



## Original Contribution

# Soy Intake and Urinary Sex Hormone Levels in Preschool Japanese Children

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The authors investigated whether soy intake is associated with sex steroid levels in Japanese children. This cross-sectional study was conducted in autumn 2006. Subjects were substantially healthy preschoolers, 230 boys and 198 girls, aged 3–6 years. Dietary data, including soy intake, were assessed using 3-day dietary records. Each child's dietary intake was controlled for total energy intake using the Willett method (*Nutritional Epidemiology*. Oxford, United Kingdom: Oxford University Press; 1990:245–271). Urinary estrone, estradiol, testosterone, and 5-androstene-3 $\beta$ ,17 $\alpha$  diol levels measured using liquid chromatography–electrospray ionization tandem mass spectrometry, and urinary dehydroepiandrosterone level measured with a radioimmunoassay, were adjusted for urinary creatinine levels. In the analysis of covariance for sex steroids after adjustments for age and body mass index, soy intake was significantly negatively related to estrone and estradiol in boys and positively related to testosterone and 5-androstene-3 $\beta$ ,17 $\alpha$  diol in girls. Isoflavone had a significant tendency to be negatively associated with estradiol in boys and to be positively associated with testosterone in girls. Total energy intake was not associated with any sex steroids in boys or girls. These results suggest that soy intake might affect the secretion or metabolism of sex steroids in childhood and that the effects might differ by sex.

child, preschool; diet records; gonadal steroid hormones; sex characteristics; soy foods

Abbreviations: DHEA, dehydroepiandrosterone; 3 $\beta$ ,17 $\alpha$ -AED, 5-androstene-3 $\beta$ ,17 $\alpha$ -diol.

Soy foods are traditional in Asian diets and are consumed in higher quantities in Asia than in the Western world (1). Since the incidence of breast cancer and prostate cancer is traditionally much lower in Asia than in the West (2, 3), it has been proposed that high intakes of soy or isoflavone might reduce the risk of breast cancer and prostate cancer. Indeed, several epidemiologic studies have reported inverse associations between soy intake and these cancers (4–10), especially among Asian populations.

Evidence that explains the effects of soy intake on breast or prostate cancer is that soybeans are a rich source of isoflavone, which is structurally similar to estrogens. Isoflavone may act as an estrogen agonist or antagonist by binding to estrogen receptors and may affect the human hormone metabolism (11), leading to beneficial health effects. Several epidemiologic studies have investigated the association of soy intake with sex steroid levels in adults (12–28). However, the association has been different for

men and women and, for women, before and after menopause.

Recent studies reported that soy intake during childhood was associated with decreased adult breast cancer risk (29–32). Soy intake early in life may protect against breast cancer later in life, a finding also supported by several experimental studies (33–35). It is possible that soy intake alters the sex hormone metabolism, resulting in the protection against breast cancer. However, even though there have been numerous investigations involving adults, only one pilot study (36) is known to have examined the relation of soy consumption to sex steroid levels in children.

In this study, we assessed the association between dietary soy intake and urinary levels of sex steroids in young Japanese children. Included were estrone, estradiol, testosterone, 5-androstene-3 $\beta$ ,17 $\alpha$  diol (3 $\beta$ ,17 $\alpha$ -AED), and dehydroepiandrosterone (DHEA).

## MATERIALS AND METHODS

### Subjects and design

Subjects were preschool children aged 3–6 years who attended 1 of 2 preschools in Aichi Prefecture, Japan. In autumn 2006, a parent-administered questionnaire about their children's health status, behavior, and lifestyles, including diet, physical activity, and sleeping status, was distributed to parents. Children's height and weight were obtained with this questionnaire. The parents were also asked to record the children's dietary intake for 3 consecutive days; at the same time, they were asked to provide first-void morning urine for the measurement of sex steroids. Of 533 preschool children, 459 (86.1%) agreed to enroll in the study, with their parents providing written informed consent. Ultimately, first-void morning urine, complete dietary records, and parent-reported height and weight were obtained for 428 of the children. This study protocol and the informed consent procedure were approved by the ethical board of Gifu University Graduate School of Medicine, Gifu, Japan.

### Dietary data

To collect nutritional data, diet including soy intake was assessed using 3-day dietary records covering 2 consecutive weekdays and 1 weekend day. The parents received written instructions on recording the food intakes of the children, and they indicated the amount and kinds of foods, beverages, and dishes consumed by their children over 3 days. Because the subjects usually ate a school-provided lunch, we obtained the menus from each kindergarten and checked the quantity of food left over after each meal. Individual nutrient intake was estimated by using the Japanese Standard Table of Food Composition (5th revised and enlarged edition) (37). We calculated daily energy intake in kilocalories and soy consumption in grams per day. Isoflavone intake (milligrams per day) from soy products was estimated on the basis of previously published data on isoflavone concentrations in soy foods summarized by Wakai et al. (38). Isoflavone intake was defined as the sum of genistein and daidzein.

### Sex steroids and other measurements

First-void morning urine samples were collected and kept frozen at  $-80^{\circ}\text{C}$  until sex hormone levels were measured. In 2008, we used the urine to measure estrone, estradiol, testosterone,  $3\beta,17\alpha\text{-AED}$ , and DHEA. Estrone, estradiol, testosterone, and  $3\beta,17\alpha\text{-AED}$  levels were measured with liquid chromatography–electrospray ionization tandem mass spectrometry. DHEA level was measured by radioimmunoassay. All samples were analyzed at the Teikoku Hormone Medical Research Center Co., Ltd. (Tokyo, Japan). The inter-assay coefficients of variation for estrone, estradiol, testosterone,  $3\beta,17\alpha\text{-AED}$ , and DHEA reported by the laboratory were less than 15.3%, 13.1%, 4.3%, 9.3%, and 7.2%, respectively; the minimum levels that the assay could detect for these 5 sex steroids were 1.6 pg/mL, 0.2 pg/mL, 0.2 pg/mL, 1.2 pg/mL, and 0.2 pg/mL, respectively. Each sex steroid was adjusted for urinary creatinine levels.

The height and weight of children were based on parent reports. Body mass index was calculated as weight in kilograms divided by height in meters squared. However, we measured the height and weight of 110 children. For them, intraclass correlation coefficients between measured and parent-reported measurements were 0.90, 0.96, and 0.77 for height, weight, and body mass index, respectively.

### Statistical analysis

We performed all analyses separately for each sex. We controlled soy and isoflavone intake level for total energy intake by using the residual method proposed by Willett (39) after logarithmic transformation. The distributions of sex steroids were skewed and hence were logarithmically transformed.

The numbers and percentages of subjects whose hormone levels were lower than the detectable value were 2 (0.5%), 28 (6.5%), 91 (21.3%), 3 (0.7%), and 8 (1.9%) for estrone, estradiol, testosterone,  $3\beta,17\alpha\text{-AED}$ , and DHEA, respectively. We compensated for the undetectable values by filling those that were less than 1 unit; that is, 1.5 pg/mL, 0.1 pg/mL, 1.1 pg/mL, and 0.1 pg/mL were replaced for the undetectable values of estrone, estradiol,  $3\beta,17\alpha\text{-AED}$ , and DHEA, respectively. Concerning testosterone, we applied the multiple imputation method (40) for the missing data because many subjects had an undetectable testosterone level. The other 4 hormone values (those for estrone, estradiol,  $3\beta,17\alpha\text{-AED}$ , and DHEA) were included in the imputation model to estimate the missing testosterone values, and 5 data sets were created computationally by using the “proc mi” procedure in SAS software (SAS Institute, Inc., Cary, North Carolina).

Characteristics of subjects by sex were calculated as mean (standard deviation). Geometric means and 95% confidence intervals were computed on the log-transformed values and were converted to the original scale of measurement. We divided the subjects into 4 groups according to quartile category (1, 2, 3, or 4) of intake of total energy, soy, and isoflavone. To elucidate the associations of these intakes with sex steroids, we used analysis of covariance for estrone, estradiol,  $3\beta,17\alpha\text{-AED}$ , and DHEA. For testosterone, we used multiple regression analyses for each data set and then the “proc mianalyze” procedure in SAS software (SAS Institute). The analyses were adjusted for age and body mass index as continuous variables. Tests for linear trend were performed on multiple regression analyses using the median values of each category of intake of total energy, soy, and isoflavone.

All analyses were conducted by using the SAS computer program, version 9.1 (SAS Institute). All *P* values were calculated by a 2-sided test. A *P* value of less than 0.05 was considered statistically significant in all analyses.

## RESULTS

The characteristics of the studied subjects (230 boys and 198 girls) are shown in Table 1. The average levels of estrone, estradiol, testosterone, and DHEA in girls were higher than those in boys. Total energy intake was greater

**Table 1.** Characteristics of 428 Studied Preschool Children From Aichi, Japan, 2006

	Boys (n = 230)			Girls (n = 198)		
	Mean (SD)	Geometric Mean	95% CI	Mean (SD)	Geometric Mean	95% CI
Age, years	5.12 (0.89)			5.14 (0.90)		
Height, cm	106.8 (7.1)			106.5 (7.4)		
Weight, kg	17.4 (2.6)			17.4 (2.7)		
Body mass index, kg/m <sup>2</sup>	15.2 (1.3)			15.3 (1.5)		
Urinary sex steroid level, pg/mg creatinine						
Estrone		30.9	27.6, 34.6		85.8	77.4, 95.1
Estradiol		5.8	4.8, 7.0		21.7	18.4, 25.7
Testosterone		53.0	38.9, 72.3		69.9	53.9, 90.8
5-Androstene-3 $\beta$ , 17 $\alpha$ -diol		78.7	69.3, 89.4		71.0	60.0, 84.1
Dehydroepiandrosterone		64.9	50.8, 82.9		179.3	146.4, 219.5
Diet						
Total energy intake, kcal/day		1,432.5	1,397.9, 1,468.0		1,350.9	1,316.9, 1,385.7
Soy intake, g/day		24.4	21.7, 27.4		22.8	20.0, 26.0
Isoflavone intake, mg/day		12.2	11.0, 13.6		11.4	10.2, 12.8

Abbreviations: CI, confidence interval; SD, standard deviation.

for boys than for girls. The geometric means of soy intake were 24.4 g/day for boys and 22.8 g/day for girls. Isoflavone intake was highly correlated with soy intake (Spearman's correlation coefficients = 0.91 for both boys and girls).

Table 2 shows the estimated means of urinary sex steroid levels according to quartile category of dietary intakes of total energy, soy, and isoflavone after adjustments for age and body mass index. Total energy intake was not associated with any sex steroids in boys or girls. Soy intake was negatively associated with estrone (linear-trend  $P = 0.013$ ) in boys. Similarly, the estradiol values for the boys with higher soy intake were significantly lower (linear-trend  $P = 0.026$ ), and there was also an association between isoflavone and estradiol with a significant trend (linear-trend  $P = 0.037$ ). For boys, testosterone, 3 $\beta$ ,17 $\alpha$ -AED, and DHEA were not associated with soy intake. For girls, those with higher soy intake had statistically significantly higher testosterone levels (linear-trend  $P = 0.003$ ). Isoflavone had a similar association with testosterone (linear-trend  $P = 0.002$ ). The 3 $\beta$ ,17 $\alpha$ -AED values for the girls with higher soy intake were significantly higher (linear-trend  $P = 0.027$ ), and the linear association between isoflavone and 3 $\beta$ ,17 $\alpha$ -AED was borderline significant (linear-trend  $P = 0.088$ ). Estrone, estradiol, and DHEA were not associated with soy intake by girls. Separate analyses for 2 major isoflavones, genistein and daidzein, showed similar results; the negative association of intake of each isoflavone with estrone and estradiol was shown for boys, and the positive association of intake of each isoflavone with testosterone and 3 $\beta$ ,17 $\alpha$ -AED was shown for girls.

To determine whether imputations for limit of detection influenced the results, we replaced limit of detection/2 and 0.0001 for the values below the limit of detection. The associations between soy intake and sex steroids were not substantially altered, although the replacement of 0.0001 weakened the negative association of soy intake with estrone (linear-trend  $P = 0.20$ ) and estradiol (linear-trend  $P = 0.065$ ) for boys. In addition, we conducted subanalysis after excluding 63 boys and 28 girls whose testosterone levels were less than the detectable value. The association of soy and isoflavone intakes with testosterone was not altered substantially for boys or girls. We also excluded 12 boys and 6 girls whose testosterone values were greater than the absolute value of the 75 percentile plus 3 times the interquartile range (>574.1 pg/mL for boys and >869.2 pg/mL for girls). The positive association of soy and isoflavone intakes with testosterone for girls remained.

We repeated the same analyses by including kindergarten as a confounder. After the additional adjustment, a negative association between isoflavone and estrone with a significant trend emerged for boys (linear-trend  $P = 0.015$ ). The results for girls were not altered. In addition, when height was replaced with body mass index as the confounder, the associations between soy intake and sex steroids were not altered substantially for either boys or girls.

## DISCUSSION

To our knowledge, this study is the first to demonstrate the association between dietary soy intake and urinary sex

**Table 2.** Urinary Sex Steroid Levels According to Quartile of Dietary Intake of Total Energy, Soy, and Isoflavone Among 428 Preschool Children From Aichi, Japan, 2006

Dietary Item and Intake Quartile	Boys							Girls						
	No.	Median Intake <sup>a</sup>	E1 <sup>b</sup>	E2 <sup>b</sup>	T <sup>b</sup>	3 $\beta$ ,17 $\alpha$ -AED <sup>b</sup>	DHEA <sup>b</sup>	No.	Median Intake <sup>a</sup>	E1 <sup>b</sup>	E2 <sup>b</sup>	T <sup>b</sup>	3 $\beta$ ,17 $\alpha$ -AED <sup>b</sup>	DHEA <sup>b</sup>
Total energy														
1	58	1,156	33.3	5.5	104.2	99.3	60.8	50	1,118	83.7	19.8	65.1	69.8	169.3
2	57	1,384	26.0	5.5	69.5	64.3	58.0	49	1,298	75.3	21.1	66.0	70.5	182.6
3	58	1,551	33.1	5.0	74.7	83.7	74.0	50	1,454	87.8	20.7	73.7	59.8	151.6
4	57	1,735	31.8	7.4	55.8	71.5	67.9	49	1,642	97.9	25.8	75.7	86.6	221.4
<i>P</i> -trend			0.91	0.42	0.18	0.19	0.62			0.24	0.33	0.68	0.53	0.49
Soy <sup>c</sup>														
1	58	8.3	37.7	7.5	61.1	95.4	58.4	50	7.9	88.7	19.9	39.5	54.4	148.0
2	57	19.9	34.1	7.1	55.6	78.6	61.0	49	19.7	81.3	24.3	62.6	66.4	140.8
3	58	34.9	26.1	4.9	33.7	64.6	54.5	50	33.1	79.9	21.5	79.4	76.4	232.3
4	57	60.9	27.2	4.3	69.5	79.3	91.7	49	53.7	94.2	21.5	123.0	92.4	213.2
<i>P</i> -trend			0.013	0.026	0.81	0.15	0.31			0.86	0.79	0.003	0.027	0.096
Isoflavone <sup>c</sup>														
1	58	5.1	35.6	7.1	74.5	97.9	62.9	50	4.7	87.2	18.9	36.8	56.6	158.0
2	57	10.6	33.0	7.4	49.6	76.9	61.4	49	10.2	85.9	25.1	69.1	67.7	163.8
3	58	15.6	28.0	5.1	53.4	71.9	62.1	50	16.4	86.1	23.1	78.7	81.5	193.2
4	57	26.5	27.7	4.1	39.9	70.7	74.0	49	24.0	84.0	20.4	120.7	81.5	206.9
<i>P</i> -trend			0.078	0.037	0.60	0.063	0.67			0.82	0.70	0.002	0.088	0.30

Abbreviations: DHEA, dehydroepiandrosterone; E1, estrone; E2, estradiol; T, testosterone; 3 $\beta$ ,17 $\alpha$ -AED, 5-androstene-3 $\beta$ ,17 $\alpha$  diol.

<sup>a</sup> Total energy: kcal/day; soy, g/day; isoflavone, mg/day.

<sup>b</sup> Estimated geometric mean after adjustments for age and body mass index.

<sup>c</sup> Soy and isoflavone intakes were adjusted for total energy intake by the Willett method (39).

steroid levels in young, substantially healthy children. Our results showed that, for Japanese children aged 3–6 years, soy intake was significantly negatively related to estrone and estradiol in boys and positively related to testosterone and 3 $\beta$ ,17 $\alpha$ -AED in girls. This finding suggests that soy intake might affect the secretion or metabolism of sex steroids in childhood. The effects of soy on sex steroids might differ in boys and girls.

The association of soy intake with sex steroid levels remains equivocal, even for adults. Some studies have demonstrated the association among premenopausal women (12–17), postmenopausal women (18), and men (19, 20). However, others have failed to find it (21–27). Recently, Hooper et al. (28) systematically reviewed 47 intervention studies. Their meta-analysis suggested that soy or isoflavone consumption did not affect estrone and estradiol in premenopausal women and that there was a small, statistically nonsignificant increase in estradiol related to soy or isoflavone in postmenopausal women.

Only the pilot study by Maskarinec et al. (36) evaluated the possible effects of soy on sex steroid levels in 20 young girls aged 8–14 years. These authors conducted a soy intervention (approximately 30 mg of isoflavones per day) for 8 weeks and observed nonsignificant increases in total androgens and total estrogens and a nonsignificant decrease in pregnanediol during the study period. The discrepancy in the results between our study and theirs may be partly

explained by the characteristics of the subjects or the sample size, but habitual intake of soy for a long time may influence sex hormone levels, which was different from the short-term effects.

It is possible that soy inhibits the action in aromatase, which promotes conversion of androgens to estrogens, resulting in decreased levels of estrogens in boys with a high soy intake and increased levels of androgens in girls with a high soy intake. The isoflavone has been shown to have weak inhibitory activity on aromatase in experimental studies (41, 42). The effects of soy on sex steroid metabolism might vary according to background steroid level, leading to the different association between soy and sex steroids by sex. Indeed, the levels of estrogens and androgens other than 3 $\beta$ ,17 $\alpha$ -AED were much higher in girls than in boys in our study. Although the results for girls might be contrary to what we had expected regarding a protective effect against breast cancer, it is not currently clear whether sex hormone levels in childhood play a role in developing breast cancer later in life. Actually, the biologic meaning of variability in these hormones at these ages is not known, and the function of the hormones themselves is still obscure.

In addition, the association with sex hormones seemed to be weaker for isoflavone intake than for soy intake. This finding might suggest the possibility that certain components other than isoflavone are involved in the effects of soy. It is also possible that the estimates of isoflavone from

soy were accompanied by relatively more error than those for soy intakes.

A strength of our study is that we tried to measure minute amounts of sex hormones in prepubertal children. Sex hormone levels in latency-age children are so low that most hospital laboratories do not offer routine measurement. Therefore, we used liquid chromatography–electrospray ionization tandem mass spectrometry, which is also highly compound specific, to measure estrone, estradiol, testosterone, and  $3\beta,17\alpha$ -AED. The interassay coefficients of variation for estrogens and androgens were, at most, 15%, which reflects good accuracy. This very sensitive and expensive method made it possible to measure minute amounts under the detectable level in a commercially routine assessment. When we applied 10 pg/mL, 10 pg/mL, and 40 pg/mL as the limits of detection of estrone, estradiol, and testosterone (43, 44), respectively, which are common in routine assessments, the numbers and percentages of subjects under the detectable value became 30 (7.0%), 182 (42.5%), and 208 (48.6%), respectively. In this situation, we could not find the association between soy intake and sex steroid levels. Our results showed higher levels of androgens than estrogens during prepuberty in boys and girls. This finding was concordant with those of 2 previous studies, which showed hyperandrogenicity in early puberty among girls (36, 45).

One limitation of our study is the use of spot urine to measure sex steroids. However, this was a more practical and noninvasive way to collect samples from healthy preschool children than obtaining blood samples or 24-hour urine samples. Another limitation was that height and weight were reported by the parents. However, the differences between parent-reported and measured values ranged from 0.08 cm to 0.76 cm for height and from  $-0.11$  kg to 0.27 kg for weight among 170 first-grade and 206 fourth-grade Japanese children (46). The correlation coefficients ranged from 0.90 to 0.96 for height and from 0.95 to 0.99 for weight. In our supplementary analysis of 110 children, the intraclass correlation between parent-reported and measured height and weight was high. Therefore, these differences would not greatly change the associations we observed. Finally, generalizability of our study is limited because our subjects were ethnically homogeneous Japanese children, whose diets differ from those of Western children. Our subjects consumed relatively high levels of soy foods compared with many other populations. Infants in Japan are weaned onto soy products at 6–12 months of age, after which they continue to receive soy foods (47). The observed associations between current soy intake and urinary hormone levels may be partially ascribed to their relatively high prior exposure to soy.

In conclusion, we demonstrated the association between dietary soy intake and urinary sex steroid levels among substantially healthy young children. This association suggested the possible effects of soy foods on the secretion or metabolism of sex steroids in childhood and that the effects of soy on sex steroids might differ in boys and girls. Although soy foods are traditional in Asian diets, the incidence of breast cancer has been rapidly increasing in Asia; concurrently, there has been an extraordinary change in dietary habits. Further studies will be needed to confirm the

effects of soy on sex steroids early in life as well as on the development of breast and prostate cancers later in life.

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