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Influence of estrus status on urinary chemical parameters related to urolithiasis

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Abstract The present study examines the urinary chemical parameters related to urolithiasis in healthy female volunteers during premenopause and menopause, and discusses the role of menopause in stone formation. We investigated 24-h urine parameters associated with urinary stones and focused upon estrus status. Participants comprised 30 healthy women, 15 childless, premenopausal women and 15 menopausal women without a history of urolithiasis. Our results showed that 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.

Keywords Urolithiasis · Chemical parameters · Menopause · Premenopause

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 in calcium oxalate (CaOx) stone formation, and that androgen (testosterone) play an important role in its metabolism. In studies using stone forming animal models, such as rats treated with ethylene glycol, several authors have shown that the administration of testosterone increases urinary oxalate excretion and enhances the formation of CaOx stones [2, 3, 4]. Moreover, the urinary concentration of lithogenic factors, such as oxalate, is higher in males than in females [5]. On the other hand, it has been shown that the administration of estrogen inhibits the formation of calcium oxalate in in vivo studies [3, 4, 6]. Thus, female sex hormones may play an important role in protection against stone formation.

Estrogen physiologically affects calcium metabolism by inhibiting bone resorption, increasing calcium absorption from the intestine and enhancing reabsorption in the 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, thereby decreasing urinary CaOx saturation. Urinary calcium concentrations are lower in female than in male 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, this 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 premenopause and menopause and discusses the role of menopause in stone formation.

Materials and methods

Participants

Participants comprised 15 childless, premenopausal women and 15 menopausal women without a history of urolithiasis who provided written informed consent to participate in this study. The premenopausal women had normal, regular menstrual cycles of about 28 days. Menopause was defined as absence menstruation for 2 years or longer. All participants had normal physical and laboratory findings, and were without a history of ovariectomy, peptic ulcer, chronic diarrhea, cardiac

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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 min of arising every day using on oral digital BBT thermometer (C502, TERUMO, 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 premenopausal women collected 24-h urine output every other day during one menstrual cycle, and the menopausal women collected 24-h urine every other day for about 1 month. During menstruation, all premenopausal women used tampons to avoid blood contaminating the urine. The menstrual cycle was divided into four phases according to the BBT: 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 corresponded essentially 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 1,500 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-h urine collection

Methods of 24-h 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 6 N hydrochloric acid to determine oxalate, and the other half was mixed with 10 ml 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 min at 750 *g* before analysis.

Sample analysis

Urinary citrate and oxalate values were determined using a commercial kit (R-Biopharm, 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-h 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 statistically analyzed using commercially available software (Stat View 5.0 for Windows, SAS Institute, Cary N.C.). Values are presented as means ± standard error. The Mann-Whitney U-test compared the two groups and each phase within and between the two groups. $P < 0.05$ was considered significant.

Results

All premenopausal 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 1. Although body weight did not differ between the two groups, body mass index was higher in the menopausal than in the premenopausal group. Hypocitraturia was found in two premenopausal and in five menopausal women. Seven of the premenopausal and nine of the menopausal group had hypomagnesuria. Hypercalciuria was found in five menopausal women, but was not found in the premenopausal

Table 1 Characteristics and urinary parameters in the premenopausal and menopausal groups. Significance of the differences was determined by Mann-Whitney U-test NA not applicable

	Premenopause	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-h 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*

*$P < 0.01$ compared with pre-menopause

group. None of the women was hyperoxaluric or hyperuricosuric. Calcium, sodium, chloride and potassium excretion were higher, whereas citrate, magnesium and creatinine excretion were 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 premenopausal group. Serum creatinine was significantly higher in the premenopausal than in the menopausal group (0.71 ± 0.03 vs 0.56 ± 0.01 mg/dl). Other serum parameters did not differ significantly between the two groups (data not shown).

For comparison with the premenopausal group, the study period of the menopausal women group was equally divided into four phases, I–IV. Mean values of citrate excretion in the premenopausal group were 389.9 ± 20.8, 367.8 ± 17.3, 506.7 ± 23.8 and 472.9 ± 22.9 mg/day, for phases I–IV, respectively. Citrate excretion in the menopause group did not change with phase, range 348.4 ± 19.2–370.3 ± 23.8 mg/day. Although citrate excretion did not differ between the groups during phases I and II, the premenopausal 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. 1). 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 premenopausal group. The level of excretion remained high until the end of phase III then gradually decreased during phase IV. Other urinary parameters did not vary in either 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 postmenopausal women has been investigated in detail, but most studies have focused on calcium metabolism with respect to osteoporosis. Few reports have compared urinary parameters between premenopausal and menopausal women without a history of urinary stones. Therefore, we investigated 24-h urine parameters associated with urinary stones and focused upon estrus status in healthy female volunteers.

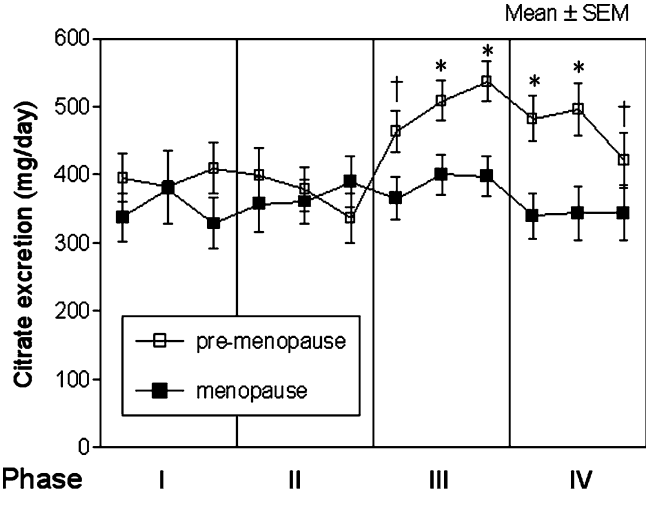

Fig 1 Citrate excretion increased sharply at the beginning of the high BBT phase in the premenopausal group, and decreased gradually closer to menstruation. Mann-Whitney U-test: an *asterisk* indicates $P < 0.01$, a cross $P < 0.05$

We found that urinary calcium was higher and urinary citrate was lower in the menopausal than in the premenopausal women. We also found that only citrate excretion among the various parameters varied during the menstrual cycle in the premenopausal women. Citrate excretion was higher during the high than 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 women (below 45 years). These results indicated that female sex hormones play as important a 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 is correlated with creatinine clearance [18]. However, in this study 24-h creatinine clearance did not significantly differ between the two groups. Therefore, we consider that renal function did not affect the excretion of urinary citrate in our cases. We showed that the curve of citrate excretion (Fig.1) resembled that of progesterone rather than of estrogen secretion during the menstrual cycle. The influence of progesterone on urinary parameters related to urinary stones remains 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] which show that testosterone enhances glycolate oxidase activity, which catalyzes 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 rats treated with ethylene

glycol. 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 a rapid decrease of female hormone level 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 premenopausal women. However, all indexes in both groups were within the normal range. This may be related to our subjects who had no history of urolithiasis and were not 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 affects stone formation in female 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 was significantly decreased, and that citrate was not significantly 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, while 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 urinary parameter value in our study may be lower than that in other studies. The reason for lower urine volume may be due to the cool climate in our region. Moreover, the small physique of our subjects may be relevant to these differences. Our study has limitations in that we examined a small population of normal women without a history of urolithiasis and that we assessed estrogen status only by measurement of BBT without measurement of sex hormones in the 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, play important roles in stone formation. Recently, Maroclo et al. [30] revealed that urinary glycosaminoglycan excretion has a biphasic pattern during the 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 premenopausal women. 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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