The Journal of Nutrition
Nutritional Epidemiology

Dietary Soy Intake Is Not Associated with Risk of Cardiovascular Disease Mortality in Singapore Chinese Adults[1–3]

Mohammad Talaei,[4] Woon-Puay Koh,[4,6] Rob M. van Dam,[4,5,7] Jian-Min Yuan,[8,9] and An Pan[4,5]*

[4]Saw Swee Hock School of Public Health and [5]Yong Loo Lin School of Medicine, National University of Singapore and National University Health System, Singapore; [6]Duke-NUS Graduate Medical School, Singapore; [7]Department of Nutrition, Harvard School of Public Health, Boston, MA; [8]Division of Cancer Control and Population Sciences, University of Pittsburgh Cancer Institute, Pittsburgh, PA; and [9]Department of Epidemiology, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA

Abstract

Although soy food has been recommended because of its presumed cardiovascular benefits, the long-term prospective association between habitual soy food intake and cardiovascular disease mortality remains unclear. This study aimed to evaluate the relation of soy protein and isoflavone intake with the risk of cardiovascular disease mortality in middle-aged and older Chinese adults residing in Singapore. The Singapore Chinese Health Study is a population-based study that recruited 63,257 Chinese adults aged 45–74 y from 1993 to 1998. Usual diet was measured at recruitment by using a validated semiquantitative food-frequency questionnaire, and mortality information was identified via registry linkage until 31 December 2011. Cox proportional hazards models were used to calculate HRs, with adjustment for potential confounders. The median intake was 5.2 g/d for soy protein, 15.8 mg/d for soy isoflavones, and 87.4 g/d for soy expressed as tofu equivalents. We documented 4780 cardiovascular deaths during 890,473 person-years of follow-up. After adjustment for sociodemographic, lifestyle, and other dietary factors, soy protein intake was not significantly associated with cardiovascular disease mortality: HRs (95% CIs) were 1.00 (reference), 1.02 (0.94, 1.11), 1.02 (0.93, 1.11), and 1.06 (0.97, 1.17) for increasing quartiles of soy protein (*P*-trend = 0.24). Similarly, no significant association was observed for soy isoflavones and total tofu equivalents and when deaths from coronary heart disease (n = 2697) and stroke (n = 1298) were considered separately. When stratified by sex, HRs for cardiovascular disease mortality across quartiles of soy protein were 1.00, 1.00, 1.05, and 1.16 (95% CI: 1.03, 1.31) in men (*P*-trend = 0.02) and 1.00, 1.01, 0.96, and 0.95 (95% CI: 0.81, 1.10) in women (*P*-trend = 0.31), although the interaction was not significant (*P*-interaction = 0.12). In conclusion, soy intake was not significantly associated with risk of cardiovascular disease mortality in the Chinese population. However, a slightly increased risk associated with high soy protein intake in men cannot be excluded and requires further investigation. J. Nutr. 144: 921–928, 2014.

Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide (1), and the disease burden has increased dramatically in the past several decades in Asian countries (2). Among the many risk factors, diet plays an important role in the development and prognosis of CVD (3,4). There has been a long-standing interest in the potential cardiovascular benefits of soy protein and isoflavones on the basis of results from short-term intervention studies on several cardiometabolic pathways [e.g., lipid profiles (5), glycemic control (6), arterial stiffness (7), blood pressure (8), and endothelial function (9)].

Several prospective cohort studies have also assessed the association between soy intake and the risk of CVD. Dietary isoflavone intake was not significantly associated with risk of coronary heart disease (CHD) or stroke in Dutch women (10) or CVD mortality in Spanish adults (11). A possible reason for the inconsistency could be that the consumption of soy is too low in the Western population (12). In an early investigation in the Shanghai Women's Health Study, higher soy protein intake was associated with a lower risk of incident CHD in Chinese women (13). However, the investigators recently reported a positive association between soy intake and risk of incident CHD in the Shanghai Men's Health Study (14). They further measured urinary isoflavone metabolites as a biomarker of soy intake in a

[1] Supported by the NIH (RO1 CA055069, R35 CA053890, RO1 CA080205, RO1 CA098497, and RO1 CA144034). M.T. is supported by a Singapore International Graduate Award scholarship. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.
[2] Author disclosures: M. Talaei, W.-P. Koh, R. M. van Dam, J.-M. Yuan, and A. Pan, no conflicts of interest.
[3] Supplemental Tables 1–6 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
* To whom correspondence should be addressed. E-mail: ephanp@nus.edu.sg.

Manuscript received January 6, 2014. Initial review completed January 31, 2014. Revision accepted March 10, 2014.
First published online April 3, 2014; doi:10.3945/jn.114.190454.

nested case-control study from these 2 cohorts and found no significant association between urinary concentrations of isoflavones and incident CHD (15). In a Japanese cohort study, dietary intakes of total soy and soy isoflavones were associated with a lower risk of incident CHD and stroke in women but not in men (16). However, no significant association between soy intake and CVD mortality was reported in another cohort study in Japanese men and women aged >35 y (17). Thus, the current evidence between soy intake and risk of incident CVD remains inconsistent, and no significant association for CVD mortality was reported in Spanish or Japanese adults (11,17).

Previous epidemiologic findings on soy consumption and CVD may have been limited by the low consumption in Western populations (10,11), short-term follow-up in the Chinese population (13,14), or small sample size in some studies (13,14,16,17). Therefore, to address the question of whether long-term intakes of soy and related isoflavones are associated with a lower risk of CVD mortality, we used data from the Singapore Chinese Health Study, a population-based prospective cohort of ~63,000 Chinese men and women in Singapore. We also examined this relation separately in CVD-free individuals (primary prevention) and in persons with CVD (secondary prevention) at baseline, in men and women, and for CHD and stroke mortality.

Participants and Methods

Study population. In this population-based cohort study, 35,303 Chinese women and 27,954 Chinese men aged 45–74 y were enrolled between April 1993 and December 1998. The study participants were recruited from 2 major dialect groups in Singapore, Hokkiens and Cantonese, who originated from Fujian and Guangdong provinces in southern China, respectively. During the enrollment period, all of the study participants were residents of government housing estates, where 86% of the Singapore population resided at the time of recruitment. This study was approved by the institutional review board at the National University of Singapore, and all enrolled participants provided informed consent. Full details of the Singapore Chinese Health Study design have been described previously (18).

We obtained information on demographic characteristics, lifestyle factors (physical activity, tobacco use, and alcohol intake), usual diet, and medical history at recruitment through in-person interviews by using structured questionnaires. We excluded individuals who had cancer at baseline identified either by self-report or via linkage with the nationwide Singapore Cancer Registry (n = 1936) and those who reported extreme energy intakes (<600 or >3000 kcal/d for women and <700 or >3700 kcal/d for men; n = 1023). Finally, 60,298 participants were included in the current analysis.

Assessment of diet and covariates. During the baseline interview, we administered a semiquantitative FFQ including 165 commonly consumed food items in this population. The respondents were instructed to select from 8 food-frequency categories (ranging from "never or hardly ever" to "two or more times a day") and 3 portion sizes (small, medium, large) with the aid of photographs. The FFQ listed 7 nonfermented soy items that are commonly consumed in the local Chinese population: plain tofu, *tau pok*, *tau kwa*, *foo pei*, *foo jook*, tofu *far*, and soybean drink (19). Equivalent amounts of tofu per day were calculated to facilitate comparison with a known dietary item while taking into account the varying water contents across the 7 soy foods: the water

TABLE 1 Participant characteristics according to quartile of soy protein intake in the Singapore Chinese Health Study[1]

	Quartile of soy protein intake			
Characteristic	1 (n = 15,182)	2 (n = 15,119)	3 (n = 14,943)	4 (n = 15,054)
Age, *y*	56.4 ± 8.0	57.0 ± 8.1	56.4 ± 8.0	55.7 ± 7.8
Female sex, *n (%)*	5918 (39.0)	8812 (58.3)	9464 (63.3)	9270 (51.6)
Dialect group, *n (%)*				
Hokkien	7567 (49.8)	8264 (54.7)	8337 (55.8)	8207 (54.5)
Cantonese	7615 (50.2)	6855 (45.3)	6606 (44.2)	6847 (45.5)
Education more than secondary school, *n (%)*	4474 (29.5)	3869 (25.6)	4099 (27.4)	4738 (31.5)
Ever smoker, *n (%)*	6281 (41.4)	4438 (29.3)	3842 (25.7)	3827 (25.4)
Weekly/daily alcohol drinker, *n (%)*	2706 (17.8)	1550 (10.2)	1292 (8.6)	1461 (9.7)
Hypertension, *n (%)*	3485 (22.9)	3530 (23.3)	3646 (24.4)	3627 (24.0)
Diabetes, *n (%)*	1246 (8.2)	1344 (8.9)	1357 (9.1)	1406 (9.3)
Coronary heart disease, *n (%)*	592 (3.9)	607 (4.0)	621 (4.1)	645 (4.3)
Stroke, *n (%)*	248 (1.6)	235 (1.5)	202 (1.3)	203 (1.3)
Weekly moderate activity, *n (%)*				
<0.5 h/wk	11,977 (78.9)	11,940 (79.0)	11,639 (77.9)	11,405 (75.8)
0.5–3.4 h/wk	2074 (13.7)	1981 (13.1)	2106 (14.1)	2239 (14.9)
≥3.5 h/wk	1131 (7.4)	1198 (7.9)	1198 (8.0)	1410 (9.4)
BMI, *kg/m²*	23.0 ± 3.3	23.0 ± 3.2	23.2 ± 3.2	23.2 ± 3.3
Total energy intake, *kcal/d*	1770 ± 508	1396 ± 461	1394 ± 470	1629 ± 535
Tofu equivalents,[2] *g/d*	24.0 ± 27.6	71.9 ± 13.2	108 ± 18.0	204 ± 88.2
Soy protein,[2] *g/d*	1.8 ± 1.4	4.3 ± 0.5	6.2 ± 0.6	11.2 ± 4.2
Soy isoflavones,[2] *mg/d*	5.4 ± 5.1	13.5 ± 3.4	19.6 ± 4.7	36.1 ± 17.5
SFAs,[2] *g/d*	13.8 ± 5.1	15.4 ± 3.9	16.0 ± 3.9	16.8 ± 4.7
MUFAs,[2] *g/d*	13.2 ± 4.4	15.4 ± 3.9	16.0 ± 3.9	16.8 ± 4.7
ω-3 PUFAs,[2] *g/d*	0.7 ± 0.3	0.9 ± 0.2	0.9 ± 0.2	1.1 ± 0.3
ω-6 PUFAs,[2] *g/d*	6.4 ± 2.9	7.5 ± 2.5	8.2 ± 2.6	9.5 ± 3.6
Fiber,[2] *g/d*	11.2 ± 4.4	12.3 ± 3.6	12.9 ± 3.5	14.2 ± 4.1

[1] Values are means ± SDs unless otherwise indicated.
[2] Dietary intakes were adjusted for energy using the residual methods.

content was 54% for cooked *foo jook*, 58% for cooked *foo pei*, 69% for cooked *tau kwa* and *tau pok*, 89% for cooked plain tofu, and 92% for tofu *far* and soybean drink. For example, we determined that 1 g of cooked *foo jook* is equivalent to 4.2 g (by dividing the percentage of nonwater content: 46/11) of plain tofu. The total soy intake for each participant was estimated as the sum of all soy foods expressed in units of plain tofu equivalents. We further assessed soy components by deriving soy protein (in g) and soy isoflavones (in mg) from the Singapore Food Composition Database (18). This database was developed specifically for this cohort study and lists values for ~100 dietary components per 100 g of the edible raw and cooked foods. We previously measured concentrations of genistein, daidzein, and glycitein in market samples of common soy foods in Singapore (20). The FFQ was validated subsequently by using two 24-h recalls including 1 weekday and 1 weekend among a subset of 810 participants from this cohort (18). The correlation coefficients were 0.32 and 0.39 for soy protein and isoflavones, respectively. In another validation study, intakes of soy foods were also significantly, monotonously associated with urinary excretions of genistein, daidzein, and glycitein (21).

Self-reported information about age, body weight, height, educational level, smoking status, and physical activity was also assessed through the baseline questionnaire. BMI (in kg/m^2) was calculated by body weight in kilograms divided by height squared in meters. Participants also self-reported their history of medical conditions diagnosed by physicians, including diabetes, hypertension, CHD, and stroke.

Assessment of mortality. Date and cause of death of study participants were obtained through linkage with the nationwide registry of birth and death in Singapore. The primary cause of death was used for analysis. Vital status for cohort participants was updated through 31 December 2011. As of 31 December 2011, only 47 participants from this cohort were known to be lost to follow-up due to migration out of Singapore or for other reasons. Accordingly, emigration among participants seems to be negligible in this cohort, and vital statistical information during follow-up was nearly complete. The *International Classification of Diseases, Ninth Revision*, was used to code underlying causes of death using codes 390–459 for cardiovascular deaths, codes 410–414 for CHD deaths, and codes 430–438 for stroke deaths.

Statistical analysis. Soy product intake was analyzed in quartiles, with the lowest quartile as the reference category. We adjusted food and nutrient intakes (including soy protein, isoflavones, and tofu equivalents) for total energy by using the residual method (22). Person-years for each participant were calculated from the date of recruitment until time of death, loss to follow-up, or 31 December 2011, whichever came first. Cox proportional hazards were used to examine associations between soy food intake and CVD mortality risk. In the multivariate model, we adjusted for age (continuous), sex, interview year (1993–1995, 1996–1998), dialect group (Hokkien, Cantonese), cigarette smoking (years of smoking and number of cigarettes per day), alcohol consumption (never or monthly, weekly, daily), level of education (none, primary school, secondary school or more), physical activity level (<0.5, 0.5–3.4, ≥3.5 h/wk), BMI (<20.0, 20.0–23.9, 24.0–27.9, ≥28.0 kg/m^2), baseline history of comorbidities (diabetes, hypertension, CHD, and stroke as 4 adjusting factors) and total energy intake (continuous), and dietary intakes of fiber, SFAs, MUFAs, and omega-3 and ω-6 PUFAs (quartiles). *P* values for trend were tested by including quartiles of soy protein or isoflavone intake as continuous variables in the models.

Stratified analysis was decided a priori by self-reported history of physician-diagnosed CHD and stroke at baseline. The rationale was to test the hypothesis for primary prevention (participants without history of CVD) and secondary prevention (patients with CVD). We further stratified the analysis in participants with or without baseline history of diabetes/hypertension among those free of CVD to evaluate whether the association persisted in the high-risk group. Some previous studies suggested sex differences in the health effects of soy intake; therefore, we also stratified the analysis by sex. The likelihood ratio test of the cross-product terms was used to test for interactions. All statistical analyses were conducted by using SAS 9.1 (SAS Institute), with 2-sided *P* values <5% as the threshold for significance.

Results

The median (IQR) intake of soy protein in the study population was 5.2 (3.4–7.5) g/d; it was 15.8 (10.1–23.7) mg/d for isoflavones and 87.4 (53.8–132) g/d for units of plain tofu equivalents. **Table 1** shows participants' baseline characteristics

TABLE 2 HRs (95% CIs) of cardiovascular disease mortality according to intakes of soy and soy components[1]

	Quartile of intake				
	1	2	3	4	*P*-trend[2]
Soy protein					
Median intake, *g/d*	2.2	4.3	6.2	9.8	
Cases/person-years, *n/n*	1224/219,813	1275/222,748	1204/223,036	1077/224,876	
Multivariate model 1	1.00	1.02 (0.94, 1.11)	1.03 (0.95, 1.11)	1.00 (0.92, 1.08)	0.99
Multivariate model 2	1.00	1.00 (0.92, 1.08)	0.98 (0.90, 1.07)	0.99 (0.91, 1.07)	0.67
Multivariate model 3	1.00	1.02 (0.94, 1.11)	1.02 (0.93, 1.11)	1.06 (0.97, 1.17)	0.24
Soy isoflavones					
Median intake, *mg/d*	6.2	13.1	19.2	32.0	
Cases/person-years, *n/n*	1261/219,398	1314/221,609	1154/222,939	1051/226,528	
Multivariate model 1	1.00	1.01 (0.93, 1.09)	0.96 (0.89, 1.05)	0.93 (0.86, 1.01)	0.06
Multivariate model 2	1.00	1.00 (0.92, 1.08)	0.95 (0.88, 1.04)	0.94 (0.86, 1.02)	0.08
Multivariate model 3	1.00	1.01 (0.93, 1.10)	0.98 (0.90, 1.07)	1.00 (0.91, 1.10)	0.83
Tofu equivalents					
Median intake, *g/d*	42.8	61.2	99.9	197	
Cases/person-years, *n/n*	1216/217,915	1284/221,868	1215/224,836	1065/225,855	
Multivariate model 1	1.00	1.04 (0.96, 1.12)	1.04 (0.96, 1.12)	0.99 (0.91, 1.07)	0.77
Multivariate model 2	1.00	1.02 (0.94, 1.10)	1.02 (0.94, 1.11)	0.98 (0.90, 1.06)	0.62
Multivariate model 3	1.00	1.03 (0.95, 1.12)	1.06 (0.97, 1.15)	1.05 (0.95, 1.15)	0.27

[1] Multivariate model 1 adjusted for age, sex, dialect, and year of interview; multivariate model 2 further adjusted for educational level, BMI, physical activity, smoking duration, cigarette smoking per day, alcohol use, baseline history of self-reported diabetes, hypertension, coronary heart disease, stroke, and total energy intake; multivariate model 3 further adjusted for dietary fiber, SFAs, MUFAs, ω-3 PUFAs, and ω-6 PUFAs.

[2] Linear trend was tested by treating the quartile as a continuous variable.

according to quartiles of soy protein intake. Participants in the highest quartile of soy protein intake were more likely to be women and to be physically active but less likely to be smokers and regular alcohol drinkers compared with those with low soy protein intake. They also had a slightly higher prevalence of diabetes and hypertension but a similar prevalence of CHD and stroke. Soy protein was also associated with higher intakes of dietary fiber and PUFAs (P-trend < 0.001). No significant differences in soy protein intake were found by age, BMI, and educational level. A similar pattern was found for intake of soy isoflavones, except for a lack of association with prevalence of diabetes and hypertension (**Supplemental Table 1**). Similar patterns were observed in those participants without diabetes and hypertension at baseline (**Supplemental Table 2**).

We documented a total of 4780 cardiovascular deaths (including 2697 CHD deaths and 1298 stroke deaths) during an average of 14.7 y of follow-up (890,473 person-years). In the multivariate model, soy protein intake was not significantly associated with a risk of cardiovascular deaths, even after adjustment for established CVD risk factors and other dietary variables (HR for the highest vs. lowest quartile: 1.06; 95% CI: 0.97, 1.17; P-trend = 0.24) (**Table 2**). Similarly, intakes of soy isoflavones and soy expressed as plain tofu equivalents were not significantly associated with CVD mortality. In addition, no significant association was found for any of the soy-related exposures with either CHD or stroke mortality (**Table 3**).

We further conducted a stratified analysis according to baseline history of CVD (**Table 4**). No significant associations between soy-related exposures and CVD mortality were found for participants with or without CVD at baseline. Among individuals without baseline CVD, we further stratified the analysis by baseline diabetes and/or hypertension (**Supplemental Table 3**). No significant associations for CVD mortality were found for the subgroups, except for participants without baseline diabetes/hypertension in whom a significant association was found for soy protein (HR comparing extreme quartiles: 1.16; 95% CI: 1.00, 1.34; P-trend = 0.05) and tofu equivalents (HR comparing extreme quartiles: 1.18; 95% CI: 1.02, 1.36; P-trend = 0.04); however, these were only significant after further adjustment for dietary covariates.

We did not find a significant interaction between sex and soy protein intake (P-interaction = 0.12) for CVD mortality (**Table 5**). However, compared with the lowest quartile, a significantly increased risk of CVD mortality was observed in the highest quartile in men (HR: 1.16; 95% CI: 1.03, 1.31; P-trend = 0.02), but this was not found in women (HR: 0.95; 95% CI: 0.81, 1.10; P-trend = 0.31). However, the increased risk in the highest quartile among men was observed only after adjustment for dietary covariates. For CVD mortality, the interaction between sex and soy isoflavones was borderline significant (P-interaction = 0.07), but the interaction between sex and tofu equivalents was not statistically significant (P-interaction = 0.35). No significant associations were observed for risk of CHD and stroke mortality in either men or women (**Supplemental Tables 4** and **5**).

For CVD mortality, no significant interaction was found between soy protein intake and several lifestyle factors (smoking

TABLE 3 HRs (95% CIs) of CHD mortality and stroke mortality according to intakes of soy and soy components[1]

	Quartile of soy product intake				
	1	2	3	4	P-trend[2]
CHD mortality					
Soy protein					
Cases/person-years, *n/n*	701/219,813	709/222,748	656/223,036	631/22,4876	
Multivariate model 1	1.00	1.00 (0.90, 1.11)	0.95 (0.85, 1.06)	1.02 (0.92, 1.14)	0.96
Multivariate model 2	1.00	1.00 (0.90, 1.12)	0.96 (0.86, 1.08)	1.06 (0.93, 1.20)	0.58
Soy isoflavones					
Cases/person-years, *n/n*	719/219,398	715/221,609	656/222,939	607/226,528	
Multivariate model 1	1.00	0.98 (0.88, 1.09)	0.98 (0.88, 1.10)	0.96 (0.86, 1.08)	0.57
Multivariate model 2	1.00	0.98 (0.88, 1.09)	0.99 (0.88, 1.11)	0.99 (0.88, 1.12)	0.97
Tofu equivalents					
Cases/person-years, *n/n*	686/217,915	717/221,868	679/224,836	615/225,855	
Multivariate model 1	1.00	1.04 (0.93, 1.15)	1.04 (0.94, 1.17)	1.01 (0.90, 1.12)	0.88
Multivariate model 2	1.00	1.04 (0.93, 1.16)	1.06 (0.94, 1.19)	1.04 (0.92, 1.18)	0.49
Stroke mortality					
Soy protein					
Cases/person-years, *n/n*	321/219,813	374/222,748	333/223,036	270/224,876	
Multivariate model 1	1.00	1.05 (0.90, 1.23)	0.99 (0.84, 1.16)	0.91 (0.78, 1.08)	0.20
Multivariate model 2	1.00	1.09 (0.93, 1.27)	1.05 (0.89, 1.24)	1.02 (0.85, 1.23)	0.96
Soy isoflavones					
Cases/person-years, *n/n*	335/219,398	386/221,609	307/222,939	270/226,528	
Multivariate model 1	1.00	1.05 (0.90, 1.22)	0.90 (0.76, 1.06)	0.88 (0.75, 1.04)	0.04
Multivariate model 2	1.00	1.07 (0.92, 1.26)	0.94 (0.80, 1.12)	0.97 (0.81, 1.16)	0.41
Tofu equivalents					
Cases/person-years, *n/n*	328/217,915	375/221,868	323/224,836	272/225,855	
Multivariate model 1	1.00	1.04 (0.89, 1.21)	0.95 (0.80, 1.11)	0.90 (0.77, 1.06)	0.12
Multivariate model 2	1.00	1.07 (0.91, 1.25)	1.00 (0.84, 1.18)	1.00 (0.83, 1.20)	0.78

[1] Multivariate model 1 adjusted for age, sex, dialect, year of interview, educational level, BMI, physical activity, smoking duration, cigarette smoking per day, alcohol use, baseline history of self-reported diabetes, hypertension, CHD, stroke, and total energy intake; multivariate model 2 further adjusted for dietary fiber, SFAs, MUFAs, ω-3 PUFAs, and ω-6 PUFAs. CHD, coronary heart disease.

[2] Linear trend was tested by treating the quartile as a continuous variable;

TABLE 4 HRs (95% CIs) of CVD mortality according to intakes of soy and soy components, stratified by self-reported history of CVD at baseline[1]

	Quartile of intake				
	1	2	3	4	*P*-trend[2]
Soy protein					
No prior CVD history					
Cases/person-years, *n/n*	1020/210,183	1047/213,352	966/213,558	887/214,581	
Multivariate model 1	1.00	0.97 (0.89, 1.06)	0.95 (0.87, 1.04)	1.00 (0.91, 1.09)	0.81
Multivariate model 2	1.00	1.00 (0.91, 1.10)	1.00 (0.91, 1.11)	1.10 (0.99, 1.22)	0.10
Prior CVD history					
Cases/person-years, *n/n*	204/9631	228/9396	238/9478	190/10,295	
Multivariate model 1	1.00	1.15 (0.94, 1.40)	1.17 (0.96, 1.42)	0.92 (0.76, 1.13)	0.51
Multivariate model 2	1.00	1.13 (0.92, 1.38)	1.15 (0.94, 1.40)	0.90 (0.72, 1.13)	0.46
Soy isoflavones					
No prior CVD history					
Cases/person-years, *n/n*	1047/209,809	1077/211,718	928/213,837	868/216,310	
Multivariate model 1	1.00	0.97 (0.88, 1.06)	0.91 (0.83, 1.00)	0.96 (0.88, 1.05)	0.19
Multivariate model 2	1.00	0.99 (0.90, 1.08)	0.95 (0.86, 1.05)	1.04 (0.94, 1.16)	0.65
Prior CVD history					
Cases/person-years, *n/n*	214/9590	237/9890	226/9102	183/10,218	
Multivariate model 1	1.00	1.10 (0.91, 1.34)	1.19 (0.98, 1.45)	0.87 (0.71, 1.06)	0.26
Multivariate model 2	1.00	1.09 (0.90, 1.32)	1.17 (0.95, 1.43)	0.85 (0.68, 1.06)	0.26
Tofu equivalents					
No prior CVD history					
Cases/person-years, *n/n*	1006/208,298	1057/212,404	975/215,427	882/215,545	
Multivariate model 1	1.00	1.00 (0.91, 1.09)	0.97 (0.89, 1.07)	1.01 (0.92, 1.10)	0.93
Multivariate model 2	1.00	1.03 (0.94, 1.13)	1.03 (0.93, 1.13)	1.11 (1.00, 1.23)	0.07
Prior CVD history					
Cases/person-years, *n/n*	210/9617	227/9464	240/9409	183/10,310	
Multivariate model 1	1.00	1.11 (0.91, 1.35)	1.24 (1.02, 1.50)	0.86 (0.70, 1.05)	0.31
Multivariate model 2	1.00	1.09 (0.89, 1.32)	1.21 (0.99, 1.47)	0.83 (0.67, 1.04)	0.28

[1] Multivariate model 1 adjusted for age, sex, dialect, year of interview, educational level, BMI, physical activity, smoking duration, cigarette smoking per day, alcohol use, baseline history of self-reported diabetes and hypertension, and total energy intake; multivariate model 2 further adjusted for dietary fiber, SFAs, MUFAs, ω-3 PUFAs, and ω-6 PUFAs. CVD, cardiovascular disease.
[2] Linear trend was tested by treating the quartile as a continuous variable.

status, drinking status, physical activity, and polyunsaturated fat intake; all *P*-interaction > 0.10). The interaction between soy protein and fiber intake (below/above median intake) was significant (*P*-interaction = 0.01), and soy protein and tofu intakes were related to increased risk of CVD mortality when dietary fiber was low (**Supplemental Table 6**).

Discussion

In this large cohort study in Chinese men and women, dietary soy consumption (soy protein, isoflavones, tofu equivalents) was not significantly associated with CVD mortality. This result persisted in subgroups according to sex and baseline disease history (CVD, diabetes, hypertension) and for CHD and stroke mortality. Among men, the highest quartile of soy protein intake was associated with a slightly increased risk of CVD mortality.

Soy products have several bioactive components that may possess cardiovascular benefits, including plant protein, isoflavones, water-soluble fiber, and unsaturated FAs (23–26). Therefore, there has been a long-standing interest in the relation between soy foods and cardiovascular risk. Experimental studies have shown antiatherosclerotic effects of soy components through various mechanisms such as cellular vasodilation and anti–platelet aggregation, inhibition of proliferation and migration of vascular smooth muscle cells, and reduction in adhesion molecules as an early sign of endothelial dysfunction (23). The beneficial effects of soy products on lipid profiles (5), glycemic control (6), arterial stiffness (7), blood pressure (8), and endothelial function (9) have been shown in some short-term clinical trials. Thus, a preventive effect of soy intake against CVD risk is biologically plausible.

Although the evidence from animal studies and short-term clinical trials provides a strong foundation for the dietary recommendation to increase soy intake, the results from long-term prospective cohort studies are mixed. A cohort study in 16,132 Dutch women with >6 y of follow-up did not find a significant association between isoflavone intake (mainly from soy foods) and risk of CVD (10). This lack of association with CVD mortality was also reported from a large Spanish cohort study (11). Studies in Asian populations, in whom soy product intake is much higher than in Western populations, also reported inconsistent results. In an early analysis in a cohort of 64,915 Chinese women with a mean follow-up of 2.5 y, soy protein intake was significantly associated with a lower risk of CHD (only 62 incident cases) (13). There is no updated report of these findings after 10 y of further follow-up. In their recent report in a cohort of 55,474 Chinese men with a mean follow-up of 5.4 y, soy intake showed a positive, independent, and dose-response association with risk of CHD (217 incident cases) (14). However, the authors measured urinary isoflavones as a biomarker of soy intake and found no significant association of total isoflavones and major isoflavone metabolites (daidzein,

TABLE 5 HRs (95% CIs) of cardiovascular disease mortality according to intakes of soy and soy components, stratified by sex[1]

	Quartile of intake				
	1	2	3	4	*P*-trend[2]
Soy protein					
Males					
Cases/person-years, *n/n*	857/131,278	653/89,344	603/78,379	589/82,393	
Multivariate model 1	1.00	0.97 (0.87, 1.08)	0.99 (0.89, 1.10)	1.04 (0.93, 1.15)	0.50
Multivariate model 2	1.00	1.00 (0.87, 1.12)	1.05 (0.93, 1.17)	1.16 (1.03, 1.31)	0.02
Females					
Cases/person-years, *n/n*	367/88,536	622/133,404	601/144,657	488/142,483	
Multivariate model 1	1.00	1.02 (0.89, 1.16)	0.96 (0.84, 1.10)	0.94 (0.82, 1.08)	0.23
Multivariate model 2	1.00	1.01 (0.88, 1.16)	0.96 (0.84, 1.11)	0.95 (0.81, 1.10)	0.31
Soy isoflavones					
Males					
Cases/person-years, *n/n*	871/131,194	684/88,965	592/78,275	555/82,961	
Multivariate model 1	1.00	0.99 (0.89, 1.09)	1.02 (0.92, 1.14)	0.96 (0.86, 1.07)	0.61
Multivariate model 2	1.00	1.01 (0.91, 1.12)	1.07 (0.96, 1.20)	1.05 (0.93, 1.19)	0.27
Females					
Cases/person-years, *n/n*	390/88,205	630/132,644	562/144,664	496/143,567	
Multivariate model 1	1.00	0.98 (0.86, 1.12)	0.86 (0.75, 0.98)	0.92 (0.81, 1.05)	0.07
Multivariate model 2	1.00	0.97 (0.85, 1.11)	0.86 (0.75, 0.99)	0.92 (0.80, 1.07)	0.11
Tofu equivalents					
Males					
Cases/person-years, *n/n*	840/129,314	667/88,650	616/79,424	579/84,007	
Multivariate model 1	1.00	1.01 (0.91, 1.12)	1.06 (0.95, 1.18)	1.00 (0.90, 1.12)	0.70
Multivariate model 2	1.00	1.04 (0.93, 1.15)	1.11 (1.00, 1.24)	1.11 (0.98, 1.25)	0.04
Females					
Cases/person-years, *n/n*	376/88,602	617/133,218	599/145,412	486/141,848	
Multivariate model 1	1.00	1.00 (0.87, 1.14)	0.96 (0.84, 1.09)	0.95 (0.83, 1.09)	0.36
Multivariate model 2	1.00	1.00 (0.87, 1.14)	0.96 (0.84, 1.10)	0.96 (0.83, 1.12)	0.50

[1] Multivariate model 1 adjusted for age, dialect, year of interview, educational level, BMI, physical activity, smoking duration, cigarette smoking per day, alcohol use, baseline history of self-reported diabetes, hypertension, coronary heart disease, stroke, and total energy intake; multivariate model 2 further adjusted for dietary fiber, SFAs, MUFAs, ω-3 PUFAs, and ω-6 PUFAs.
[2] Linear trend was tested by treating the quartile as a continuous variable.

genistein, and glycitein) with incident CHD for either men or women in a case-control study including 377 cases nested within these 2 cohorts (15). In a cohort study in 40,462 Japanese adults with an average of 12.5 y of follow-up, total soy and soy isoflavone intakes were associated with a lower risk of cerebral and myocardial infarctions in postmenopausal women but not in men or premenopausal women (16). However, no significant association between soy intake and CVD mortality was reported in another cohort study in 13,355 male and 15,724 female Japanese adults with a mean follow-up of 7 y (17). Therefore, the current evidence for effects of soy intake on CVD is inconsistent for both Western and Asian populations.

There are several possible reasons for the lack of association between soy intake and CVD mortality in our study. First, despite some evidence of soy products improving surrogate outcomes in short-term trials (5–9), other studies have not found beneficial effects [as summarized in meta-analyses (27–29)]. Particularly in long-term interventions with large sample sizes, no significant effects of soy protein or isoflavones on blood lipid profiles were found (30–32). Therefore, it is possible that the null association with CVD mortality reflects the fact that "the direct cardiovascular health benefit of soy protein or isoflavone supplements is minimal at best," as stated in the American Heart Association recommendation (28). Second, animal studies and clinical trials usually provide very controlled exposure situation (high dosage, continuous contrast between 2 groups, and control of other lifestyles), and the findings may not be directly translatable to free-living individuals. Third, the consumption amount of soy protein is far lower in cohort studies (usually <10 g/d) compared with that in clinical trials (usually >25 g/d) (28). Fourth, the isoflavone content of foods and its bioavailability are influenced by growth conditions, postharvest processing, and storage (24,33,34). Interindividual variations due to gut microflora and genotype add further complexity to the metabolic fate of isoflavones (35). In addition, soy products can be cooked in many different ways and with different combinations of other foods in Chinese cuisine, which may also influence the nutrient contents and effects on health. FFQs cannot fully capture all these sources of variability, and some measurement error is inevitable.

In our study, soy protein intake was associated with a slightly higher risk of cardiovascular mortality in men but not in women. The potential interaction between soy and sex has been reported previously, and a Japanese study (16) found an inverse association between soy intake and CVD in postmenopausal women but not in men. The 2 cohort studies in Chinese adults reported an inverse association between soy intake and incident CHD in women (13) but a positive association in men (14). In a Chinese cross-sectional study, soy protein intake significantly interacted with sex in relation to the metabolic syndrome, with a trend of a positive association in men and an inverse association in women (36). These studies suggested that the effect of soy and isoflavones on cardiovascular risk may be sex-dependent. Some

studies reported inverse associations between dietary soy intake and serum testosterone concentrations in men (37,38), although the results were not consistent (39,40). Lower testosterone concentrations in men have been shown to be related to increased risk of diabetes (41) and metabolic syndrome (42). Moreover, the statistically significant associations were observed only after adjustment for other dietary factors, and we cannot exclude the possibility that this might be a chance finding, particularly given the lack of association with CHD or stroke mortality when stratified by sex and a significant association only in the highest quartile in men. We observed an unexpected interaction with fiber intake in that dietary soy was associated with a modest increased risk of CVD mortality in participants with lower fiber intake. The significant interaction between dietary soy and fiber intake was not found in the Shanghai cohorts (13,14), and further studies are still needed to confirm our results.

Our study has several strengths. First, to our knowledge, it is thus far the largest such study, with 4780 CVD deaths and a long duration of follow-up. Second, soy food consumption was comparable to other studies in Chinese and Japanese populations (13,14,16,17), although much higher compared with that in Western populations (10,11). Third, our study had a high response and follow-up rate, collection of detailed data on diet and potential confounders through face-to-face interviews, and nearly complete mortality assessment with accurate records on time and cause of death. Finally, dietary intake was assessed by an FFQ that was specifically developed and validated in this population and that has been shown to be reasonably accurate and reproducible (18,21).

We are also aware of several limitations of our study. First, some degree of measurement error in the assessment of soy intake was inevitable due to the self-report assessment of dietary intake, which most likely resulted in some nondifferential misclassification and underestimation of the strength of the association. The self-reported lifestyle-related data may have resulted in some misclassification and residual confounding. Second, we had only a single assessment of diet, and repeated assessments would have allowed us to capture potential changes in exposure during follow-up and reduce measurement errors. Third, we do not know whether soy products have benefits on nonfatal cardiovascular events, and future efforts should be made to validate and confirm the nonfatal cases. Finally, these findings may not be generalizable to other ethnic groups or populations with different amounts of soy intake.

In conclusion, our results from a large well-established prospective cohort study in a Chinese population do not support a protective effect of habitual consumption of soy foods on cardiovascular death. Our results cannot exclude that high doses of soy protein intake may be associated with a slightly increased risk of CVD mortality in men, but further studies are needed to confirm or refute this possibility. Taken together, our results do not provide evidence for recommendations of increasing soy food consumption as a CVD prevention strategy.

Acknowledgments

The authors thank Siew-Hong Low of the National University of Singapore for supervising the fieldwork of the Singapore Chinese Health Study. Finally, the authors acknowledge the founding, long-standing principal investigator of the Singapore Chinese Health Study, Mimi C. Yu. W.-P.K. and A.P. designed and conducted the research and analyzed the data; M.T. and A.P. wrote the manuscript; W.-P.K., R.M.v.D., and J.-M.Y. assisted in interpreting the data and edited the manuscript; and A.P. had primary responsibility for final content. All authors read and approved the final manuscript.

Literature Cited

1. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012;380:2095–128. Erratum in: Lancet. 2013;381(9867):628.
2. Ohira T, Iso H. Cardiovascular disease epidemiology in Asia: an overview. Circ J. 2013;77:1646–52.
3. Mente A, de Koning L, Shannon HS, Anand SS. A systematic review of the evidence supporting a causal link between dietary factors and coronary heart disease. Arch Intern Med. 2009;169:659–69.
4. Heidemann C, Schulze MB, Franco OH, van Dam RM, Mantzoros CS, Hu FB. Dietary patterns and risk of mortality from cardiovascular disease, cancer, and all causes in a prospective cohort of women. Circulation. 2008;118:230–7.
5. Anderson JW, Bush HM. Soy protein effects on serum lipoproteins: a quality assessment and meta-analysis of randomized, controlled studies. J Am Coll Nutr. 2011;30:79–91.
6. Kwon DY, Daily JW III, Kim HJ, Park S. Antidiabetic effects of fermented soybean products on type 2 diabetes. Nutr Res. 2010;30:1–13.
7. Pase MP, Grima NA, Sarris J. The effects of dietary and nutrient interventions on arterial stiffness: a systematic review. Am J Clin Nutr. 2011;93:446–54.
8. Liu XX, Li SH, Chen JZ, Sun K, Wang XJ, Wang XG, Hui RT. Effect of soy isoflavones on blood pressure: a meta-analysis of randomized controlled trials. Nutr Metab Cardiovasc Dis. 2012;22:463–70.
9. Beavers DP, Beavers KM, Miller M, Stamey J, Messina MJ. Exposure to isoflavone-containing soy products and endothelial function: a Bayesian meta-analysis of randomized controlled trials. Nutr Metab Cardiovasc Dis. 2012;22:182–91.
10. van der Schouw YT, Kreijkamp-Kaspers S, Peeters PH, Keinan-Boker L, Rimm EB, Grobbee DE. Prospective study on usual dietary phytoestrogen intake and cardiovascular disease risk in Western women. Circulation. 2005;111:465–71.
11. Zamora-Ros R, Jimenez C, Cleries R, Agudo A, Sanchez MJ, Sanchez-Cantalejo E, Molina-Montes E, Navarro C, Chirlaque MD, Maria Huerta J, et al. Dietary flavonoid and lignan intake and mortality in a Spanish cohort. Epidemiology. 2013;24:726–33.
12. Peterson JJ, Dwyer JT, Jacques PF, McCullough ML. Associations between flavonoids and cardiovascular disease incidence or mortality in European and US populations. Nutr Rev. 2012;70:491–508.
13. Zhang X, Shu XO, Gao YT, Yang G, Li Q, Li H, Jin F, Zheng W. Soy food consumption is associated with lower risk of coronary heart disease in Chinese women. J Nutr. 2003;133:2874–8.
14. Yu D, Zhang X, Xiang YB, Yang G, Li H, Fazio S, Linton M, Cai Q, Zheng W, Gao YT, et al. Association of soy food intake with risk and biomarkers of coronary heart disease in Chinese men. Int J Cardiol. 2014;172:e285-7.
15. Zhang X, Gao YT, Yang G, Li H, Cai Q, Xiang YB, Ji BT, Franke AA, Zheng W, Shu XO. Urinary isoflavonoids and risk of coronary heart disease. Int J Epidemiol. 2012;41:1367–75.
16. Kokubo Y, Iso H, Ishihara J, Okada K, Inoue M, Tsugane S. Association of dietary intake of soy, beans, and isoflavones with risk of cerebral and myocardial infarctions in Japanese populations: the Japan Public Health Center-based (JPHC) Study Cohort I. Circulation. 2007;116:2553–62.
17. Nagata C, Takatsuka N, Shimizu H. Soy and fish oil intake and mortality in a Japanese community. Am J Epidemiol. 2002;156:824–31.
18. Hankin JH, Stram DO, Arakawa K, Park S, Low SH, Lee HP, Yu MC. Singapore Chinese Health Study: development, validation, and calibration of the quantitative food frequency questionnaire. Nutr Cancer. 2001;39:187–95.
19. Mueller NT, Odegaard AO, Gross MD, Koh WP, Yu MC, Yuan JM, Pereira MA. Soy intake and risk of type 2 diabetes in Chinese Singaporeans. Eur J Nutr. 2012;51:1033–40.
20. Franke AA, Hankin JH, Yu MC, Maskarinec G, Low SH, Custer LJ. Isoflavone levels in soy foods consumed by multiethnic populations in Singapore and Hawaii. J Agric Food Chem. 1999;47:977–86.
21. Seow A, Shi CY, Franke AA, Hankin JH, Lee HP, Yu MC. Isoflavonoid levels in spot urine are associated with frequency of dietary soy intake in a population-based sample of middle-aged and older Chinese in Singapore. Cancer Epidemiol Biomarkers Prev. 1998;7:135–40.

22. Willett W, Stampfer MJ. Implications of total energy intake for epidemiologic analysis. In: Willett W, editor. Nutritional epidemiology. 3rd ed. Oxford (UK): Oxford University Press; 2013.
23. Cano A, Garcia-Perez MA, Tarin JJ. Isoflavones and cardiovascular disease. Maturitas. 2010;67:219–26.
24. Cederroth CR, Nef S. Soy, phytoestrogens and metabolism: a review. Mol Cell Endocrinol. 2009;304:30–42.
25. Rimbach G, Boesch-Saadatmandi C, Frank J, Fuchs D, Wenzel U, Daniel H, Hall WL, Weinberg PD. Dietary isoflavones in the prevention of cardiovascular disease—a molecular perspective. Food Chem Toxicol. 2008;46:1308–19.
26. van Ee JH. Soy constituents: modes of action in low-density lipoprotein management. Nutr Rev. 2009;67:222–34.
27. Liu ZM, Chen YM, Ho SC. Effects of soy intake on glycemic control: a meta-analysis of randomized controlled trials. Am J Clin Nutr. 2011;93:1092–101.
28. Sacks FM, Lichtenstein A, Van Horn L, Harris W, Kris-Etherton P, Winston M. Soy protein, isoflavones, and cardiovascular health: an American Heart Association science advisory for professionals from the Nutrition Committee. Circulation. 2006;113:1034–44.
29. Beavers KM, Jonnalagadda SS, Messina MJ. Soy consumption, adhesion molecules, and pro-inflammatory cytokines: a brief review of the literature. Nutr Rev. 2009;67:213–21.
30. Atteritano M, Marini H, Minutoli L, Polito F, Bitto A, Altavilla D, Mazzaferro S, D'Anna R, Cannata ML, Gaudio A, et al. Effects of the phytoestrogen genistein on some predictors of cardiovascular risk in osteopenic, postmenopausal women: a two-year randomized, double-blind, placebo-controlled study. J Clin Endocrinol Metab. 2007;92: 3068–75.
31. Kreijkamp-Kaspers S, Kok L, Grobbee DE, de Haan EH, Aleman A, Lampe JW, van der Schouw YT. Effect of soy protein containing isoflavones on cognitive function, bone mineral density, and plasma lipids in postmenopausal women: a randomized controlled trial. JAMA. 2004;292:65–74.
32. Ho SC, Chen YM, Ho SS, Woo JL. Soy isoflavone supplementation and fasting serum glucose and lipid profile among postmenopausal Chinese women: a double-blind, randomized, placebo-controlled trial. Menopause. 2007;14:905–12.
33. Cermak R, Durazzo A, Maiani G, Bohm V, Kammerer DR, Carle R, Wiczkowski W, Piskula MK, Galensa R. The influence of postharvest processing and storage of foodstuffs on the bioavailability of flavonoids and phenolic acids. Mol Nutr Food Res. 2009;53 Suppl 2:S184–93.
34. Faraj A, Vasanthan T. Soybean isoflavones: effects of processing and health benefits. Food Rev Int. 2004;20:51–75.
35. Franke AA, Halm BM, Kakazu K, Li X, Custer LJ. Phytoestrogenic isoflavonoids in epidemiologic and clinical research. Drug Test Anal. 2009;1:14–21.
36. Pan A, Franco OH, Ye J, Demark-Wahnefried W, Ye X, Yu Z, Li H, Lin X. Soy protein intake has sex-specific effects on the risk of metabolic syndrome in middle-aged and elderly Chinese. J Nutr. 2008;138:2413–21.
37. Nagata C, Inaba S, Kawakami N, Kakizoe T, Shimizu H. Inverse association of soy product intake with serum androgen and estrogen concentrations in Japanese men. Nutr Cancer. 2000;36:14–8.
38. Goodin S, Shen F, Shih WJ, Dave N, Kane MP, Medina P, Lambert GH, Aisner J, Gallo M, DiPaola RS. Clinical and biological activity of soy protein powder supplementation in healthy male volunteers. Cancer Epidemiol Biomarkers Prev. 2007;16:829–33.
39. Kurzer MS. Hormonal effects of soy in premenopausal women and men. J Nutr. 2002;132 Suppl:570S–3S.
40. Hamilton-Reeves JM, Vazquez G, Duval SJ, Phipps WR, Kurzer MS, Messina MJ. Clinical studies show no effects of soy protein or isoflavones on reproductive hormones in men: results of a meta-analysis. Fertil Steril. 2010;94:997–1007.
41. Ding EL, Song Y, Malik VS, Liu S. Sex differences of endogenous sex hormones and risk of type 2 diabetes: a systematic review and meta-analysis. JAMA. 2006;295:1288–99.
42. Kweon SS, Shin MH, Nam HS, Jeong SK, Park KS, Choi JS, Lee YH. Sex differences in the associations of testosterone and sex hormone-binding globulin with metabolic syndrome in middle-aged and elderly Koreans: the Namwon study. Circ J. 2013;77:734–40.