/l with an average value of 5.92 U/l during the follicular phase, and 4.81 to 8.87 U/l with an average value of 6.25 U/l during the luteal phase. Takagi et al. [22] have previously reported that serum DAO levels in healthy 18–21 year old Japanese subjects range from 4.1 to 5.7 U/l, resulting in a mean level of 4.9±0.5 U/l. Klocker et al. evaluated basal DAO levels in 13 men and 15 women and reported no gender-related differences in DAO levels [29]. In contrast, García-Martín et al. reported that mean±SD serum DAO level for 25 women was 7.59±3.67 U/l (range, 1.59–14.0) and that for 25 men was 2.38±0.71 U/l (range, 0.90–4.32) [18]. Consistent with the results of the study conducted by Garcia-Martin et al., a wide range in serum DAO levels in healthy Japanese women was observed in the present study. Our results suggest that the menstrual cycle partially influences serum DAO levels. However, the menstrual cycle by itself is not responsible for the wide range. Differences in endometrium thickening in each subject may influence serum DAO levels.

Our study had some limitations. First, the sample size was small to make definite conclusions, although significant differences in serum DAO levels between the luteal and follicular phases were demonstrated. Based on the present results, sample size for further studies was calculated using JMP version 9 software. Four hundred and eighteen pairs of subjects will be needed for further studies, where the power is 0.8 and probability of Type I error (α) is 0.05. Second, the accuracy of the luteal and follicular phases was verified by serum levels of female hormones. However, the day when blood samples were taken was not compared with the days in each phase. Third, some substances which affect serum DAO levels, such as thyroid hormones and polyamines, were not evaluated. Instead, we confirmed that all participants have no abnormal findings through medical examination. Polyamines are cations involved in the division and regulation of cells and their sources are diet, gut microflora, and cells themselves. Food intake and bowel habits

were at least similar between the luteal and follicular phases. Further research is needed to clarify the cause of this wide range of values in healthy women.

In conclusion, although serum DAO levels is used as a marker to evaluate intestinal mucosa maturation and integrity [10], little information is available regarding basal values in healthy subjects. In addition, it has been suggested that abnormal serum DAO levels in women is not always associated with a pathological status [18]. Our results confirmed that serum DAO levels are closely associated with the menstrual cycle in healthy premenopausal women. Therefore, serum DAO levels should be interpreted cautiously in premenopausal women.

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