

ORIGINAL**Variation of urinary follicle-stimulating hormone level after menopause : From the results of Japan Nurses' Health Study**

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Abstract : The change in follicle-stimulating hormone (FSH) during the menopausal transition and associations of FSH with various diseases have been assessed by using blood samples. We examined cross-sectionally the variation of FSH levels, associations of estrone and estradiol with FSH, and associations of BMI with these hormones by using urinary samples from peri- and postmenopausal women in Japan. Of 4472 participants in the Urinary Isoflavone Concentration Survey of the Japan Nurses' Health Study, we analyzed urinary levels of estrone, estradiol and FSH in 547 women aged from 45 to 54 years. Urinary FSH levels varied widely in postmenopausal women and the pattern of change in urinary FSH levels seems to be similar to that in blood FSH levels in previous studies. There were no significant differences in age, body mass index (BMI), estradiol, estrone and estradiol/estrone ratio among three groups according to the tertile of FSH. In postmenopausal women, there were significant associations of BMI with levels of estrone and estradiol, but there was no significant association of BMI with FSH. Studies using urinary samples will allow us to establish a study project as a large-scale population-based study to determine associations between FSH and various diseases after menopause. *J. Med. Invest.* 66:297-302, August, 2019

Keywords : Urinary FSH, urinary estrogen, menopause, Japanese women

INTRODUCTION

Estradiol level shows a drastic decrease throughout the menopausal transition and is less than the sensitivity level after menopause. Follicle-stimulating hormone (FSH), which is necessary for follicular growth initiation and regulation of ovarian estrogen synthesis, increases in women during the menopausal transition and postmenopausal period through a feedback mechanism, but the level varies from low to high. Tepper *et al.* showed in a longitudinal observation study that the patterns of increase in FSH have three unique trajectories : high rising, medium rising and low rising (1). FSH may play important roles other than follicular growth initiation and regulation of ovarian estrogen synthesis. Recently, various studies have shown extragonadal effects of FSH. A higher FSH level was shown to be associated with an increase in frequencies of hot flashes and night sweats (2) and with an increase in subclinical atherosclerosis progression in women at midlife (3). On the other hand, low FSH levels were shown to be significantly associated with diabetes (4), metabolic syndrome (5) and non-alcoholic fatty liver disease in postmenopausal women (6).

Although changes in estradiol and FSH and associations of these hormonal levels with menopausal symptoms and various diseases have usually been assessed by using blood samples, it is easier to collect urinary samples for large population-based studies. In epidemiological studies, the collection of urinary samples has several advantages compared with blood samples. The

collection of urinary samples is noninvasive and can be conducted by the subjects without the health professional assistance, and urinary samples can be easily stored. There were several studies regarding estrogen and estrogen metabolites by measuring urinary samples (7, 8, 9). However, there have been very few reports on changes in FSH determined by using urinary samples (10). In addition, it has been suggested that differences in race and body mass index (BMI) are involved in the differences in FSH trajectories (1). The variation of FSH levels in Japanese women may be different from that in the previous report in the previous report (1). The change in FSH level and the association of BMI with FSH during the menopausal transition and postmenopausal period in Japanese women have not been clarified.

We have been conducting a survey of the Japan Nurses' Health Study (JNHS) since 2001 as a nationwide prospective cohort study, in which reproductive health-related conditions have been investigated in female nurses (11). A cross-sectional baseline survey was conducted from 2001 to 2007. As a project for determining the associations between FSH and various diseases, we have planned to measure levels of estrogen and FSH by using urinary samples in peri- and postmenopausal women. In the present study, we examined the urinary levels of estradiol, estrone and FSH in women, associations of estrone and estradiol with FSH, and associations of BMI with these hormones.

SUBJECTS AND METHODS*Data collection*

Of 4472 participants in the Urinary Isoflavone Concentration Survey of the JNHS in 2015, we identified 1,752 women whose ages ranged from 45 to 54 years. After excluding 92 women who were receiving hormone therapy, we decided to use data for one third of the remaining women. Thus, urinary levels of estrone,

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estradiol and FSH in 570 non-hormone users who were randomly selected were measured. The details of the study design for JNHS were shown previously (11). We asked about menopausal status, age at the final menstrual period (FMP), cause of menopause (natural menopause, surgical menopause, menopause secondary to radiation therapy or chemotherapy and other therapies), and past history of unilateral/bilateral oophorectomy and hysterectomy. Women in whom the duration from the FMP was less than 1 year were defined as premenopausal women, and women in whom the duration from the FMP was more than 1 year were defined as postmenopausal women. The Ethics Committee of Gunma University reviewed and approved the study.

Measurements of the concentrations of estradiol, estrone and FSH in urine

Participants were given urine collection kits and asked to provide early morning urine specimens immediately after waking up. Urine specimens were basically fasted samples. Participants collected urine by themselves by using the urine collection kits in the morning at home and mailed a urinary specimen to our laboratory at Gunma University. The urine aliquots were stored at -70°C until using in assays. Urinary concentrations of estrone and estradiol were measured by a radioimmunoassay (12). It has been reported that urinary estradiol concentrations were correlated with serum estradiol concentrations, and urinary estrone concentrations were also correlated with serum estrone concentrations (12). Intra-assay and inter-assay coefficients of variation ranged from 7.3% to 11.5% and from 6.9% to 10.6%, respectively, for the estradiol assay, and they ranged from 6.5% to 9.7% and from 9.6% to 11.6%, respectively, for the estrone assay. Sensitivities in the estradiol and estrone assays were 0.20 and 0.40 ng/ml, respectively. Urinary FSH concentrations were measured by a chemiluminescence immunoassay. The concentrations of estradiol, estrone and FSH were adjusted by urinary creatinine. Urinary samples (each 25 µl) were reacted with mouse anti-FSH monoclonal antibodies, which were immobilized magnetism microparticles (50 µl). After removing unreacted materials, acridinium-labeled mouse anti-FSH monoclonal antibodies (50 µl) were added. Unreacted materials were removed and fluorescence intensity at 400-500 nm was mea-

sured after addition of hydrogen peroxide (300 µl). Calibrators were also reacted by the same methods as that used for urinary samples, and we obtained values of FSH based on the obtained standard curve. In a previous study, urinary FSH level was measured by an immunoenzymometric assay (13). The results of a chemiluminescence immunoassay correlate well with the results of an immunoenzymometric assay.

Data analysis

Of the 570 women, 7 women who had received hysterectomy, one woman who had received bilateral oophorectomy, one woman with breast-feeding, and 22 women in whom periods from the FMP were unknown were excluded, and we analyzed data for 547 women. We compared urinary FSH concentrations in premenopausal women and postmenopausal women by using the Mann-Whitney test. We examined differences in age, years since menopause, BMI, urinary estrone and estradiol levels, and estradiol/estrone ratio between the three groups of urinary FSH levels by using the Kruskal-Wallis test. Spearman's correlation coefficient was used to examine the correlations between estradiol level and estrone level, between BMI and estradiol level, between BMI and estrone level, and between BMI and FSH level. $P < 0.05$ was considered statistically significant. All statistical analyses were carried out using SAS ver 9.4 (SAS Institute Inc., Cary, NC).

RESULTS

Ages, BMIs, levels of estrone, levels of estradiol, estradiol/estrone ratios and levels of FSH in pre- and postmenopausal women (< 3 years, 3-5 years and ≥ 6 years) are shown in Table 1. Estradiol levels in postmenopausal women were extremely low, about 1 ng/mg creatinine. The median (25-75 percentiles) of estrone levels in postmenopausal women was 4.47 (3.37-6.17) ng/mg creatinine, which was lower than the estrone level in premenopausal women. The median (25-75 percentiles) of estradiol/estrone ratios in postmenopausal women was 0.27 (0.21-0.36), which was lower than that in premenopausal women. The median FSH level in postmenopausal women was significantly higher

Table 1. Levels of estrone, estradiol, estradiol/estrone and FSH in pre- and postmenopausal women

	Premenopause	Postmenopause		
		< 3 years	3-5 years	≥ 6 years
Number	248	154	102	43
Age (years)	48 (46 - 50)	52 (50 - 53)	53 (51 - 54)	53 (52 - 54)
Years since menopause (years)		1.8 (1.1 - 2.0)	4.0 (3.0 - 4.5)	8.0 (6.2 - 8.0)
BMI	20.9 (19.6 - 22.9)	21.3 (19.6 - 23.6)	21.2 (19.5 - 23.2)	21.1 (19.5 - 22.4)
Estrone (ng/mg creatinine)	11.46 (5.86 - 20.41)	4.84 (3.47 - 6.50)	4.21 (3.46 - 6.13)	3.82 (3.03 - 4.87)
Estradiol (ng/mg creatinine)	6.38 (3.37 - 10.08)	1.29 (0.88 - 1.65)	1.18 (0.87 - 1.64)	0.94 (0.77 - 1.43)
E2/E1 ratio	0.51 (0.37 - 0.64)	0.27 (0.21 - 0.36)	0.28 (0.22 - 0.36)	0.26 (0.20 - 0.32)
FSH (mIU/mg creatinine)	5.1 (2.1 - 13.0)	33.2 (11.5 - 64.9)	40.3 (12.1 - 67.0)	19.3 (7.9 - 53.3)

Values are indicated as medians (25-75%).

E1 : estrone, E2 : estradiol, FSH : follicle-stimulating hormone, BMI : body mass index

than that in premenopausal women ($p < 0.001$). Changes in levels of estradiol, estrone and FSH according to duration since the FMP are shown in Figure 1. In postmenopausal women, FSH levels varied widely from 0.2 to 172.8 mIU/mg creatinine, though levels of estrone and estradiol remained low in most postmenopausal women. Estradiol levels showed significant positive correlations with estrone levels in premenopausal women ($r = 0.93$, $p < 0.01$) and postmenopausal women ($r = 0.67$, $p < 0.01$).

The correlations between estradiol and FSH and between estrone and FSH in postmenopausal women are shown in Figure 2. There was no significant correlation between estrone and FSH ($r = -0.02$, $p = 0.72$) or between estradiol and FSH ($r = -0.01$, $p = 0.92$) in postmenopausal women. FSH levels varied widely even in postmenopausal women with low estradiol and estrone

levels.

We divided the postmenopausal women into three groups, a low FSH group, a middle FSH group and a high FSH group, according to the tertile of FSH (Table 2). There were no significant differences in age, years since the FMP, BMI, estradiol level, estrone level and estradiol/estrone ratio among the three groups in postmenopausal women.

In postmenopausal women, there were significant associations of BMI with estrone ($r = 0.14$, $p = 0.02$) and estradiol ($r = 0.19$, $p < 0.01$), but there was no significant association of BMI with FSH ($r = -0.07$, $p = 0.21$) (Figure 3). In premenopausal women, there was no significant association of BMI with estrone ($r = 0.06$, $p = 0.38$), estradiol ($r = 0.02$, $p = 0.77$) or FSH ($r = -0.08$, $p = 0.24$).

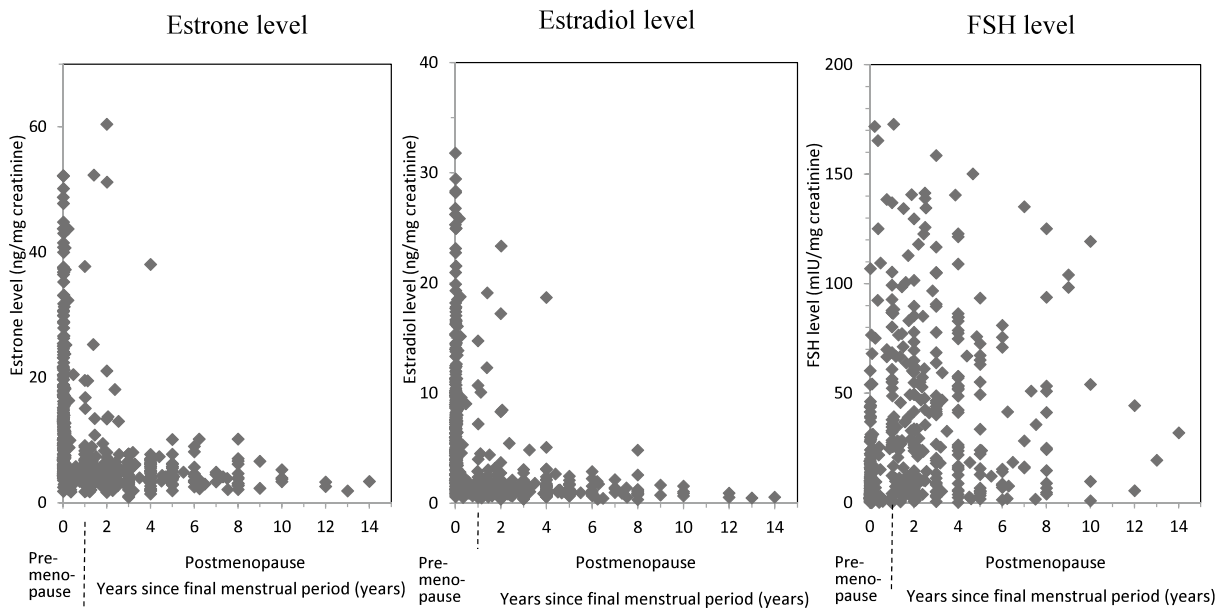


Figure 1. Urinary levels of estradiol, estrone and FSH in pre- and postmenopausal women
Left panel : estrone level, Middle panel : estradiol level, Right panel : FSH level

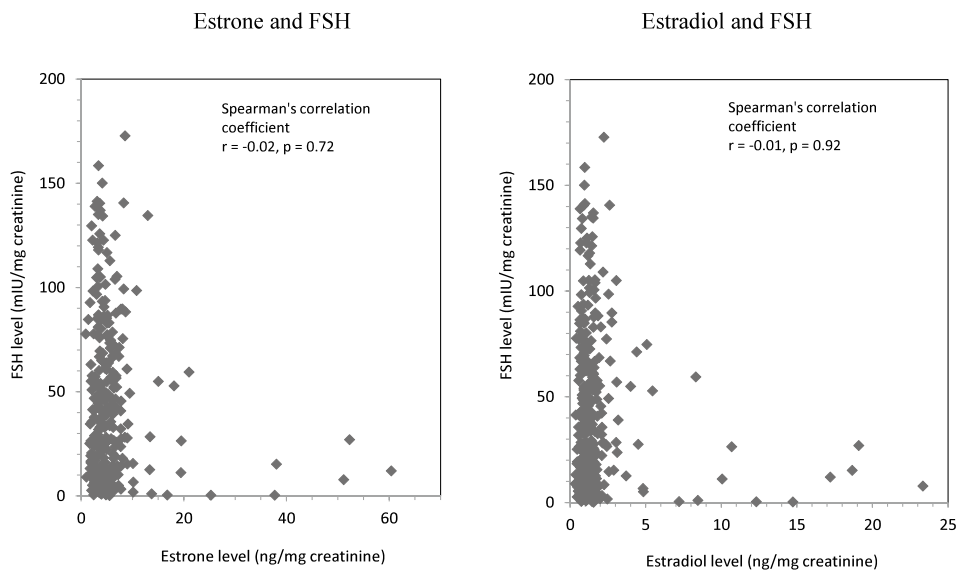


Figure 2. Correlations of urinary FSH levels with urinary levels of estradiol and estrone in postmenopausal women
Left panel : correlation of FSH with estrone, Right panel : correlation of FSH with estradiol

Table 2. Estrone, estradiol and estradiol/estrone ratio according to tertile of FSH levels in postmenopausal women

	Low FSH group (< 16.0) n = 99	Middle FSH group (16.0-53.9) n = 103	High FSH group (≥ 54.0) n = 97	p value*
Age (years)	52 (51 – 53)	52 (51 – 54)	53 (51 – 54)	0.27
Years since menopause (years)	3.0 (2.0 – 5.0)	2.5 (1.4 – 4.0)	2.5 (1.7 – 4.0)	0.13
BMI	21.2 (19.6 – 23.2)	21.0 (19.6 – 24.1)	21.2 (19.2 – 22.9)	0.51
Estrone (ng/mg creatinine)	4.55 (3.33 – 6.17)	4.31 (3.39 – 6.25)	4.47 (3.37 – 6.14)	0.72
Estradiol (ng/mg creatinine)	1.20 (0.81 – 1.70)	1.20 (0.87 – 1.59)	1.20 (0.88 – 1.59)	0.98
E2/E1 ratio	0.27 (0.20 – 0.37)	0.27 (0.22 – 0.36)	0.27 (0.21 – 0.34)	0.78
FSH (mIU/mg creatinine)	6.7 (3.2 – 11.2)	32.9 (25.2 – 42.5)	84.7 (66.7 – 105.0)	

Values are indicated as medians (25-75%).

E1 : estrone, E2 : estradiol, FSH : follicle-stimulating hormone, BMI : body mass index

*Kruskal-Wallis test

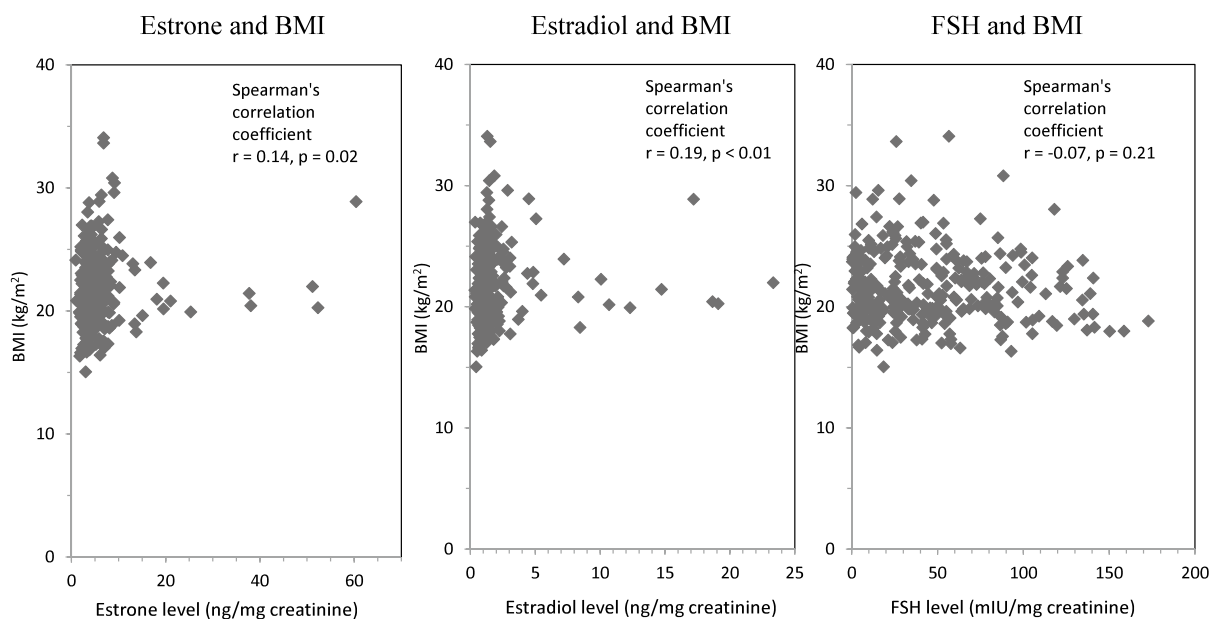


Figure 3. Correlations of urinary levels of estrone, estradiol and FSH with BMI in postmenopausal women
 Left panel : correlation of estrone with BMI, Middle panel : correlation of estradiol with BMI
 Right panel : correlation of FSH with BMI

DISCUSSION

In the present study, we showed that urinary FSH levels in Japanese postmenopausal women varied widely as was shown for blood FSH levels in previous studies, though estradiol levels were lower than the sensitivity level. There have been very few studies in which urinary FSH levels in postmenopausal women were used for research. It has been suggested that urine collection might be preferable in large population-based surveys which study participation rates have been reduced by the requirement of a blood draw (7). It has been reported that measurements using urinary samples were effective and efficient

in a population-level study (10) based on the validations and stability of FSH measurement in urine (13).

Randolph *et al.* reported that increase in FSH from 8 years before the FMP to 8 years after the FMP could be divided into 5 segments : no change, a gradual increase, a marked increase, a slower increase and stabilization (14). Sowers *et al.* reported that FSH level had an acute increase from 2 years before the FMP to one year after the FMP and showed a plateau thereafter (15). In the present study, we showed that urinary FSH levels increased until about 3-5 years after the FMP and that FSH levels in women whose years after the FMP was more than 6 years were lower than those in women whose years after the FMP were

less than 6 years. The pattern of changes in urinary FSH levels seems to be similar with the pattern of changes in FSH levels in blood samples. Tepper *et al.* reported that the variation in FSH trajectories was evident after the FMP and that the FSH levels varied widely from about 25 to 120 mIU/ml (1). In the present study, urinary FSH levels also varied widely from 0.2 to 172.8 mIU/mg creatinine.

Estradiol secretion shifts from the ovary to a compensatory source in fat in postmenopausal women (16). Even if estradiol level is less than the sensitivity level, estrogenic activity may be high in women with high BMI. Obese women tend to have a higher estrone level, lower sex hormone-binding globulin level and higher free estradiol level (5). A more significant correlation between estrone and BMI than that between estradiol and BMI has been found (17). In the present study, the proportion of women whose BMI was more than 25 was only 11.4% and there were significant associations of BMI with urinary levels of estrone and estradiol even in these women. However, we did not find a significant association between FSH level and BMI in postmenopausal women, although urinary FSH levels also varied widely. Tepper *et al.* reported that BMI affected the trajectory of FSH changes over the menopausal transition (1). It has been reported that the change in FSH levels was markedly less pronounced in obese women than in non-obese women (14). Also, it has been reported that weight loss led to an increase in FSH level in overweight postmenopausal women (18). A possible reason for the difference in the results between our study and previous studies is due to difference in BMIs in the subjects. In those previous studies, the proportion of women with high BMI was large. In the SWAN study, the proportions of women in whom BMI ranged from 25 to 29.9 and women in whom BMI was more than 30 were 25.3% and 32.1%, respectively (1). Randolph *et al.* reported that a difference in FSH levels was found in women with BMI between less than 30 and more than 30 (14). Estrone and estradiol may be very sensitive to only a small amount of adipose tissue in women with normal BMI, but FSH may not be sensitive to a small amount of adipose tissue. Another reason is due to the difference in the measurement between blood samples and urinary samples.

In the present study, FSH levels varied from low to high and FSH levels according to tertile were not associated with levels of estrone and estradiol in postmenopausal women. Low FSH levels were shown to be associated with risk of prevalent prediabetes and diabetes (4), metabolic syndrome (5), non-alcoholic fatty liver disease (6) and 10-year atherosclerotic cardiovascular diseases in postmenopausal women (19). The associations of FSH with these diseases may be involved in BMI. On the other hand, it has been reported that a higher level of FSH was associated with an increase in subclinical atherosclerosis progression in women at midlife (3), and women in high and medium FSH trajectory groups had significantly larger intima-media thickness (20). In addition, Randolph *et al.* suggested that FSH levels may be predictive of the frequency of hot flashes in women experiencing vasomotor symptoms (2). A longitudinal study suggested that women with higher BMI have a higher risk of vasomotor symptoms (21). Based on the associations of high FSH with these diseases, the variation of FSH after menopause may be involved in not only BMI but also other factors.

An increase in FSH levels may not indicate only the response to a decrease in estrogen in the pituitary, and FSH may have an independent role. There have been studies on the role of FSH in extragonadal tissues. At first, FSH was reported to have non-gonadal effects such as direct regulation of bone mass (22). It has also been shown that FSH receptors are expressed in the liver (23), adipose tissue (24) and blood vessels (25), providing the basis for the extrareproductive function of FSH. Recently,

the ratio of FSH to estradiol has been used to screen for the occurrence of mild cognitive function in postmenopausal women (26). Extragonadal effects of FSH should be further examined in a large epidemiological study.

We found that urinary FSH levels in postmenopausal women varied widely as do levels in serum FSH samples. Also, the changes in and the pattern of changes in urinary FSH levels were similar to those in serum FSH levels. Based on the results, it is possible to examine the associations between the occurrence of various diseases and urinary FSH levels. It is easier to collect urine samples for large population-based epidemiological studies, and results of studies using urinary samples may contribute to elucidation of the roles of FSH.

A limitation of this study is the use of spot urine samples for hormonal measurements rather than samples of total urine output over 24 hours, although morning first-void urine was used to obtain concentrated urine. The period of urinary collection in all premenopausal women could not be fixed at 1 to 10 days of menstruation. Since the design of the study was cross-sectional, investigation of longitudinal changes in urinary hormonal levels may be needed.

In conclusion, we showed that urinary FSH levels in Japanese women varied widely. Studies using urinary samples will allow us to establish a study project as a large population study to determine associations between FSH and various diseases after menopause.

DISCLOSURE STATEMENT

The authors declare that there is no conflict of interest for this work.

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