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Influence of estrus status on urinary chemical parameters related to urolithiasis

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Introduction

The epidemiological incidence of urolithiasis is two to three-fold higher in men than in women [1], but the reason for this male predominance is obscure. It is well-known that urinary oxalate is one of the most important culprits related to calcium oxalate (CaOx) stone formation and androgen (testosterone) has important roles for its metabolism. Studies of stone forming model, such as rats treated with ethylene glycol, several authors have revealed that administration of testosterone increases urinary oxalate excretion and enhances formation of CaOx stones [2, 3, 4]. Moreover, urinary concentration of lithogenic factors, such as oxalate, is higher in male than in female [5]. On the other hand, it has been indicated that administration of estrogen inhibits the formation of calcium oxalate in vivo study [3, 4, 6]. Thus, female sex hormones may play important roles in protection against stone formation.

Estrogen physiologically affects calcium metabolism by inhibiting bone resorption, increasing calcium absorption from the intestine and enhancing reabsorption in renal tubules. Therefore, urinary calcium excretion increases after menopause [7] and ovariectomy [8]. Moreover, estrogen might be involved in renal citrate handling. Urinary citrate inhibits the development of CaOx stones by forming soluble complexes with urinary calcium and thereby decreasing urinary CaOx saturation. Urinary calcium concentrations are lower in women than in men with stone formers, while urinary citrate concentrations are higher [9]. However, Curhan et al. [10] showed that urinary calcium levels are slightly lower in women than in men with urinary stones, and that urinary citrate did not appear to be affected by gender. However, that study did not consider the age of patients or the estrus status of women. Therefore, the present study examines the urinary chemical parameters related to urolithiasis

in healthy female volunteers during pre-menopause and menopause and discusses the role of menopause in stone formation.

Materials and methods

Participants

Participants comprised 15 childless, pre-menopausal women and 15 menopausal women without a history of urolithiasis provided written informed consent to participate in this study. The pre-menopausal women had normal regular menstrual cycles of about 28 days. Menopause was defined as absent menstruation for 2 years or greater. All participants had normal physical findings and routine laboratory tests without a history of ovariectomy, peptic ulcer, chronic diarrhea, cardiac disease, hyperkalemia, hypermagnesemia or renal dysfunction. None of them were under medication, including estrogen replacement therapy.

Study protocol

All participants measured their basal body temperature (BBT) within 5 minutes of arising every day, using on oral digital BBT thermometer (C502, TERUMO, Inc., Japan). They recorded the BBT on a calendar along with possible influences such as menstrual period, insomnia and symptoms of illness. Regular menstrual cycle was defined as a luteal phase that continued for at least 14 days on the BBT. The pre-menopausal women collected

24-hour urine output every other day during one menstrual cycle, and the menopausal women collected 24-hour urine every other day for about 1 month. During menstruation, all pre-menopausal women used tampons to avoid blood contaminating the urine. The menstrual cycle was divided into 4 phases according to the BBT as follows: Phase I, first half of the lower BBT phase; Phase II, last half of the lower BBT phase; Phase III, first half of the higher BBT phase and Phase IV, last half of the higher BBT phase. We considered that phases I and II essentially corresponded to the follicular phase, whereas phases III and IV corresponded to the luteal phase. The women were instructed to maintain their usual diets, to consume over 1500 mL of water daily and to sleep well. Drinking alcohol and strenuous exercise were strictly prohibited during urine collection. Serum samples after fasting were also obtained once each morning during the study.

24-Hour urine collection

Methods of 24-hour urine collection were described previously [11]. Participants collected the entire void volume at each urination. For analysis, about half of the urine was mixed with 10 ml of 6N hydrochloric acid to determine oxalate, and the other half was mixed with 10 ml of sodium azide to measure urinary pH and determine factors other than oxalate. The samples were stored at 4°C during the experimental period, and total urine volume was determined. All urine samples were clarified by centrifugation for 10 minutes at 750 x g before analysis.

Sample analysis

Urinary citrate and oxalate values were determined using a commercial kit (R-Biopharm GmbH, Inc., Germany) based on an enzymatic method and by capillary electrophoresis, respectively. Urinary pH was measured using a pH meter. Urinary creatinine, calcium, uric acid, phosphorus, sodium, potassium, chloride and magnesium were also determined. Based on 24-hour urinary excretion, we defined hyperoxaluria as > 45 mg excreted, hypercalciuria as > 250 mg, hyperuricosuria as > 750 mg, hypocitraturia as < 320 mg, hypomagnesuria as < 75 mg. The ion-activity product indexes of CaOx (AP (CaOx) index) and calcium phosphate (AP (CaP) index) in urine were calculated according to the formula of Tiselius [12]. Serum creatinine, magnesium, calcium, uric acid and phosphorus were also determined.

Statistical analysis

All data were statistical analyzed using commercially available software (Stat View 5.0 for Windows, SAS Institute Inc., Cary NC). Values are presented as means plus or minus standard error. The Mann-Whitney U-test compared the two groups and each phase between the two groups. A P value of < 0.05 was considered significant.

Results

All pre-menopausal women had a biphasic BBT cycle of which the high phase continued

for at least 14 days. The BBT of the menopausal women did not change for the entire month. The characteristics and urinary parameters in the two groups are listed in Table. Although body weight did not differ between the two groups, body mass index was higher in the menopausal, than in the pre-menopausal group. Hypocitraturia was found in 2 pre-menopausal and in 5 menopausal women. Seven of the pre-menopausal and 9 of the menopausal group had hypomagnesuria. Hypercalciuria was found in 5 menopausal women, but was not found in the pre-menopausal group. None of the women was hyperoxaluric or hyperuricosuric. Calcium, sodium, chloride and potassium excretion was higher, whereas citrate, magnesium and creatinine excretion was lower in the menopausal group. Oxalate and uric acid excretion did not differ between the two groups. The AP (CaOx) and AP (CaP) indexes in the menopausal group were significantly higher than those of the pre-menopausal group. Serum creatinine was significantly higher in the pre-menopausal than in the menopausal group (0.71 ± 0.03 vs. 0.56 ± 0.01 mg / dl). Other serum parameters did not significantly differ between the two groups (data not shown).

To compare with the pre-menopausal group, the study period of the menopausal women group was equally divided into phases I to IV. Mean values of citrate excretion in the pre-menopausal group were 389.9 ± 20.8, 367.8 ± 17.3, 506.7 ± 23.8 and 472.9 ± 22.9 mg/day, during phase I to IV, respectively. On the other hand, citrate excretion in the menopause group did not change during each phase, range 348.4 ± 19.2 to 370.3 ± 23.8 mg/day. Although citrate excretion did not differ between the groups during phases I and II, the pre-menopausal group excreted significantly more citrate than the menopausal group during phases III and IV. We divided the study period into 12 time points to show the curve of citrate excretion (Fig.). Citrate excretion did not change at any point in either group during

phases I and II, but sharply increased at the start of phase III in the pre-menopausal group. The level of excretion remained high until the end of phase III, and gradually decreased during phase IV. Other urinary parameters did not vary during each phase in both groups.

Discussion

Urinary stones predominantly occur in males, indicating that sex hormones are involved in their formation. Although estrus status changes during the menstrual cycle and after menopause, its impact on stone formation has not been clarified. Urinary chemistry in post-menopausal women has been investigated in detail, but most studies focused on calcium metabolism with respect to osteoporosis. Furthermore, few reports have compared urinary parameters between pre-menopausal and menopausal women without a history of urinary stones. Therefore, we investigated 24-hour urine parameters associated with urinary stones and focused upon estrus status in healthy female volunteers.

We found that urinary calcium was higher and urinary citrate was lower in the menopausal, than in the pre-menopausal women. We also found that only citrate excretion among the various parameters varied during the menstrual cycle in the pre-menopausal women. Citrate excretion was higher during the high, than during the low BBT phase. This finding is consistent with previous reports [13, 14, 15]. The comparison between the two groups showed that urinary citrate was identical during the low BBT phase. Yagisawa et al. [16] showed that hypocitraturia is more frequent and citrate excretion is lower in elderly women with urinary stones (above 61 years) than in younger (below 45 years). These results indicated that female sex hormones play an important role in citrate excretion as they do for

calcium excretion. Thus, the age and estrus status of female patients should be considered when investigating urinary chemistry related to urolithiasis.

The reduction in urinary citrate after menopause might be attributable to the estrogenic effect on renal handling. However, this mechanism has not been investigated in detail. Alpern and Sakhaee [17] proposed that renal function decreases with age, causing impaired renal acid excretion and subsequently decreased urinary citrate excretion. It has been reported that citrate excretion was correlated with creatinine clearance [18]. However, in this study 24-hour creatinine clearance did not significantly differ between two groups. Therefore, we considered that renal function was not affect the excretion of urinary citrate in our cases. We showed that the curve of citrate excretion (Fig.) resembled that of progesterone rather than of estrogen secretion during the menstrual cycle. The influence of progesterone on urinary parameters related to urinary stones remain unknown, therefore, the effect of progesterone on citrate metabolism requires investigation.

Oxalate excretion did not differ between the groups and did not change during the menstrual cycle. Yagisawa et al. [19] found in a study of stone patients that urinary oxalate values did not differ between younger (below 59 years) and older (above 60 years) women. These results are supported by the findings of Yoshihara et al. [20] that showed that testosterone enhances glycolate oxidase activity, which catalyzed the synthesis of oxalate in the rat liver, whereas estrogen has little effect on this activity. Conversely, Fan et al. [21] demonstrated that estrogen administration decreases oxalate excretion and the deposition of CaOx crystals in the rat treated with ethylene glycol. Although whether female sex hormones influence oxalate metabolism under experimental conditions remains obscure, our data indicate that female sex hormones do not affect oxalate metabolism under clinical

conditions.

Since stone formation is a multifactorial process, changes in estrus status alone would not affect urolithiasis. Hall et al.[22] suggested that a history of hypertension, low dietary magnesium and insufficient calcium supplementation are risk factors for urinary stones in menopausal women. Mattix Kramer et al.[23] revealed that surgical, rather than natural menopause is associated with an increased risk of stone formation. Probably, rapid decrease of female hormone due to surgery may affect urinary calcium excretion. The present study found that the AP(CaOx) and AP(CaP) indexes were higher in the menopausal, than in the pre-menopausal women. However, all indexes in both groups were within the normal range. This reason may be related to our subjects without history of urolithiasis and without hyperoxaluric or hypercalciuric and we could not directly demonstrate that menopause increases the risk of urinary stone formation. Therefore, further study is needed to determine whether menopause affect stone formation in women with stone formers.

Hormone replacement therapy (HRT) is widely prescribed to reduce symptoms of estrogen deficiency during menopause, and to prevent bone loss and osteoporosis. Although many reports have described the effects of HRT on bone metabolism, few have addressed its influence on urinary parameters related to urinary stones and its preventive effect. Dey et al.[24] showed that urinary citrate and calcium increased, whereas urinary oxalate did not change in menopausal women with urolithiasis given HRT. Heller et al.[25] indicated that urinary calcium significantly decreased, and that citrate insignificantly increased in patients with stones who received HRT compared with those who did not. However, Mattix Kramer et al.[23] revealed that HRT is not associated with incident urinary stones in a cohort study and another author reported that urinary calcium increases after estrogen administration in

menopausal women without urolithiasis [26, 27]. Accordingly, the prophylactic effect of HRT for urinary stones remains controversial. Moreover, because HRT increases the risk of breast cancer and events associated with cardiovascular disease [28, 29], HRT prevention of urinary stones has limitations.

Each value of urinary parameter in our study may be lower than that in other studies. The reason of lower urine volume may be due to cool climate in our region. Moreover, small physique of our subjects may be relevant to these differences. The study limitations are that we examined a small population of normal women without a history of urolithiasis and we assessed estrogen status by only measurement of basal body temperature without measurement of sex hormone in serum or urine. Moreover, we investigated only urinary electrolytes related to urolithiasis and did not examine urinary macromolecules. Urinary macromolecules, such as glycosaminoglycan and osteopontin, has important role for stone formation. Recently, Maroclo et al.[30] revealed that urinary glycosaminoglycan excretion had a biphasic pattern during normal menstrual cycle. Therefore, further examination is required to clarify whether the estrus status of patients with urolithiasis influences associated urinary parameters, particularly urinary macromolecules.

In conclusion, menopausal women might have an increased potential for urinary stone formation compared with pre-menopausal women. Namely, menopausal women have lower citrate and higher calcium excretion, which might enhance calcium stone crystallization. We propose that the estrus status of female patients should be considered when evaluating metabolic abnormalities.

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Figure Legend

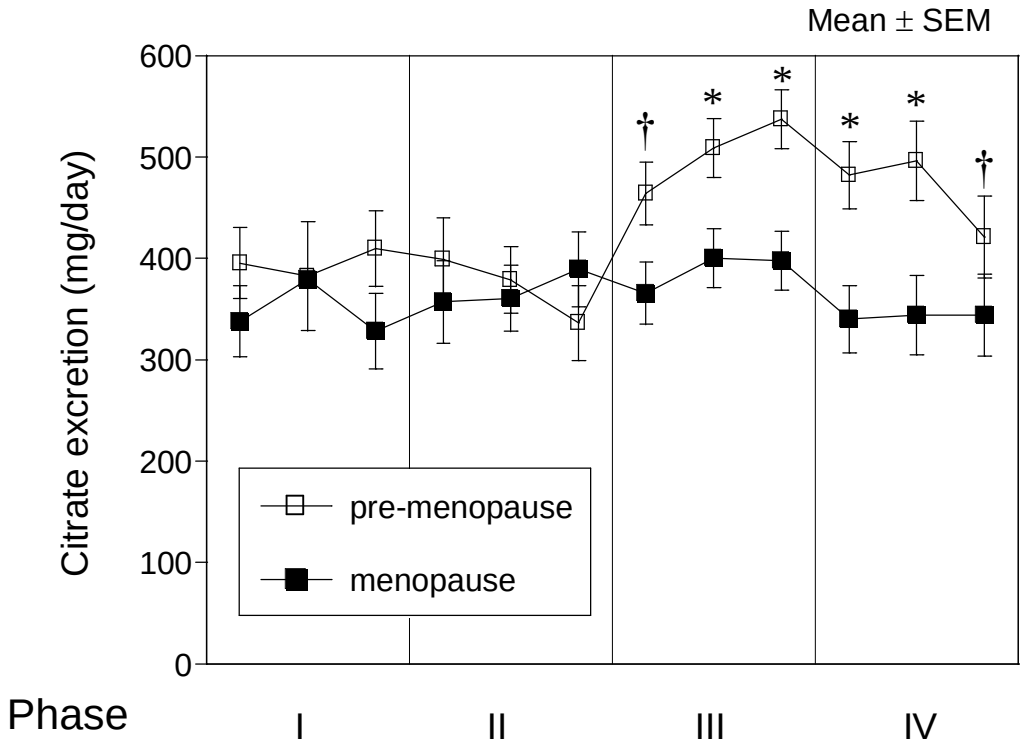
Fig.:

Citrate excretion sharply increased at the beginning of high BBT phase in the pre-menopausal group, and gradually decreased closer to menstruation. Significance of differences determined by Mann-Whitney U-test. *: $P < 0.01$, †: $P < 0.05$

Table: Characteristics and urinary parameters in the pre-menopausal and menopausal groups. Significance of differences was determined by Mann-Whitney U-test. * : $P < 0.01$ compared with pre-menopause. NA : not applicable

	Pre-menopause	**Menopause**
Age (y)	21.7 ± 0.65	54.2 ± 0.56*
Body Weight (kg)	50.2 ± 1.3	51.5 ± 2.2
Height (cm)	159.1 ± 1.4	148.4 ± 1.9*
BMI (kg/m^2)	19.8 ± 0.36	23.3 ± 0.48*
Follicular phase (day)	14.7 ± 0.33	NA
Luteal phase (day)	14.5 ± 0.19	NA
Sample number	188	170
Total volume (l/day)	1.08 ± 0.02	1.21 ± 0.03*
Urine pH	6.26 ± 0.04	6.14 ± 0.03*
Sodium (mEq/day)	129.0 ± 6.2	159.7 ± 4.0*
Potassium (mEq/day)	35.4 ± 0.83	40.6 ± 0.87*
Chrolide (mEq/day)	127.6 ± 3.4	169.6 ± 3.9*
Calcium (mg/day)	115.5 ± 3.3	139.8 ± 3.8*
Phosphorus (mg/day)	546.3 ± 13.3	588.2 ± 13.4
Uric acid (mg/day)	473.2 ± 11.0	431.8 ± 8.0
Magnesium (mg/day)	76.7 ± 2.0	69.8 ± 2.1*
Citrate (mg/day)	430.0 ± 11.2	367.5 ± 9.4*
Oxalate (mg/day)	15.2 ± 0.42	14.5 ± 0.34
Creatinine (mg/day)	987.8 ± 14.5	681.4 ± 10.0*
24-hour creatinine clearance (g/day)	150.2 ± 5.6	147.2 ± 6.2
Magnesium / calcium ratio	0.72 ± 0.02	0.59 ± 0.03*
AP (CaOx) index	0.45 ± 0.02	0.59 ± 0.02*
AP (CaP) index	15.8 ± 0.59	17.5 ± 0.54*

Fig.


Mean ± SEM
Citrate excretion (mg/day)
600
500
400
300
200
100
0
pre-menopause
menopause
Phase
I
II
III
IV