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Urinary isoflavonoids and risk of coronary heart disease

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Background Whether soy food consumption may protect against coronary heart disease (CHD) remains controversial. No previous study has used biomarkers of soy intake in assessing the relationship between soy consumption and CHD. Biomarkers that reflect both intake and metabolism may be more informative than self-reports of dietary intake.

Methods We examined associations of urinary isoflavonoids, a biomarker of soy or soy isoflavone intake, with risk of CHD in a case–control study nested within two prospective cohort studies of Chinese adults in Shanghai. Cases were defined as subjects with no history of CHD at baseline who developed incident CHD during follow-up. Control subjects were randomly selected from those who remained free of CHD and matched to cases by sex, age, date and time of sample collection and antibiotic use. Baseline urinary isoflavonoids (daidzein, genistein, glycitein, equol, O-desmethylangolensin, dihydrodaidzein and dihydrogenistein) were compared between cases ($n = 377$) and control subjects ($n = 753$). Conditional logistic regression was used to evaluate the associations.

Results Total urinary isoflavonoids were not associated with CHD in either women or men. However, urinary equol excretion showed a significant inverse association with CHD in women. The adjusted odds ratios (95% confidence intervals) for CHD across increasing quartiles of equol levels in women were 1 (reference), 0.61 (0.32, 1.15), 0.51 (0.26, 0.98) and 0.46 (0.24, 0.89) ($P = 0.02$ for trend).

Conclusions Our study suggests for the first time that equol, a bioactive metabolite of soy isoflavone daidzein, may be inversely associated with risk of CHD in women.

Keywords coronary disease, isoflavones, soy foods

Controversy remains over the US Food and Drug Administration-approved health claim on soy protein and coronary heart disease (CHD) prevention.[1] The health claim approval was made in 1999 primarily on the basis of data from clinical trials demonstrating cholesterol-lowering effects of soy protein products.[2] A review of more recent trials on soy products, however, concluded that the lipid benefits conferred by soy are small.[3] In addition to lipids, soy products have also been shown in some, but not all, clinical

studies to have potential beneficial effects on blood pressure, vascular function, glucose metabolism, inflammatory biomarkers and metabolic syndrome.[4–13] Very few prospective epidemiological studies have directly evaluated the association between soy consumption and incidence of cardiovascular disease (CVD), and the findings have not been consistent across studies.[14–16] Furthermore, previous studies have exclusively relied on food-frequency questionnaires (FFQs) for dietary assessment; none of these studies has used biomarkers in the assessment of soy exposure.

Soy foods are rich in isoflavones, a major class of phytoestrogens known to have a range of hormonal and non-hormonal activities that may be protective against CVD.[17] Studies have shown that there is substantial inter-individual variability in the absorption and metabolism of soy isoflavones.[18,19] Because biomarkers of soy intake, such as urinary isoflavonoids, provide an integrated measure of intake, absorption and metabolism, they may be more informative than FFQs.[18,19] We measured urinary isoflavonoids and examined their associations with risk of CHD in a nested case–control study within the Shanghai Women's Health Study (SWHS) and the Shanghai Men's Health Study (SMHS).

Methods

Study population

The SWHS and the SMHS are prospective, population-based cohort studies conducted in typical urban communities of Shanghai, China. Both studies were approved by the institutional review boards of all institutes involved, and informed consent was obtained from all participants. The designs and methods used in the two studies are similar and have been described in detail previously.[20,21] Briefly, the SWHS recruited 74 941 women aged 40–70 years (participation rate 92.7%) from 1996 to 2000, and the SMHS recruited 61 491 men aged 40–74 years (participation rate 74.1%) from 2002 to 2006. In both studies, recruitment activities, including baseline surveys and anthropometric measurements, were carried out at participants' homes by trained interviewers. Information was obtained using structured questionnaires on demographics, diet and other lifestyle habits, medical history and other characteristics. Biospecimens were also collected at baseline in both studies, including blood samples (sample collection rates 76% in SWHS and 75% in SMHS) and spot urine samples (sample collection rates 88% in SWHS and 89% in SMHS). All samples were processed within 6 h of collection and stored at −70°C. Cohort members were followed through biennial home visits (overall response rate 96% in both cohorts).

Selection of CHD cases and control subjects

Subjects who were eligible for this nested case–control study included members of both cohorts who provided biospecimens and reported no history of CHD, stroke or cancer at baseline. Cases were defined as subjects with incident CHD, including both non-fatal myocardial infarction and fatal CHD, that occurred after the baseline survey but before the end of 2009. Potential cases were identified through follow-up interviews. The diagnosis of myocardial infarction was confirmed through medical record review, using the WHO criteria (i.e. symptoms plus either diagnostic electrocardiographic changes or elevation of cardiac enzyme levels).[22] Death from CHD was confirmed by reviewing medical records whenever possible, referring to death certificates and interviewing the next of kin. During a mean follow-up of 10 years, 536 cases were identified in the SWHS cohort, and during a mean follow-up of 5 years, 559 cases were identified in the SMHS cohort. After excluding subjects who had no urine samples for assays, reported history of CVD or cancer at baseline, or for whom medical records were not available, 195 incident cases of CHD from the SWHS and 182 cases from the SMHS were finally included in the present study. Cases included in the present study were slightly older than those not included. No difference was found between cases included in the present study and those not included with regard to the level of education, BMI, cigarette smoking or history of hypertension or diabetes. For each incident case of CHD, two control subjects were selected from the eligible cohort members who were free of CHD at the time of diagnosis of the index case. Control subjects were matched to cases by sex, age, menopausal status (women only), date and time of sample collection and antibiotic use in the past week. Using these criteria, 389 control subjects were selected from the SWHS cohort and 364 control subjects were selected from the SMHS cohort. All cases had two matched control subjects, except for one case with only one control subject.

Laboratory analyses

Isoflavonoids, i.e. daidzein, genistein, glycitein, equol, O-desmethylangolensin (O-DMA), dihydrodaidzein and dihydrogenistein, were measured in urine samples collected at baseline, using high-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (model TSQ Ultra Thermo Fisher, Waltham, MA).[19] The intra-assay and inter-assay coefficients of variation for the analytes were as follows: 6.7% and 10.2% for daidzein, 9.2% and 10.9% for genistein, 9.1% and 13.4% for glycitein, 2.4% and 3.1% for equol, 10.1% and 6.7% for O-DMA, 11.8% and 20.3% for dihydrodaidzein and 9.3% and 8.2% for dihydrogenistein, respectively. The detection limits were 10 nM for equol and 5 nM for all other analytes. Concentrations of urinary isoflavonoids

were adjusted for urinary creatinine concentration and expressed in nanomoles per mg of creatinine. Urinary creatinine concentrations were measured on a Roche Cobas Mira Plus chemistry analyser, using a test kit based on kinetic modification of the Jaffé reaction (Randox Laboratories, Crumlin, UK). Plasma total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides were measured on ACE Clinical Chemistry System (Alfa Wassermann, West Caldwell, NJ). Low-density lipoprotein (LDL) levels were calculated by using the Friedwald equation. To control for batch-to-batch variation, samples for each case–control set were analysed in the same laboratory run. All analyses were performed in a blinded fashion.

Statistical analysis

Analyses were conducted separately for women and men. Of the total 1130 subjects, one subject had undetectable value for dihydrodaidzein, five subjects for dihydrogenistein, eight subjects for O-DMA and 110 subjects for equol. These undetectable values were replaced by a random number between zero and the lower limit of detection of the analytes. Total urinary isoflavonoids were estimated by summing the individual isoflavonoids measured. Urinary isoflavonoid data were highly skewed and thus were logtransformed. Generalized linear models were used to examine differences in mean values of logtransformed isoflavonoids between cases and control subjects. Conditional logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) of CHD associated with levels of urinary isoflavonoids and to adjust for potential confounders. Subjects were divided into quartiles using batch-specific cut points, which were based on the distribution among control subjects in each batch. The lowest quartile was used as the reference group. Covariates included in the analyses were age, education level, family income, cigarette smoking, alcohol consumption, BMI, waist-hip ratio, amount of regular exercise, intakes of total energy, saturated fat, fruit and vegetables, hormone therapy (women only) and history of hypertension or diabetes. Additional adjustment for urinary creatinine levels did not materially alter the results; creatinine levels were not included in the final models. Analyses were also conducted to additionally adjust for cholesterol levels in subjects with blood lipid measurements ($n = 515$, 88% of total women; $n = 461$, 84% of total men). Tests for linear trend in risk across categories of urinary isoflavonoids were performed by modelling the categories as ordinal variables in the models. Statistical analyses were performed by using SAS statistical software (version 9.2; SAS Institute Inc, Cary, NC). All statistical tests were based on 2-sided probability.

Results

The baseline characteristics of study participants by case–control status are shown in Table 1. In women, the prevalence of smoking, hypertension and diabetes at baseline was higher among individuals who developed CHD (cases) during follow-up compared with those who did not (control subjects). BMI, waist-hip ratio, total cholesterol and LDL cholesterol at baseline were also higher in cases than in control subjects, whereas total energy intake and HDL cholesterol level were lower in cases than in control subjects. No difference was found between cases and control subjects with respect to levels of education or family income, exercise or dietary intakes of saturated fat or soy protein. Similar results were found for men.

No significant differences in geometric means of urinary excretion of total and individual isoflavonoids were found between cases and control subjects in either women or men (Table 2). Excretion of several isoflavonoids, particularly equol, appeared to be somewhat lower in cases than in control subjects. The geometric mean ratios of equol for cases vs control subjects were 0.77 in women and 0.70 in men.

Adjusted ORs and 95% CIs of CHD associated with urinary excretion of total isoflavonoids and individual isoflavonoids are summarized in Tables 3 and 4 for women and men, respectively. Total urinary isoflavonoid excretion was not associated with the development of CHD in either women or men. Among individual isoflavonoids, equol excretion was significantly and inversely associated with CHD in women. The fully adjusted ORs (95% CIs) for CHD across increasing quartiles of equol levels in women were 1 (reference), 0.61 (0.32, 1.15), 0.51 (0.26, 0.98) and 0.46 (0.24, 0.89) ($P = 0.02$ for trend). In men, none of the individual isoflavonoids showed significant associations with CHD.

Discussion

In this nested case–control study conducted within two prospective, population-based cohort studies, total urinary isoflavonoids did not appear to be related to the risk of CHD in either women or men. Urinary excretion of equol, however, showed a significant inverse association with risk of CHD in women.

To our knowledge, this is the first epidemiological study that has examined urinary isoflavonoid excretion in relation to risk of CHD. Our results on urinary isoflavonoids and CHD do not provide evidence to support a role for total soy isoflavones in CHD prevention. However, the finding of a significant inverse association between equol and CHD in women, although this needs to be further confirmed, provides support for the hypothesis that equol may be the key to the potential health effects of soy.[23–25] Equol is a product of intestinal bacterial metabolism of isoflavone daidzein.[23–25] Equol has been shown to be

Table 1 Baseline characteristics of cases and control subjects

	Cases	Control subjects	P^a
Women			
No. subjects	195	389	
Age (years), mean ± SD	61.6 ± 7.4	61.4 ± 7.3	Matched
Education ⩾ high school, %	21.0	23.9	0.40
Annual family income ⩾ 20 000 yuan, %	31.8	35.7	0.32
Cigarette smoking, %	9.7	4.9	0.03
Alcohol consumption, %	3.6	3.1	0.75
Hormone therapy, %	1.0	0.8	0.75
Body mass index (kg/m^2), mean ± SD	25.7 ± 3.8	24.9 ± 3.6	0.01
Waist-hip ratio, mean ± SD	0.85 ± 0.06	0.83 ± 0.06	0.0001
Regular exercise (hour/week), mean ± SD	3.2 ± 4.5	2.8 ± 3.7	0.30
Hypertension, %	51.3	31.1	< 0.0001
Diabetes mellitus, %	17.4	9.0	0.003
Total energy intake (kcal/day), mean ± SD	1595 ± 426	1669 ± 415	0.05
Saturated fat intake (g/day), mean ± SD	7.6 ± 4.3	8.0 ± 4.4	0.33
Fruit and vegetable intake (g/day), mean ± SD	463 ± 274	519 ± 311	0.03
Soy protein intake (g/day), mean ± SD	9.3 ± 6.9	9.7 ± 7.5	0.55
Total cholesterol (mg/dl), mean ± SD	194 ± 42	183 ± 34	0.005
LDL cholesterol (mg/dl), mean ± SD	110 ± 35	102 ± 27	0.007
HDL cholesterol (mg/dl), mean ± SD	42.8 ± 9.5	45.1 ± 11.2	0.02
Men			
No. subjects	182	364	
Age (years), mean ± SD	62.9 ± 8.4	62.8 ± 8.4	Matched
Education ⩾ high school, %	49.5	54.4	0.24
Annual per capita family income ⩾ 12 000 yuan, %	44.5	46.7	0.63
Cigarette smoking, %	67.0	58.0	0.03
Alcohol consumption, %	35.2	31.9	0.45
Body mass index (kg/m^2), mean ± SD	24.5 ± 3.2	23.8 ± 3.1	0.008
Waist-hip ratio, mean ± SD	0.91 ± 0.05	0.90 ± 0.06	0.03
Regular exercise (hour/week), mean ± SD	3.3 ± 5.3	3.4 ± 4.9	0.88
Hypertension, %	64.8	38.2	<0.0001
Diabetes mellitus, %	13.7	9.6	0.14
Total energy intake (kcal/day), mean ± SD	1828 ± 448	1927 ± 479	0.02
Saturated fat intake (g/day), mean ± SD	10.2 ± 6.2	10.6 ± 6.1	0.44
Fruit and vegetable intake (g/day), mean ± SD	480 ± 263	487 ± 282	0.77
Soy protein intake (g/day), mean ± SD	11.4 ± 7.5	11.2 ± 8.0	0.75
Total cholesterol (mg/dl), mean ± SD	185 ± 38	173 ± 33	0.0002
LDL cholesterol (mg/dl), mean ± SD	108 ± 32	97.8 ± 26.2	0.0002
HDL cholesterol (mg/dl), mean ± SD	37.3 ± 9.0	38.7 ± 9.7	0.09

[a] P values: paired t test for continuous variables and χ^2 test for categorical variables.

more biologically active than its parent compound daidzein.[23–25] The formation of equol varies substantially among individuals.[23–25] Inter-individual variation in equol formation has been suggested as one possible explanation for some inconsistent results on the health effects of soy.[23–25] Many factors, such as intestinal microflora profile, host genetics and dietary composition, may influence equol formation.[23–25]

Table 2 Geometric means[a] (95% CIs) of urinary isoflavonoids and creatinine among cases and control subjects

	Cases	Control subjects	Ratios[b]	*P*[c]
Women				
Total isoflavonoids	19.4 (16.1, 23.3)	18.2 (16.0, 20.8)	1.07	0.59
Daidzein	7.09 (5.89, 8.54)	6.76 (5.93, 7.72)	1.05	0.68
Genistein	3.35 (2.72, 4.13)	3.12 (2.69, 3.61)	1.08	0.58
Glycitein	1.12 (0.91, 1.37)	1.15 (0.99, 1.32)	0.98	0.85
Equol	0.11 (0.07, 0.16)	0.14 (0.11, 0.19)	0.77	0.31
O-DMA[d]	0.68 (0.50, 0.93)	0.73 (0.58, 0.90)	0.94	0.76
Dihydrodaidzein	0.66 (0.48, 0.90)	0.82 (0.65, 1.02)	0.81	0.28
Dihydrogenistein	0.20 (0.15, 0.28)	0.25 (0.20, 0.31)	0.81	0.28
Creatinine	74.1 (67.1, 81.8)	66.2 (61.7, 71.0)	1.12	0.07
Men				
Total isoflavonoids	13.5 (11.0, 16.6)	14.8 (12.8, 17.2)	0.91	0.46
Daidzein	5.24 (4.26, 6.44)	5.60 (4.84, 6.48)	0.94	0.61
Genistein	2.42 (1.93, 3.04)	2.61 (2.23, 3.07)	0.93	0.59
Glycitein	0.78 (0.62, 0.98)	0.83 (0.70, 0.97)	0.94	0.68
Equol	0.05 (0.03, 0.07)	0.07 (0.05, 0.09)	0.70	0.16
O-DMA	0.41 (0.29, 0.57)	0.46 (0.36, 0.58)	0.88	0.57
Dihydrodaidzein	0.74 (0.54, 1.03)	0.66 (0.53, 0.83)	1.13	0.55
Dihydrogenistein	0.19 (0.14, 0.26)	0.21 (0.17, 0.25)	0.95	0.79
Creatinine	70.9 (64.4, 78.1)	74.2 (69.3, 79.4)	0.96	0.45

[a]Expressed as nmol/mg creatinine for isoflavonoids, and mg/dl for creatinine.
[b]Ratios of geometric means for cases vs control subjects.
[c]*P* values for case–control differences in means of log transformed isoflavonoids.
[d]O-DMA: O-desmethylangolensin.

Whether it is equol per se or the ability of individuals to produce equol (the equol-producing phenotype) that is relevant to soy's health effects remains to be determined.[23–25] Using a method of defining equol producers based on a urinary equol/daidzein ratio of 0.018, we found that ~57% of women and 48% of men in our study were equol producers, which was similar to the frequency of equol producers reported for other Asian populations.[26,27] Our study was not designed and thus not adequately powered to formally assess the potential modifying effect of equol-producing status on the relationship between urinary isoflavonoids and CHD. Future studies are needed to address this important question.

It is not clear why men had lower urinary excretion of equol and lower prevalence of equol producers than women in our study or whether this difference may account for the different associations between equol and CHD in men and women. It is possible that differences in colonic function, dietary habits and other lifestyle factors such as smoking and drinking, as well as sex hormones, between men and women may contribute to some of the divergent results observed between sexes.[18,28]

There have been limited prospective epidemiological studies investigating the relationship between soy intake assessed by FFQs and incidence of CVD. In a previous analysis of the SWHS cohort, we found that a high dietary intake of soy foods was associated with a reduced risk of CHD in Chinese women.[14] Similarly, in the Japan Public Health Center-Based Study, an inverse association between soy intake and CHD was also found in Japanese women, but no association was found in men.[15] In contrast, a prospective cohort study of Dutch women showed no evidence that dietary phytoestrogen intake was related to the risk of CVD, including CHD.[16] However, the low range or low level of phytoestrogen intake in typical Western diets might account for the lack of association in the Dutch study.

The present study extended previous investigations of soy foods or isoflavones and CVD by evaluating the association of urinary excretion of isoflavones and their metabolites with CHD risk. Although urinary isoflavonoids may provide a more objective estimate of intake than FFQs and a more direct measure of the bioavailability of isoflavone exposure, they reflect primarily recent exposure (over 48–72 hours). As with many large-scale epidemiological studies, urinary isoflavonoids were measured in spot urine samples collected at baseline in our study, and this could be a concern because of the presence of within-person

Table 3 Odds ratios of CHD by quartiles of urinary isoflavonoids in women

	Quartile of urinary isoflavonoids				
	Q1	Q2	Q3	Q4	*P* for trend
Total isoflavonoids					
Median (IQR[a])	3.79 (1.93, 5.58)	13.9 (10.7, 17.1)	31.9 (26.7, 37.4)	86.2 (61.9, 124)	
Cases/control subjects	40/99	64/96	44/97	47/97	
Model 1[b]	1.00	1.52 (0.86, 2.68)	0.97 (0.52, 1.79)	1.03 (0.57, 1.86)	0.70
Model 2[c]	1.00	1.64 (0.83, 3.25)	1.56 (0.79, 3.08)	1.20 (0.60, 2.38)	0.72
Daidzein					
Median (IQR)	1.31 (0.74, 1.88)	4.92 (3.85, 6.46)	12.13 (9.76, 15.1)	29.5 (22.8, 47.4)	
Cases/control subjects	38/97	63/98	47/96	47/98	
Model 1	1.00	1.61 (0.90, 2.88)	1.20 (0.66, 2.18)	1.09 (0.59, 2.04)	0.80
Model 2	1.00	2.11 (1.05, 4.26)	1.78 (0.89, 3.54)	1.29 (0.62, 2.67)	0.86
Genistein					
Median (IQR)	0.44 (0.22, 0.81)	2.11 (1.54, 2.90)	5.67 (4.52, 7.34)	16.8 (11.9, 29.5)	
Cases/control subjects	38/97	53/98	56/97	48/97	
Model 1	1.00	1.15 (0.66, 2.02)	1.24 (0.70, 2.19)	1.08 (0.60, 1.93)	0.77
Model 2	1.00	1.30 (0.69, 2.46)	1.64 (0.86, 3.13)	1.18 (0.61, 2.29)	0.50
Glycitein					
Median (IQR)	0.18 (0.09, 0.31)	0.81 (0.61, 1.02)	1.86 (1.60, 2.35)	6.24 (4.34, 9.41)	
Cases/control subjects	46/97	55/98	43/95	51/99	
Model 1	1.00	1.10 (0.63, 1.94)	0.80 (0.45, 1.42)	1.01 (0.56, 1.81)	0.78
Model 2	1.00	1.26 (0.66, 2.40)	0.88 (0.47, 1.66)	1.17 (0.59, 2.31)	0.97
Equol					
Median (IQR)	0.01 (0.00, 0.03)	0.07 (0.03, 0.13)	0.34 (0.15, 0.74)	7.18 (3.34, 16.0)	
Cases/control subjects	61/97	48/97	44/96	42/99	
Model 1	1.00	0.59 (0.34, 1.04)	0.62 (0.35, 1.10)	0.49 (0.27, 0.89)	0.03
Model 2	1.00	0.61 (0.32, 1.15)	0.51 (0.26, 0.98)	0.46 (0.24, 0.89)	0.02
O-DMA					
Median (IQR)	0.07 (0.03, 0.16)	0.53 (0.35, 0.73)	1.80 (1.29, 2.35)	6.99 (4.45, 12.9)	
Cases/control subjects	60/98	39/96	39/96	57/98	
Model 1	1.00	0.64 (0.35, 1.16)	0.71 (0.39, 1.28)	0.84 (0.46, 1.51)	0.67
Model 2	1.00	0.46 (0.23, 0.92)	0.74 (0.39, 1.40)	1.15 (0.60, 2.21)	0.41
Dihydrodaidzein					
Median (IQR)	0.06 (0.02, 0.12)	0.50 (0.31, 0.72)	2.02 (1.42, 2.75)	8.21 (5.09, 15.3)	
Cases/control subjects	59/98	28/96	50/96	58/99	
Model 1	1.00	0.40 (0.21, 0.75)	0.88 (0.51, 1.51)	0.93 (0.54, 1.61)	0.69
Model 2	1.00	0.27 (0.13, 0.56)	0.75 (0.41, 1.36)	1.00 (0.54, 1.88)	0.69
Dihydrogenistein					
Median (IQR)	0.02 (0.01, 0.05)	0.13 (0.07, 0.19)	0.42 (0.31, 0.61)	3.22 (1.48, 9.42)	
Cases/control subjects	56/98	45/96	46/97	48/98	
Model 1	1.00	0.74 (0.43, 1.27)	0.80 (0.45, 1.44)	0.82 (0.47, 1.42)	0.58
Model 2	1.00	0.66 (0.36, 1.22)	0.62 (0.33, 1.19)	0.66 (0.35, 1.23)	0.20

[a]IQR: Interquartile range.
[b]Model 1: Conditional logistic regression model: conditioned on matching variables and adjusted for age, education, family income, cigarette smoking, alcohol consumption, BMI, waist-hip ratio, amount of regular exercise, intakes of total energy, saturated fat, total fruits and vegetables, menopausal status, hormone therapy use and history of hypertension or diabetes.
[c]Model 2: Additionally adjusted for total cholesterol, LDL cholesterol and HDL cholesterol.

Table 4 Odds ratios of CHD by quartiles of urinary isoflavonoids in men

	Quartile of urinary isoflavonoids				
	Q1	Q2	Q3	Q4	*P* for trend
Total isoflavonoids					
Median (IQR[a])	2.67 (1.33, 4.26)	10.8 (8.29, 14.5)	28.8 (22.5, 36.6)	74.4 (56.9, 118)	
Cases/control subjects	57/91	41/91	47/91	37/91	
Model 1[b]	1.00	0.95 (0.53, 1.73)	0.80 (0.45, 1.41)	0.62 (0.35, 1.10)	0.09
Model 2[c]	1.00	0.76 (0.39, 1.46)	0.80 (0.42, 1.52)	0.80 (0.43, 1.51)	0.49
Daidzein					
Median (IQR)	0.88 (0.49, 1.55)	3.75 (2.72, 5.03)	10.3 (8.21, 13.2)	29.3 (21.4, 44.2)	
Cases/control subjects	48/91	47/91	42/91	45/91	
Model 1	1.00	1.26 (0.71, 2.24)	0.89 (0.49, 1.63)	0.92 (0.50, 1.67)	0.53
Model 2	1.00	1.53 (0.81, 2.88)	1.20 (0.61, 2.38)	1.17 (0.59, 2.30)	0.82
Genistein					
Median (IQR)	0.35 (0.18, 0.63)	1.66 (1.23, 2.52)	4.96 (3.98, 6.24)	16.0 (11.2, 27.8)	
Cases/control subjects	45/91	53/91	41/91	43/91	
Model 1	1.00	1.34 (0.76, 2.36)	0.92 (0.50, 1.68)	0.90 (0.50, 1.60)	0.42
Model 2	1.00	1.30 (0.70, 2.40)	0.87 (0.44, 1.69)	1.07 (0.57, 2.02)	0.90
Glycitein					
Median (IQR)	0.11 (0.05, 0.18)	0.58 (0.41, 0.82)	1.70 (1.32, 2.12)	5.13 (3.52, 7.30)	
Cases/control subjects	44/91	55/91	39/91	44/91	
Model 1	1.00	1.24 (0.70, 2.20)	0.92 (0.50, 1.70)	0.89 (0.49, 1.59)	0.47
Model 2	1.00	1.05 (0.56, 1.96)	0.92 (0.46, 1.83)	1.04 (0.55, 1.97)	0.97
Equol					
Median (IQR)	0.00 (0.00, 0.01)	0.02 (0.01, 0.03)	0.09 (0.05, 0.20)	3.73 (1.68, 9.73)	
Cases/control subjects	49/91	49/91	45/91	39/91	
Model 1	1.00	1.02 (0.58, 1.80)	0.96 (0.52, 1.77)	0.75 (0.41, 1.39)	0.36
Model 2	1.00	0.78 (0.40, 1.51)	0.93 (0.46, 1.87)	0.76 (0.39, 1.49)	0.53
O-DMA					
Median (IQR)	0.02 (0.01, 0.05)	0.25 (0.15, 0.44)	1.41 (0.94, 2.17)	6.75 (4.69, 13.4)	
Cases/control subjects	41/91	57/91	48/91	36/91	
Model 1	1.00	1.46 (0.81, 2.62)	1.25 (0.69, 2.27)	0.98 (0.52, 1.82)	0.85
Model 2	1.00	1.28 (0.64, 2.54)	1.47 (0.76, 2.84)	1.00 (0.49, 2.06)	0.87
Dihydrodaidzein					
Median (IQR)	0.03 (0.01, 0.08)	0.42 (0.26, 0.65)	1.92 (1.42, 3.04)	7.99 (5.37, 12.2)	
Cases/control subjects	38/91	58/91	41/91	45/91	
Model 1	1.00	1.40 (0.77, 2.55)	1.05 (0.56, 1.96)	1.02 (0.55, 1.90)	0.68
Model 2	1.00	1.68 (0.84, 3.37)	1.39 (0.68, 2.84)	1.33 (0.65, 2.75)	0.67
Dihydrogenistein					
Median (IQR)	0.02 (0.01, 0.03)	0.10 (0.06, 0.14)	0.36 (0.25, 0.55)	2.20 (1.13, 5.35)	
Cases/control subjects	39/91	57/91	36/91	50/91	
Model 1	1.00	1.37 (0.75, 2.51)	0.85 (0.44, 1.65)	1.00 (0.55, 1.83)	0.59
Model 2	1.00	1.22 (0.64, 2.31)	0.93 (0.45, 1.91)	1.24 (0.64, 2.38)	0.67

[a]IQR: Interquartile range.
[b]Model 1: Conditional logistic regression model: conditioned on matching variables and adjusted for age, education, family income, cigarette smoking, alcohol consumption, BMI, waist-hip ratio, amount of regular exercise, intakes of total energy, saturated fat, total fruits and vegetables and history of hypertension or diabetes.
[c]Model 2: Additionally adjusted for total cholesterol, LDL cholesterol and HDL cholesterol.

variation. However, studies conducted among Chinese in Singapore and Shanghai, where soy foods are regularly consumed, have shown that urinary isoflavonoids measured in spot urine or overnight urine samples were positively related to usual soy intake measured by FFQs in a dose-dependent fashion.[29,30] In our study, the average length of time between sample collection and measurements was 5 years for men and 10 years for women. Previous studies have demonstrated that isoflavonoids are highly stable in urine kept at room temperature for 14 days;[31] however, the effects of long-term sample storage at −70°C are unclear. Nevertheless, cases and control subjects were matched on date and time of sample collection and follow-up time in our study, and thus potential sample storage effects are unlikely to have biased our results. Other limitations of this study include the possibility of residual confounding due to unmeasured or inaccurately measured covariates and insufficient statistical power to conduct analyses separately for equol producers and non-producers. Finally, although the observed inverse association between equol and CHD in women is consistent with the equol hypothesis, this finding needs to be interpreted with caution because of the multiple comparisons conducted. We cannot rule out completely the possibility that this could be a chance finding.

In conclusion, this prospective, nested case–control study provides little evidence for an association between urinary excretion of total isoflavonoids and risk of CHD in either women or men. However, this study suggests an inverse association between urinary excretion of equol and risk of CHD in women. Further investigation of the potential role of equol in preventing CHD is warranted.

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KEY MESSAGES

- Total urinary isoflavonoids are not associated with risk of CHD in either men or women.
- Urinary excretion of equol, a bioactive metabolite of soy isoflavone daidzein, may be inversely associated with risk of CHD in women.

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