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ORIGINAL ARTICLE

Association between dietary intake of flavonoid and bone mineral density in middle aged and elderly Chinese women and men

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Abstract
Summary This large cross-sectional study examined the associations of dietary intakes of total flavonoids and their subtypes with bone density in women and men. We found that greater flavonoid intake was associated with higher bone density in women but not in men.
Introduction Studies in vitro and in animal models suggest a potential effect of flavonoids on bone health. Few studies have examined the association between the habitual intake of flavonoids and bone mineral density (BMD) in humans.
Methods The cross-sectional study recruited 2,239 women and 1,078 men. A semiquantitative food frequency questionnaire was administered in face-to-face interviews to assess habitual dietary flavonoid intake using food composition databases. BMD was measured over the whole body (WB) and in the femoral neck (FN) and lumbar spine (LS) by dual-energy X-ray absorptiometry (DXA).
Results After adjusting for covariates, women who consumed higher total flavonoids, and the subtypes of flavonols, flavan-3-ols, flavones, and proanthocyanidins tended to have greater BMD at the WB, LS, and FN (all P-trend<0.05). Women in the highest (vs. the lowest) quartile of total flavonoids intake had 0.020 (1.91 %), 0.021 (2.51 %), and 0.013 (1.99 %) g/cm^2 greater BMD at the whole body, LS, and FN, respectively. For the subtypes of flavonoids, the corresponding differences in BMD (in g/cm^2) were 0.012–0.021 (flavan-3-ols), 0.013–0.020 (flavonols), 0.016–0.019 (flavones), and 0.014–0.016 (proanthocyanidins), respectively. A higher intake of flavonones was associated with a greater BMD at the whole body (P-trend 0.041) and the FN (P-trend 0.022). In men, there were no significant positive associations between the consumption of total flavonoids and the subclasses and BMD at any sites.
Conclusion Dietary flavonoids intake was positively associated with BMD in women. Further large studies are needed to clarify this issue in men.

Keywords Bone mineral density · Chinese · Dietary · Flavonoid

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Introduction

Evidence is increasing for positive associations between plant-based foods like fruit and vegetable intakes and bone health [1, 2]. These associations are ascribed, at least in part, to the fiber, calcium, magnesium, and potassium contents of these foods. Flavonoids are another group of potentially chemoprotective compounds that are widely found in fruit, vegetables, tea, nuts, and seeds. A large number of studies have examined the associations between flavonoids and bone

health [3–5]; however, most of them, including randomized controlled trials (RCTs), have focused primarily on the isoflavone subclass, which is mainly contained in soy foods. Some [6] but not all [3] studies have found a positive correlation between isoflavone consumption and bone health. Other classes of flavonoids, including anthocyanidins, flavanones, flavan-3-ols, and proanthocyanidins, are also abundant in plants and contribute significantly to daily intake. Important differences in the chemical structure of subclasses affect both their biological efficacy and bioavailability [7, 8]. The antioxidant capacity, estrogenic properties as well as the antiproliferative activity of those subclassfication of flavonoid were also varied [9]. Therefore, it is possible that different type of flavonoids might exert differed impacts on bone health.

Animal models have shown that certain classes of flavonoid compounds could confer benefits on bone health [10–12]. To date, however, only two studies performed in the UK [4, 5] have investigated the relationship between the dietary intake of total flavonoids and their subclasses and bone mineral density (BMD). Asian populations ingest more soybean products than their Western counterparts. Studies performed in Asia have usually pointed to habitual consumption of isoflavonoids and bone health but yielded conflicting results [13, 14]. No research has produced findings in relation to the effects of other flavonoid subclasses on bone health in Asians. In addition, the two studies conducted in Western countries only enrolled women. Whether flavonoid intake positively modulates the bone health of men has yet to be validated.

The purpose of this study was to examine the associations between flavonoid intake (flavonols, flavanones, flavones, flavan-3-ols, isoflavones, anthocyanidins, and proanthocyanidins) and bone health in middle aged and elderly Chinese adults.

Subjects and methods

Subjects

The community-based cross-sectional study was approved by Sun Yat-sen University's School of Public Health Ethics Committee, and written informed consent was obtained from each participant. The participants in this analysis were drawn from a cohort study designed to assess the determinants of cardiometabolic outcomes and osteoporosis that started in 2008. A follow-up examination was conducted between April 2011 and March 2013. Of the 3,169 participants, 2,520 (79.5 %) participated in the first follow-up survey at a mean follow-up interval of 3.1 (SD 0.4)years. The remaining 649 participants dropped out due to refusal (419), loss-to-contact or emigration (194), or serious disease or death (36). Eight hundred seventy-nine new participants were recruited through advertisements, health talks, and referrals from community centers between July 2012 and December 2013, in urban Guangzhou, Guangdong Province, China. We excluded 4 men and 13 women who reported extreme energy intakes (<800 or >4,200 kcal/d for men and <600 or >3,500 kcal/d for women). A total of 2,239 women and 1,078 men with completed bone measures, and questionnaires were included in this cross-sectional study.

Dietary assessment

The participants were asked to recall their usual dietary consumption using a 79-item food frequency questionnaire (FFQ). The frequency and amount of usual individual foods (e.g., tomatoes, bananas, grapes) or groups of similar foods (e.g., citrus fruit: orange, grapefruit, and lemon) were reported. Berries and chocolate were not included in the FFQ because these items were infrequently consumed in this population. The validity and reproducibility of the FFQ, with six 3-day energy-adjusted diet records and 26 nutrients, have been confirmed among the local population (correlation coefficients between the FFQ and 3-day dietary records were 0.36 for vegetables and 0.56 for fruit) [15]. The consumption of foods was estimated per day, per week, per month, or per year according to the choice of the respondents. Photographs of the foods and portion sizes were provided as visual aids for the participants to judge portion size. The selected value for each food was then converted to reflect the daily intake. The dietary total energy intake was calculated according to the Chinese Food Composition Table, 2002 [16]. Habitual tea drinkers were asked to state the frequency per week and the types of tea (black, green, and oolong) usually consumed. Wine intake was measured by milliliters consumed per month.

The flavonoid values were derived from two USDA databases of flavonoids [17] and proanthocyanidins [18], and one Hong Kong database of isoflavones [19]. We derived the intake of the six main subclasses consumed in Chinese diet, specifically flavanones (hesperetin, naringenin), anthocyanins (cyanidin, delphinidin, malvidin, pelargonidin, petunidin, peonidin), flavan-3-ols ((+)-Catechin, (+)-Gallocatechin, (−)-Epicatechin, (−)-Epigallocatechin, (−)-Epicatechin 3-gallate, (−)-Epigallocatechin 3-gallate), flavonols (quercetin, kaempferol, myricetin, isohamnetin), flavones (luteolin, apigenin), and isoflavonones (daidzein, genistein, glycitein). The proanthocyanidin intake was calculated as the sum of the dimers, trimers, 4–6 oligomers, 7–10 oligomers, and polymers (>10) of flavan-3-ol units. The proanthocyanidins monomer data was removed because flavan-3-ols monomers (USDA database on flavonoids) [17] and proanthocyanidins monomers (USDA database on proanthocyanidins) [18] are the same molecules. Total flavonoid intake was derived by summarizing each component subclass (flavanones, anthocyanins, flavan-3-ols, flavonols, flavones, isoflavonones, and

proanthocyanidins). For items in the FFQ that included more than one food, we calculated the average of the flavonoid values for corresponding items in the USDA databases. If values for specific foods were not available, we imputed from similar foods if appropriate. When values for a processed food item were missing, 50 % of the raw food values were used to account for possible losses of flavonoids and proanthocyanidins during food preparation.

Outcome assessment

During the first follow-up survey, the BMDs (g/cm^2) of the whole body, lumbar spine (L1–L4), and femur neck were measured by dual-energy X-ray absorptiometry (Discovery W; Hologic Inc., Waltham, MA, USA). The scans for the lumbar spine and left hip were performed using a high-definition mode, whereas the scans for the whole body were performed in the default mode. The scans were analyzed with Hologic Discovery software version 3.2. The in-vivo coefficients of variation of the duplicated BMD measurements in 30 participants after re-positioning were 1.18, 0.87, and 1.92 % for the whole body, lumbar spine, and femur neck, respectively.

Assessment of potential confounders

The body weight and height of each participant wearing light clothing and no shoes was measured, and the body mass index calculated as weight (in kilograms)/height squared (in meters). Information on the participants' demographics, cigarette smoking habits and use of multivitamins and calcium, and their medical histories were collected from the structured questionnaires. Participants who smoked at least one cigarette per day in the past year were defined as smokers. Physical activity was estimated as described previously [20].

Statistical analysis

All of the analyses were performed separately for the male and female participants. The data are presented as medians and interquartile ranges for the continuous variables and as frequencies for the categorical variables. Square root transformation was used in the daily flavonoid intakes to achieve an approximately normal distribution. Individual and total flavonoids were adjusted for the total energy intake using the residual method after square root transformation [21]. Multivariate analyses of covariance (ANCOVAs) were used to compare the mean BMDs of the whole body, lumbar spine, and femur neck among the quartiles of energy-adjusted individual and total flavonoids after adjusted by age, BMI, energy intake, physical activity, smoking status, calcium supplements, multivitamin use, years since menopause, and estrogen use (for women only) were included in the model. Pair-wise comparisons were performed using the Bonferroni test. Both the absolute and the percentage differences of BMD between the top and bottom quintiles of flavonoid subclass intake at each skeletal region were calculated in women and men. A P value of <0.05 was considered statistically significant. The statistical analyses were carried out using SPSS Statistics 13.0 software (SPSS, Inc., Chicago, USA).

Results

The baseline characteristics of the study population are shown in Table 1. The study population comprised 2,239 women and 1,078 men with a median age of 60.2 years. The men were heavier than the women and had a higher BMD. The women smoked and drank less, and were more likely to use calcium and multivitamin supplements. Table 2 shows the median (interquartile range) intake of individual and total flavonoids in both genders in each quartile. Women tended to consume fewer total flavonoids than men, and this has persisted even after adjustments for their lower total energy intake. Based on the similarities in the food sources of flavonoids in women and men, the foods that contributed to more than 7 % of the intake for each subclass of flavonoids in the total sample are listed in Fig. 1. Overall, tea was the main contributor to total flavonoids, flavan-3-ols, and flavonols.

The associations between flavonoid intake and BMD at each measured site after adjusting for covariates are listed in Tables 3, 4, and 5. In women, there was a statistically significant, dose-dependent positive relationship with BMD at all measured sites for total flavonoid intake (P for trend: <0.001–0.004). The mean differences in BMD between the highest and lowest quartiles of total flavonoids were 0.020 g/cm^2 (1.91 %), 0.021 g/cm^2 (2.51 %), and 0.013 g/cm^2 (1.99 %) at the whole body, LS, and FN, respectively. We observed similar results of mean BMD differences at these three bone sites when the participants were classified according to the consumption of flavan-3-ols (0.012–0.021 g/cm^2, P-trend <0.001–0.025), flavonols (0.013–0.020 g/cm^2, P-trend 0.005–0.012), flavones (0.016–0.019 g/cm^2, P-trend 0.001–0.019), and proanthocyanidin (0.014–0.016 g/cm^2, P-trend 0.002–0.044). A higher intake of flavanones resulted in significantly higher BMD at whole body (P-trend 0.041) and FN (P-trend 0.022). No significant association between intake of anthocyanins and BMD at any of the sites were observed. In men, however, no significant positive associations were found between total and individual flavonoid intake and BMD at any measured sites.

Discussion

To the best of our knowledge, this is the first study to report on the potential influence of dietary flavonoids on bone health in

Table 1 Baseline characteristics in women and men*

	Women (n=2,239)	Men (n=1,078)
Age (year)	59.2 (56.0–63.3)	62.3 (58.0–66.7)
Height (cm)	154.9 (151.4–158.6)	166.0 (162.1–169.9)
Weight (kg)	55.8 (50.6–61.5)	65.8 (59.4–71.8)
BMI (kg/m^2)	23.2 (21.3–25.3)	23.9 (21.9–25.9)
Energy intake (Kcal)	1,459 (1,235–1,731)	1,719 (1,431–2,038)
Physical activities (MET·h/d)[a]	33.1 (30.2–37.0)	32.6 (29.6–36.5)
Years since menopause	9.3 (5.3–13.8)	–
Calcium supplement use (%)		
Yes	34.3	20.7
No	65.7	79.8
Multivitamin use (%)		
Yes	21.6	13.4
No	78.4	86.6
Estrogen use (%)		
Yes	6.4	–
No	93.6	–
Smoking status (%)[b]		
Yes	0.4	18.4
No	99.6	81.6
BMD (g/cm^2)		
Whole body	1.055 (0.989–1.127)	1.173 (1.109–1.246)
Lumbar spine	0.839 (0.751–0.941)	0.952 (0.848–1.060)
Femur neck	0.655 (0.592–0.727)	0.735 (0.666–0.815)

* Values are presented as medians (interquartile range)

[a] Physical activities evaluated by metabolic equivalent (*MET*) hours per day

[b] Smoking was defined as having smoked one or more cigarettes daily in the past year

Asian women and men. We found a significant positive association between dietary consumption of total flavonoids, flavan-3-ols, flavonols, flavones, proanthocyanidins and flavanones, and BMD in women. No significant favorable effects were observed in men probably due to a much smaller study size.

A previous study of 3,160 women with a mean age of 48.3 years from the UK reported that participants in the top quintile of total flavonoid intake had a 0.021 g/cm^2 higher spine BMD compared with those in the lowest quintile [5]. Hardcastle et al. also demonstrated that the intake of each additional milligram of total energy-adjusted flavonoids resulted in a 0.009-mg/cm^2 increase in femur neck BMD in 3,230 Scottish women with a mean age of 60.8 years after adjusting for confounders [4]. Consistent with the two previous studies, in our study, women who had the highest total flavonoid consumption exhibited a 20.9 (0.022 g/cm^2), 15.7 (0.023 g/cm^2), and 14.6 % (0.036 g/cm^2) greater BMD in the whole body, spine, and femur neck than those in the lowest quintile, respectively. The magnitude of the association between flavan-3-ol consumption and BMD in females was also notable. Hardcastle et al. showed that BMD was positively associated with catechin, a constituent of the flavan-3-ol subclass [4]. A universal source of flavan-3-ols is tea. In our study, flavan-3-ol was also abundantly derived from tea (>90 %). Tea catechins, epigallocatechin in particular, have positive effects on bone metabolism through a double process of promoting osteoblastic activity and inhibiting osteoclast differentiation in cell culture experiments [22]. A growing body of evidence in humans suggests that greater tea consumption may benefit bone health in terms of maintaining a higher BMD [23] and reducing the risk of fracture [24]. A recent review suggested that flavonoids from green tea may be associated with increases in BMD by mitigating bone loss through antioxidant capacity and anti-inflammatory action [25].

A higher intake of flavonols was also correlated with a higher BMD at all sites in women. This was in line with the findings in UK populations [4, 5]. Animal studies have demonstrated that quercetin inhibits bone loss in ovariectomized mice [10]. Mechanistic studies have suggested that quercetin, kaempferol, and myricetin may favorably regulate bone metabolism through a range of potential mechanisms, including effects on osteoclast differentiation involving NF-kB and AP-1 induction by RANKL [26], promoting bone morphogenetic protein-2 (BMP-2) production, stimulating alkaline phosphatase activity, and upregulating bone sialoprotein gene promoter [27–29].

Table 2 Dietary intake of energy-adjusted total flavonoids and the subclasses in each quartile (mg/d)

	Quartile of intake			
	Q1	Q2	Q3	Q4
Women				
Total flavonoids	53.5 (40.5–66.3)	110.0 (92.2–132.1)	232.4 (194.8–274.4)	486.9 (402.2–584.4)
Flavanones	1.0 (0.3–1.7)	4.0 (3.2–4.9)	8.9 (7.3–10.4)	19.9 (15.3–27.2)
Anthocyanins	2.2 (1.5–2.8)	4.5 (3.9–5.1)	7.3 (6.5–8.1)	12.9 (10.5–17.2)
Flavan-3-ols	4.4 (3.2–5.7)	10.4 (8.5–12.9)	129.2 (89.1–168.7)	345.3 (280.2–467.7)
Flavonols	5.0 (4.1–5.9)	8.3 (7.4–9.4)	14.2 (12.5–16.5)	23.5 (21.3–26.0)
Flavones	0.3 (0.2–0.4)	0.5 (0.4–0.6)	0.8 (0.7–0.9)	1.4 (1.2–1.6)
Isoflavonoid	1.4 (0.7–2.1)	4.2 (3.5–5.0)	7.9 (6.9–9.3)	16.5 (13.1–23.5)
Proanthocyanidins	19.5 (13.9–25.5)	40.0 (35.0–45.2)	62.4 (56.2–68.8)	103.3 (87.6–126.6)
Men				
Total flavonoids	63.1 (44.9–94.6)	207.9 (174.4–237.2)	351.8 (297.6–392.2)	555.3 (479.6–618.2)
Flavanones	0.7 (0.2–1.4)	3.3 (2.6–4.0)	7.0 (5.9–8.3)	16.6 (12.7–23.7)
Anthocyanins	1.8 (1.1–2.1)	3.5 (3.0–4.0)	5.7 (5.1–6.3)	9.9 (8.4–13.7)
Flavan-3-ols	5.2 (3.1–8.6)	108.0 (86.0–139.9)	248.0 (184.8–288.4)	464.1 (355.9–476.1)
Flavonols	5.9 (4.3–7.6)	13.7 (11.5–15.5)	19.9 (18.5–21.2)	26.8 (24.3–31.3)
Flavones	0.3 (0.2–0.4)	0.6 (0.5–0.7)	0.9 (0.8–1.0)	1.3 (1.5–1.8)
Isoflavonoid	1.5 (0.7–2.2)	4.4 (3.7–5.2)	8.5 (7.1–10.1)	17.7 (14.2–24.8)
Proanthocyanidins	19.1 (13.9–23.8)	36.9 (32.7–41.1)	55.4 (49.3–62.2)	97.8 (80.5–123.4)

Values are presented as medians (interquartile range)

Flavones are present in relatively large quantities in leafy vegetables. Luteolin as well as apigenin has inhibitory activities toward both osteoclast differentiation and functions through inhibition of RANKL-induced signaling pathway [30, 31]. They could prevent the decrease of bone mass induced by ovariectomy in mice [32, 33]. Consistent with these findings, we did observe that increased flavones intake was associated with an increased BMD for all skeletal sites in women. Mechanistic support also exists for a beneficial effect of higher proanthocyanidins on BMD. The inhibition of

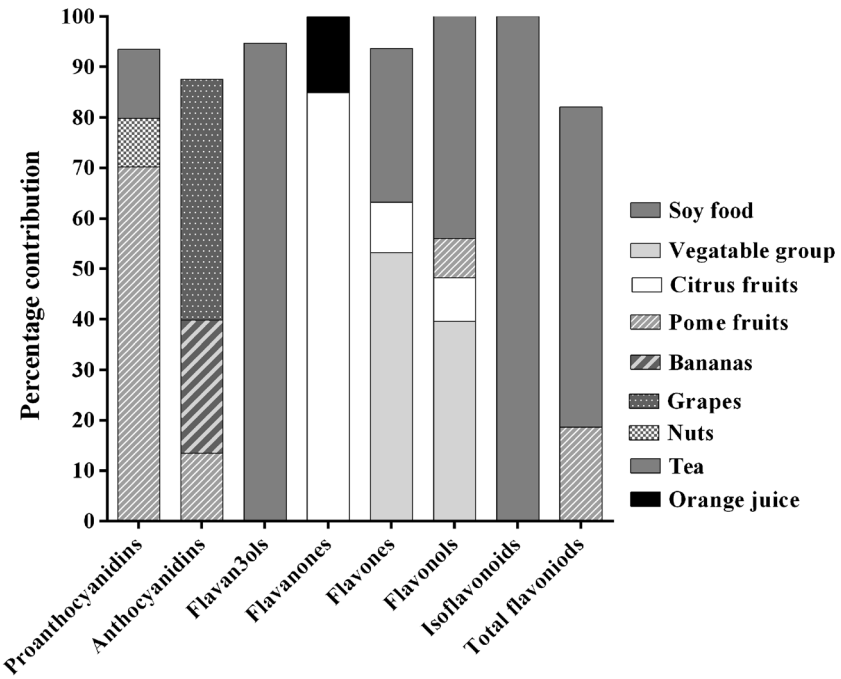

Fig. 1 Foods contributing to a greater than 7 % of intake for total and each subclass of flavonoids in the combined sample. Total flavonoids were derived by summarizing flavanones, anthocyanins, flavan-3-ols, flavonols, flavones, isoflavonones, and proanthocyanidins. Pome fruits: including apple, pear, peach, pineapple, and plum

Table 3 Mean total body bone density (g/cm^2) according to quartile of flavonoid intake

	BMD at whole body (g/cm^2)								Diff[a]	% Diff[b]	P-diff	P-trend
	Mean±SE	N	Mean±SE	N	Mean±SE	N	Mean±SE	N				
Women (*n*)												
Total flavonoids	1.046±0.004	559	1.059±0.004	560	1.073±0.004†††	560	1.066±0.004††	560	0.020	1.91	<0.001	<0.001
Flavanones	1.058±0.004	559	1.057±0.004	560	1.060±0.004	560	1.069±0.004	560	0.011	1.04	0.145	0.041
Anthocyanins	1.059±0.004	559	1.059±0.004	560	1.064±0.004	560	1.062±0.004	560	0.003	0.28	0.748	0.397
Flavan-3-ols	1.047±0.004	559	1.062±0.004	560	1.068±0.004††	560	1.068±0.004††	560	0.021	2.01	0.001	<0.001
Flavonols	1.053±0.004	559	1.058±0.004	560	1.066±0.004	560	1.068±0.004	560	0.015	1.42	0.039	0.005
Flavones	1.052±0.004	559	1.060±0.004	560	1.061±0.004	560	1.071±0.004††	560	0.019	1.81	0.010	0.001
Isoflavonoids	1.064±0.004	559	1.064±0.004	560	1.061±0.004	560	1.055±0.004	560	−0.009	−0.85	0.406	0.123
Proanthocyanidins	1.054±0.004	559	1.055±0.004	560	1.065±0.004	560	1.070±0.004†‡	560	0.016	1.52	0.011	0.002
Men (*n*)												
Total flavonoids	1.181±0.006	269	1.173±0.006	270	1.187±0.006	270	1.181±0.006	269	0.000	0.00	0.499	0.664
Flavanones	1.172±0.006	269	1.182±0.006	270	1.188±0.006	270	1.181±0.006	269	0.009	0.77	0.332	0.233
Anthocyanins	1.169±0.006	269	1.189±0.006	270	1.186±0.006	270	1.178±0.006	269	0.009	0.77	0.102	0.359
Flavan-3-ols	1.179±0.006	269	1.175±0.006	270	1.188±0.006	270	1.180±0.006	269	0.001	0.08	0.484	0.585
Flavonols	1.180±0.006	269	1.180±0.006	270	1.183±0.006	270	1.178±0.006	269	−0.002	−0.17	0.957	0.876
Flavones	1.170±0.006	269	1.190±0.006	270	1.184±0.006	270	1.178±0.006	269	0.008	0.68	0.118	0.501
Isoflavonoids	1.182±0.006	269	1.188±0.006	270	1.172±0.006	270	1.180±0.006	269	−0.002	−0.17	0.378	0.421
Proanthocyanidins	1.169±0.006	269	1.183±0.006	270	1.185±0.006	270	1.185±0.006	269	0.016	1.37	0.202	0.079

Values are adjusted for age, weight, physical activity, smoking habit, energy intake, calcium supplements, multivitamin use, years since menopause, and estrogen use (for women only)

[a] Percentage difference of BMD between Q4 and Q1=(Q4–Q1)/Q1×100

[b] Absolute difference between Q4 and Q1

† $P<0.05$, †† $P<0.01$, ††† $P<0.001$ compared with Q1

‡ $P<0.05$ compared with Q2

RANKL-dependent osteoclast differentiation caused by proanthocyanidins was indicated by studies in vitro [34]. Animal experimental results suggested that proanthocyanidins can promote bone formation [35]. Citrus fruits are the main source of flavanones. Rat model of osteoporosis indicated that feeding citrus juice positively affects serum antioxidant status and bone strength [36]. The hesperidin and naringin has also been proved to be effective in protecting against ovariectomy-induced bone loss [11, 12]. The positive associations between flavanones and bone health were detected at whole body and femur neck in women. However, the magnitude of the difference was weak when compared with that of flavan-3-ols, flavonols, flavones, and proanthocyanidins. The diversity in chemical structures of these subclasses may alter both their biological efficacy and bioavailability [7, 8]. Further researches are warranted to evaluate the relative performance of individual flavonoids in maintaining bone health.

The differences of 0.011–0.021 g/cm^2 (1.5–2.5 %) BMD in women between the highest and the lowest quartiles of the total and subtypes of flavonoids were equivalent to an age-related decrease of 1.5–3.0 year in this population (LS and FN BMD changes: about −0.0071 g/cm^2 per year). The degree of vertebral fracture risk reduction was estimated to be 3 % for a 1 % spine BMD increase [37], and the observed differences at the FN (1/9 SD) in this study could translate to a 6 % reduction in osteoporotic fractures and 17 % reduction in hip fractures in women [38]. Our magnitude of difference, although relatively small, taken together with other nutritional factors, may thus be of potential clinical and public health importance. Moreover, the favorable associations must have been underestimated due to great random errors in the assessment of dietary intakes of flavonoids in this study.

We failed to detect any obvious beneficial relationship between total flavonoid as well as those subclasses intakes and BMD in men. This raises the hypothesis that flavonoids act not only as antioxidants or anti-inflammatory factors, but possibly also as phytoestrogens. Estrogenic effects have been demonstrated for kaempferol, naringin, quercetin, and catechin [12, 39–41]. In the female participants of the Rotterdam Study, a positive association was found between tea drinking and prolactin secretion, which was used as a bioassay of estrogenic activity [42].

Table 4 Mean spine bone density (g/cm^2) according to quartile of flavonoid intake

	BMD at lumbar spine (g/cm^2)								Diff[a]	% Diff[b]	P-diff	P-trend
	Mean±SE	N	Mean±SE	N	Mean±SE	N	Mean±SE	N				
Women												
Total flavonoids	0.835±0.006	559	0.846±0.006	560	0.865±0.006[††]	560	0.856±0.006[†]	560	0.021	2.51	0.001	0.001
Flavanones	0.847±0.006	559	0.849±0.006	560	0.851±0.006	560	0.855±0.006	560	0.008	0.94	0.789	0.312
Anthocyanins	0.848±0.006	559	0.844±0.006	560	0.859±0.006	560	0.851±0.006	560	0.003	0.35	0.299	0.309
Flavan-3-ols	0.838±0.006	559	0.848±0.006	560	0.859±0.006[†]	560	0.857±0.006	560	0.019	2.27	0.030	0.006
Flavonols	0.837±0.006	559	0.853±0.006	560	0.855±0.006	560	0.857±0.006[†]	560	0.020	2.39	0.039	0.012
Flavones	0.842±0.006	559	0.849±0.006	560	0.850±0.006	560	0.861±0.006	560	0.019	2.26	0.112	0.019
Isoflavonoids	0.853±0.006	559	0.850±0.006	560	0.852±0.006	560	0.847±0.006	560	−0.006	−0.70	0.867	0.512
Proanthocyanidins	0.843±0.006	559	0.848±0.006	560	0.853±0.006	560	0.858±0.006	560	0.015	1.78	0.252	0.044
Men												
Total flavonoids	0.966±0.010	269	0.955±0.009	270	0.976±0.009	270	0.957±0.009	269	−0.009	−0.93	0.384	0.860
Flavanones	0.964±0.009	269	0.956±0.009	270	0.971±0.009	270	0.964±0.009	269	0.000	0.00	0.716	0.719
Anthocyanins	0.952±0.009	269	0.962±0.009	270	0.970±0.009	270	0.971±0.009	269	0.019	2.00	0.444	0.118
Flavan-3-ols	0.962±0.010	269	0.959±0.009	270	0.980±0.009	270	0.954±0.009	269	−0.008	−0.83	0.212	0.959
Flavonols	0.963±0.009	269	0.972±0.009	270	0.962±0.009	270	0.957±0.010	269	−0.006	−0.62	0.715	0.504
Flavones	0.952±0.009	269	0.974±0.009	270	0.973±0.009	270	0.955±0.009	269	0.003	0.32	0.188	0.828
Isoflavonoids	0.959±0.009	269	0.971±0.009	270	0.957±0.009	270	0.967±0.009	269	0.008	0.83	0.699	0.807
Proanthocyanidins	0.950±0.009	269	0.968±0.009	270	0.971±0.009	270	0.966±0.009	269	0.016	1.68	0.384	0.202

[a] See Table 3

[b] See Table 3

[†,††] See Table 3

Interestingly, men in the highest quartile of total flavonoids, flavan-3-ols, and flavonols which mainly derived from tea tended to have lower BMD in our study. In women, some BMD values were slightly lower in the highest quartile of the consumption of total flavonoids and flavan-3-ols than those in quartile 3 at the studied sites. These results might possibly be due to a potential ceiling effect, and a reduction in calcium absorption caused by high tea consumption [43]. However, further studies are needed to clarify this issue.

Unlike the previous study performed in the UK population [5], our data did not confirm an association between anthocyanidin intake and BMD regardless of gender. The median intake of anthocyanidin was only 5.8 mg/d in women and 4.6 mg/d in men, which was far below the values reported by Welchet al. (13.7 mg/d) [5]. The lack of association could be due to the very low intake of anthocyanidinin in our population, which makes it tempting to speculate that the antioxidative or phytoestrogenic potential was not sufficient to provide favorable effects on bone density.

Soy intake is part of the regular diet of Asian populations. Most previous studies linking dietary flavonoid intake and bone health in Asians have focused on isoflavones. Mei et al. reported a positive association between dietary isoflavone intake (21.9 mg/d) and spine and hip BMD in women aged 63 who were about 13 years postmenopausal, but not in premenopausal women [44]. Another study conducted by Ho et al. showed no association between isoflavone intake (18.32 mg/d) and BMD in women within the first 4 years after menopause, but they did observe a dose–response association for hip and total body BMD in women in later menopausal years [13]. We performed an analysis among the later postmenopausal women in our sample and the results were similar (data not shown). The subjects in the previous two studies consumed three times more isoflavones than our population. This difference in dietary intake may account for the conflicting results across the studies. To date, few studies have examined the effects of dietary isoflavone consumption on bone health in men. However, the lack of correlation between isoflavones intake and BMD in men in our study corroborate the results of a prospective cohort of 63,257 Chinese living in Singapore, which found no association between isoflavone intake and fractures in men [45].

The strengths of this study include the large sample size and the inclusion of both men and women. We also obtained detailed information on the important risk factors and confounders for bone health that were absent from some of the

Table 5 Mean femur neck bone density (g/cm^2) according to quartile of flavonoid intake*

	BMD at femur neck (g/cm^2)								Diff[a]	% Diff[b]	P-diff	P-trend
	Mean±SE	N	Mean±SE	N	Mean±SE	N	Mean±SE	N				
Women												
Total flavonoids	0.654±0.004	559	0.664±0.004	560	0.672±0.004††	560	0.667±0.004	560	0.013	1.99	0.011	0.004
Flavanones	0.660±0.004	559	0.660±0.004	560	0.667±0.004	560	0.671±0.004	560	0.011	1.67	0.122	0.022
Anthocyanins	0.662±0.004	559	0.661±0.004	560	0.671±0.004	560	0.663±0.004	560	0.001	0.15	0.264	0.447
Flavan-3-ols	0.657±0.004	559	0.663±0.004	560	0.668±0.004	560	0.669±0.004	560	0.012	1.83	0.144	0.025
Flavonols	0.656±0.004	559	0.660±0.004	560	0.671±0.004†	560	0.669±0.004	560	0.013	1.98	0.019	0.005
Flavones	0.656±0.004	559	0.663±0.004	560	0.666±0.004	560	0.672±0.004†	560	0.016	2.44	0.032	0.003
Isoflavonoids	0.660±0.004	559	0.664±0.004	560	0.670±0.004	560	0.663±0.004	560	0.003	0.45	0.644	0.811
Proanthocyanidins	0.657±0.004	559	0.662±0.004	560	0.667±0.004	560	0.671±0.004	560	0.014	2.13	0.094	0.012
Men												
Total flavonoids	0.753±0.006	269	0.745±0.006	270	0.746±0.006	270	0.738±0.006	269	−0.015	−1.99	0.457	0.141
Flavanones	0.739±0.006	269	0.743±0.006	270	0.751±0.006	270	0.749±0.006	269	0.010	1.35	0.469	0.159
Anthocyanins	0.735±0.006	269	0.750±0.006	270	0.744±0.006	270	0.753±0.006	269	0.018	2.45	0.221	0.100
Flavan-3-ols	0.752±0.006	269	0.745±0.006	270	0.751±0.006	270	0.733±0.006	269	−0.019	−2.53	0.149	0.100
Flavonols	0.755±0.006	269	0.748±0.006	270	0.745±0.006	270	0.735±0.006	269	−0.020	−2.65	0.178	0.030
Flavones	0.744±0.006	269	0.750±0.006	270	0.752±0.006	270	0.736±0.006	269	−0.008	−1.08	0.293	0.429
Isoflavonoids	0.748±0.006	269	0.744±0.006	270	0.745±0.006	270	0.745±0.006	269	−0.003	−0.40	0.962	0.712
Proanthocyanidins	0.736±0.006	269	0.748±0.006	270	0.745±0.006	270	0.753±0.006	269	0.017	2.31	0.267	0.085

[a] See Table 3

[b] Ssee Table 3

†,†† See Table 3

earlier studies. Several limitations of our study deserve a mention. First, as with any observational study, no causal associations can be made. Second, the dietary flavonoid intake was calculated from a database developed using the most recent USDA databases, with additional input from other sources. There is wide variability in the flavonoid content of foods, depending on the climatic, growing, soil, and harvesting conditions of plants, and their storage and preparation conditions. The measurement error caused by these factors may have led to attenuated estimates of effect size. Third, our analysis was based on a single measurement of dietary intake and the dietary questionnaires were not specifically designed to estimate the flavonoid and proanthocyanidin intake. Thus, our dietary exposure data may have been subject to measurement error related to both the dietary assessment technique and the flavonoid and proanthocyanidin database, which may have skewed any true association. Finally, we could not rule out the influence of co-existing bioactive compounds in plants food that might positively modulate bone health.

In conclusion, we found a favorable relation between the dietary intake of total flavonoid and most of its subclasses and BMD in women. Further large studies are needed to clarify this issue in men.

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Conflicts of interest None.

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