




Dietary flavonoids and flavonoid-rich foods: validity and reproducibility of FFQ-derived intake estimates

Yiyang Yue^{1,2,a} , Joshua Petimar^{1,2,3,a}, Walter C Willett^{1,2,4},
Stephanie A Smith-Warner^{1,2}, Changzheng Yuan^{1,2,5}, Sinara L Rossato^{1,6,‡},
Laura Sampson¹, Bernard Rosner^{4,5}, Aedin Cassidy⁷, Eric B Rimm^{1,2,4,b} and
Kerry L Ivey^{1,8,9,b,*}

¹Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA, USA: ²Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, USA: ³Department of Population Medicine, Harvard Medical School and Harvard Pilgrim Health Care Institute, Boston, MA, USA: ⁴Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA: ⁵Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, MA, USA: ⁶Universidade do Estado de São Paulo Júlio Mesquita Filho, Botucatu, Brazil: ⁷Institute for Global Food Security, Queen's University Belfast, Belfast, Northern Ireland: ⁸South Australian Health and Medical Research Institute, Infection and Immunity Theme, South Australia, Australia: ⁹Department of Nutrition and Dietetics, College of Nursing and Health Sciences, Flinders University, Adelaide, Australia

Submitted 7 January 2020: Final revision received 10 April 2020: Accepted 27 April 2020: First published online 23 July 2020

Abstract

Objective: To evaluate the validity and reproducibility of a 152-item semi-quantitative FFQ (SFFQ) for estimating flavonoid intakes.

Design: Over a 1-year period, participants completed two SFFQ and two weighed 7-d dietary records (7DDR). Flavonoid intakes from the SFFQ were estimated separately using Harvard (SFFQ_{Harvard}) and Phenol-Explorer (SFFQ_{PE}) food composition databases. 7DDR flavonoid intakes were derived using the Phenol-Explorer database (7DDR_{PE}). Validity was assessed using Spearman's rank correlation coefficients deattenuated for random measurement error (r_s), and reproducibility was assessed using rank intraclass correlation coefficients.

Setting: This validation study included primarily participants from two large observational cohort studies.

Participants: Six hundred forty-one men and 724 women.

Results: When compared with two 7DDR_{PE}, the validity of total flavonoid intake assessed by SFFQ_{PE} was high for both men and women ($r_s = 0.77$ and $r_s = 0.74$, respectively). The r_s for flavonoid subclasses ranged from 0.47 for flavones to 0.78 for anthocyanins in men and from 0.46 for flavonols to 0.77 for anthocyanins in women. We observed similarly moderate (0.4–0.7) to high (≥ 0.7) validity when using SFFQ_{Harvard} estimates, except for flavones_{Harvard} ($r_s = 0.25$ for men and $r_s = 0.19$ for women). The SFFQ demonstrated high reproducibility for total flavonoid and flavonoid subclass intake estimates when using either food composition database. The intraclass correlation coefficients ranged from 0.69 (flavonols_{PE}) to 0.80 (proanthocyanidins_{PE}) in men and from 0.67 (flavonols_{PE}) to 0.77 (flavan-3-ol monomers_{Harvard}) in women.

Conclusions: SFFQ-derived intakes of total flavonoids and flavonoid subclasses (except for flavones) are valid and reproducible for both men and women.

Keywords
Dietary assessment
Flavonoids
FFQ
Diet record
Validation study

^aThese authors are co-first authors.

^bThese authors are co-last authors.

[‡]The original version of this article was published with incorrect author information. A notice detailing this has been published and the error rectified in the online PDF and HTML copies.

Flavonoids comprise a diverse group of biologically active plant-derived polyphenolic compounds⁽¹⁾ that have been associated with improved health outcomes^(2–4) and reduced risk of all-cause mortality^(5–7). They are found in many foods such as red wine, blueberries and tea⁽⁸⁾.

*Corresponding author: Email kivey@hsph.harvard.edu

© The Author(s), 2020. Published by Cambridge University Press on behalf of The Nutrition Society.



Over 4000 flavonoid compounds have been identified and grouped into one of the seven subclasses: flavonols, flavan-3-ols monomers, proanthocyanidins, flavanones, flavones, anthocyanins and isoflavones⁽⁹⁾. These subclasses have demonstrated specific relationships with several health outcomes. For example, flavones and flavan-3-ols have been associated with lower cardiovascular mortality⁽¹⁰⁾, while protective associations have been observed for intake of flavonols and flavones with risk of breast cancer⁽¹¹⁾.

In order to fully understand the subclass-specific relevance of flavonoids to health and the population-level impacts of a flavonoid-rich diet, it is essential to obtain valid and reproducible intake estimates of flavonoid subclasses and the foods from which they are derived from semi-quantitative FFQ (SFFQ). This is because SFFQ are often the primary dietary assessment tool in nutritional epidemiology studies, owing to their generally valid measures of various macro- and micronutrients and the ease with which they can be administered and analysed in large-scale cohort studies^(12,13). The validity and reproducibility of SFFQ for measuring flavonoid intake are especially important because there are few appropriate biomarkers for intake due to the short half-life of flavonoids and their metabolites, as well as the complex and underdefined microbial and human metabolism of these compounds^(14,15). However, few validation studies have considered flavonoid intake, and those that have were generally small and used only a few days of diet records or 24-h recalls to compare against SFFQ^(16–20).

To address this gap, we used data from two large validation studies of men and women, including two weighed 7-d dietary records (7DDR) to evaluate the validity and reproducibility of an SFFQ to estimate intakes of flavonoids, flavonoid subclasses and flavonoid-rich foods.

Methods

Study population

The Men's Lifestyle Validation Study (MLVS) was conducted from 2011 to 2013 and consisted of 914 men aged 45–80 years randomly sampled from the Health Professionals Follow-Up Study, an ongoing, nationwide, prospective cohort of 51 529 US male health professionals established in 1986⁽²¹⁾. To be eligible for participation in MLVS, participants had to have completed a 2006/2007 SFFQ administered in Health Professionals Follow-Up Study, have previously provided blood samples, currently have internet access and never have been diagnosed with CHD, stroke, cancer or major neurological disease. The Women's Lifestyle Validation Study (WLVS) was conducted in 2010–2012 and sampled 796 women from the Nurses' Health Study and Nurses' Health Study-II, two prospective cohorts established in 1976 and 1989, respectively^(22,23). The inclusion criteria for WLVS were identical to those of MLVS.

We excluded participants who did not complete both SFFQ and at least one 7DDR over the course of the study. We additionally excluded those who reported extreme energy intake [men <800 or >4200 kcal/d (<3347.2 or > 17572.8 kJ/d); women <600 or >3500 kcal/d (<2510.4 or >14644.0 kJ/d)] or if they left seventy or more SFFQ items blank, resulting in 641 men and 724 women included in the final analysis (see online supplementary material, Supplemental Fig. 1).

The current study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving research study participants were approved by the Harvard T.H. Chan School of Public Health and Brigham and Women's Hospital. Written informed consent was obtained from all subjects/patients.

Semi-quantitative FFQ

In both the MLVS and WLVS, a 152-item SFFQ was administered at baseline⁽¹²⁾, as well as at the end of the study 1 year later. Respondents were asked how often, on average, they consumed a specified amount of each food or beverage on the SFFQ during the past year with nine possible frequency categories ranging from 'never/almost never' to '≥6 times per day'. Open-ended questions were used to obtain information on usual brand and type of margarine, cooking oil, cold breakfast cereal and multivitamins. Flavonoid intakes were calculated from the questionnaire by multiplying a weight proportional to the intake frequency by the flavonoid content for the portion size specified for each food item. Intakes were then summed across all foods to obtain overall intakes for total flavonoids and several flavonoid subclasses for every individual.

Weighed 7-d dietary records

Each participant in MLVS and WLVS completed two weighed 7DDR approximately 6 months apart in order to capture seasonal variability. Each participant, therefore, completed up to 14 d of weighed diet records. The 7DDR were completed approximately 6 months apart in order to capture seasonal variability and were completed in the time between repeated SFFQ assessments. Research dietitians provided participants with detailed instructions on keeping the 7DDR (via telephone), an instructional video on DVD, a Primo Multifunction Kitchen Scale (Model P115NB; Escali Corporation) and ruler (printed on the 7DDR booklet developed by Nutrition Coordinating Center, University of Minnesota). A computerised system was used to send reminders and encouragement emails to participants on days of diet recording. Participants measured and reported gram weights for foods before and after eating, so that actual intake could be computed. They also provided recipes of all home-prepared foods, including the number of servings



in the recipe and the portions of the recipes that they consumed, as well as labels of store-brand products.

Application of food composition databases to obtain flavonoid intake estimates

We used the Phenol-Explorer (PE) database, which includes detailed information on polyphenol content in 452 foods and beverages using estimates from published literature⁽²⁴⁾, to derive flavonoid intake estimates for the 7DDR (7DDR_{PE}). We also used PE to estimate flavonoid intake as measured by the SFFQ (SFFQ_{PE}), but additionally estimated intake using a database maintained by Harvard dietitians (available for request at <https://regepi.bwh.harvard.edu/health/nutrition.html>) that is largely based on USDA data (SFFQ_{Harvard}). We took these two approaches because they allowed us to investigate different sources of error in SFFQ-derived flavonoid intake estimation. Using PE to estimate flavonoid intake in both the SFFQ and 7DDR allowed us to isolate differences in intake estimation due to the measurement tool alone (i.e., not due to the food composition database)⁽⁹⁾. Conversely, using PE for the 7DDR and the Harvard database for the SFFQ allowed us to compare estimates of flavonoid intake from the two assessment tools without introducing the same source of errors in calculating intakes, which can inflate validity⁽¹³⁾. Methods used to apply these databases to obtain intake estimates have been previously described^(9,25). The two databases differ in terms of the composition of foods included and the analytic methods used to obtain the composition estimates. Briefly, the PE database represented glycosides or aglycones in the form that they naturally occur in food, while in the Harvard database, the flavonoid compounds were expressed largely as aglycone. For details of the differences between databases, see Ivey et al.⁽²⁶⁾.

We considered seven flavonoid subclasses: flavonols, flavan-3-ol monomers, proanthocyanidins (a class of flavan-3-ol polymers, limited to proanthocyanidin dimers and trimers to allow for comparison between Harvard and Phenol Explorer databases), flavanones, flavones, anthocyanins and isoflavones. Chalcones, dihydrochalcones and dihydroflavonols were also not included in the current analysis because they are typically considered precursors to many flavonoid compounds but are not flavonoids themselves.

Statistical analysis

All analyses were conducted separately in men and women. We calculated means and SD for absolute daily total flavonoid intakes and intakes for each subclass from the baseline SFFQ, the second SFFQ and average of two 7DDR.

In our primary analyses, we assessed the validity of SFFQ-derived flavonoid intake estimates by comparing intake from the second SFFQ, which assessed diet over

the same period as the 7DDR collection, to intake as measured by the average of the two 7DDR using Spearman's correlation coefficients (r_s). For total flavonoids and each subclass, we calculated r_s and two-sided 95 % CI that were deattenuated for random measurement error in the 7DDR using a method to account for the variable number of repeats of the 7DDR^(27–29). We adjusted r_s for total energy intake using the residual method⁽³⁰⁾ with a reference energy level of 1600 kcal for women and 2000 kcal for men. We additionally calculated r_s that were deattenuated but not adjusted for total energy intake. We determined reproducibility of the SFFQ by calculating rank intraclass correlation coefficients (ICC) comparing intakes from the first SFFQ to those from the second SFFQ. We calculated ICC that were adjusted and not adjusted for total energy intake. To reduce the influence of extreme nutrient intakes, ICC were based on the ranks of the log-transformed and energy-adjusted nutrient values^(12,27).

In secondary analyses, we determined the validity and reproducibility of the SFFQ for measuring intake of the top three food contributors to each flavonoid subclass according to the second SFFQ. For isoflavones, we considered only the top two contributors because subsequent contributors were composite group which included soya as a minor ingredient. We did this using the same analytic approach that we used for flavonoids in our primary analyses. We additionally explored whether the SFFQ adequately captured the absolute intake and variance of these top flavonoid contributors. We did this by calculating, in the 7DDR, the mean flavonoid intake and variance in intake that were captured by items listed on the SFFQ. We also summarised the mean intake and variance in the intake from foods not represented by the SFFQ that contributed to intake of each flavonoid subclass.

All analyses were conducted using SAS version 9.4.

Results

Most study participants were white (men 98%; women 90%) and had overweight (men 45%; women 30%) or obesity (men 13%; women 22%). Only 1% of men and 2% of women were current smokers. At baseline, men had a mean age of 67 (SD 8) years, and women had a mean age of 62 (SD 10) years (Table 1).

According to the 7DDR_{PE} intake estimates, the mean total flavonoid intake was 356 (SD 241) mg/d for men and 328 (SD 250) mg/d for women (Table 2). Total flavonoid intakes estimated from SFFQ_{PE} were slightly higher in women, but not in men. Intakes of most flavonoid subclasses were generally similar between SFFQ_{PE} and 7DDR_{PE}. However, estimated intakes of flavan-3-ol monomers were substantially higher when measured by the SFFQ_{PE} (58 and 68% higher in SFFQ_{PE} than 7DDR_{PE} in men and women, respectively), and intakes of flavonols were substantially higher when measured by the 7DDR_{PE}

Table 1 Baseline characteristics and dietary intake of participants in the Men's Lifestyle Validation Study and Women's Lifestyle Validation Study

	Men (n 641)			Women (n 724)		
	Value*	%	SD	Value*	%	SD
Age (years)	67		8	62		10
Geographic region						
West		20			20	
Midwest		17			24	
South		20			31	
Northeast		43			25	
BMI (kg/m ²)						
18 ≤ BMI < 25		42			47	
25 ≤ BMI < 30		45			30	
BMI ≥ 30		13			22	
Dietary intake†						
Total energy (kcal/d)‡	2109		579	1867		523
Carbohydrate (g/d)	246		80	219		70
Protein (g/d)	85		25	81		24
Total fat (g/d)	79		29	71		25
Saturated fat (g/d)	24		10	22		9
Monounsaturated fat (g/d)	31		13	27		11
Polyunsaturated fat (g/d)	17		7	15		6
Alcohol (g/d)	17		17	9		13
Dietary fibre (g/d)	27		10	24		9

*Values are presented as means and SD or percentages where appropriate.

†Dietary intake from the second semi-quantitative FFQ.

‡1 kcal = 4.184 kJ.

(39 and 38% lower in SFFQ_{PE} than 7DDR_{PE} in men and women, respectively). For total flavonoids, flavonols, flavan-3-ol monomers, flavones and isoflavones, SFFQ_{Harvard} intakes were substantially lower than SFFQ_{PE} intakes. SFFQ-estimated intake of proanthocyanidins, flavanones and anthocyanins was generally similar when using either the Harvard or PE database. For any given flavonoid subclass, the top whole food contributors to that subclass, as measured by the second SFFQ, were generally the same between men and women. However, the percentage of intake accounted for by the top three food contributors varied by subclass. In men, this ranged from 45% (flavonols) to 91% (flavanones); in women, it ranged from 43% (flavones) to 90% (flavanones).

The second SFFQ_{PE} generally demonstrated moderate (0.4–0.7) to high (≥0.7) validity as assessed by Spearman's correlation coefficients for measuring intake of total flavonoids and flavonoid subclasses compared with the average of two 7DDR_{PE} (Table 3). The deattenuated, energy-adjusted r_s for total flavonoid intake was 0.77 (95% CI 0.72, 0.82) for men and 0.74 (95% CI 0.69, 0.79) for women. The r_s for intake of flavonoid subclasses ranged from 0.47 for flavones to 0.78 for anthocyanins in men and from 0.46 for flavonols to 0.77 for anthocyanins in women.

Table 2 Mean (and SD) flavonoid intakes (mg/d) in Men's Lifestyle Validation Study and Women's Lifestyle Validation Study as estimated by two semi-quantitative FFQ (SFFQ) and two 7-d dietary records

	SFFQ _{Harvard}				SFFQ _{PE}				7DDR _{PE} *		Major food contributors (%)†
	SFFQ1		SFFQ2		SFFQ1		SFFQ2		7DDR _{PE} *		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Men											
Total flavonoids	237	132	234	132	362	234	352	225	356	241	
Flavonols	27	15	26	15	86	57	84	53	137	163	Onion (23%), tea (13%), apple (9%)
Flavan-3-ol monomers	62	62	61	62	97	134	93	131	59	85	Tea (41%), blueberry (17%), red wine (10%)
Proanthocyanidins‡	54	32	52	31	53	46	50	43	44	40	Red wine (25%), apple (18%), tea (7%)
Flavanones	38	34	38	35	46	60	44	59	42	56	Orange juice (53%), orange (30%), grapefruit (8%)
Flavones	4	3	3	3	15	10	15	10	11	8	Red wine (23%), orange juice (20%), orange (9%)
Anthocyanins	49	48	50	48	58	50	58	50	58	56	Blueberry (63%), red wine (12%), strawberry (9%)
Isoflavones	4	6	3	6	7	15	7	15	4	16	Tofu (32%), soya milk (18%)§
Women											
Total flavonoids	216	125	207	132	374	246	358	249	328	250	
Flavonols	27	16	26	16	91	59	91	61	146	172	Onion (27%), tea (19%), apple (9%)
Flavan-3-ol monomers	69	73	65	73	140	159	128	155	76	114	Tea (58%), blueberry (12%), apple (6%)
Proanthocyanidins‡	48	27	46	28	51	40	48	40	35	38	Apple (20%), red wine (15%), tea (13%)
Flavanones	27	30	23	27	28	54	26	50	21	35	Orange juice (50%), orange (32%), grapefruit (8%)
Flavones	3	2	2	2	13	8	13	8	8	5	Orange juice (18%), red wine (17%), orange (8%)
Anthocyanins	39	36	41	40	45	38	47	42	39	40	Blueberry (62%), strawberry (12%), red wine (8%)
Isoflavones	3	6	3	6	6	12	6	11	3	8	Tofu (21%), soya milk (18%)§

7DDR_{PE}, weighed 7-d diet record derived using Phenol-Explorer; SFFQ1, first semi-quantitative FFQ; SFFQ2, second semi-quantitative FFQ; SFFQ_{Harvard}, semi-quantitative FFQ derived using the Harvard database; SFFQ_{PE}, semi-quantitative FFQ derived using the Phenol-Explorer database.

*Values are for the average of 2 weeks of weighed diet records.

†Foods are as estimated from the second SFFQ using the Harvard food composition database. The numbers in parentheses are the percentages that the food contributes to mean intake of that flavonoid subclass.

‡Includes proanthocyanidin dimers and trimers.

§Other contributors were composite foods.

**Table 3** Deattenuated Spearman's correlation coefficients and 95% CI comparing flavonoid intake as measured by the second semi-quantitative FFQ with intake as measured by the average of 2 weeks of 7-d diet records in Men's Lifestyle Validation Study and Women's Lifestyle Validation Study

	SFFQ _{Harvard} v. 7DDR _{PE}				SFFQ _{PE} v. 7DDR _{PE}			
	Unadjusted		Energy-adjusted*		Unadjusted		Energy-adjusted*	
	Coefficients	95 % CI	Coefficients	95 % CI	Coefficients	95 % CI	Coefficients	95 % CI
Men								
Total flavonoids	0.65	0.59, 0.70	0.65	0.59, 0.70	0.75	0.70, 0.80	0.77	0.72–0.82
Flavonols	0.49	0.41, 0.55	0.52	0.44, 0.58	0.49	0.42, 0.56	0.53	0.46–0.59
Flavan-3-ol monomers	0.60	0.53, 0.66	0.59	0.52, 0.65	0.73	0.67, 0.79	0.74	0.68–0.79
Proanthocyanidins†	0.66	0.59, 0.72	0.69	0.62, 0.75	0.73	0.65, 0.79	0.74	0.67–0.80
Flavanones	0.73	0.67, 0.78	0.67	0.61, 0.71	0.80	0.75, 0.85	0.74	0.69–0.79
Flavones	0.26	0.17, 0.35	0.25	0.15, 0.34	0.51	0.43, 0.58	0.47	0.38–0.54
Anthocyanins	0.76	0.69, 0.82	0.76	0.68, 0.82	0.77	0.70, 0.82	0.78	0.71–0.84
Isoflavones	0.51	0.42, 0.59	0.51	0.41, 0.59	0.51	0.41, 0.59	0.48	0.38–0.57
Women								
Total flavonoids	0.63	0.57, 0.68	0.63	0.57, 0.67	0.73	0.68, 0.78	0.74	0.69–0.79
Flavonols	0.48	0.41, 0.55	0.48	0.40, 0.54	0.47	0.39, 0.53	0.46	0.39–0.53
Flavan-3-ol monomers	0.58	0.51, 0.64	0.57	0.50, 0.63	0.74	0.68, 0.79	0.73	0.67–0.79
Proanthocyanidins†	0.50	0.42, 0.57	0.51	0.43, 0.58	0.62	0.54, 0.68	0.61	0.54–0.68
Flavanones	0.60	0.54, 0.66	0.56	0.48, 0.62	0.68	0.62, 0.73	0.65	0.58–0.70
Flavones	0.20	0.11, 0.29	0.19	0.10, 0.28	0.45	0.37, 0.53	0.48	0.38–0.55
Anthocyanins	0.73	0.65, 0.80	0.74	0.66, 0.81	0.76	0.68, 0.82	0.77	0.69–0.84
Isoflavones	0.48	0.39, 0.56	0.46	0.37, 0.54	0.54	0.45, 0.61	0.49	0.40–0.57

7DDR_{PE}, weighed 7-d diet record derived using Phenol-Explorer; SFFQ_{Harvard}, semi-quantitative FFQ derived using the Harvard database; SFFQ_{PE}, semi-quantitative FFQ derived using the Phenol-Explorer database.

*Adjusted for total energy intake using the residual method.

†Includes proanthocyanidin dimers and trimers.

Results for all were similar when not adjusting for total energy intake. Results were similar or slightly weaker when using SFFQ_{Harvard}. We observed the strongest difference between databases for measuring intake of flavones ($r_s = 0.25$ in men and 0.19 in women). There was moderate to high agreement ($r_s \geq 0.43$) in the ranking of flavonoid subclass consumers between SFFQ_{PE} and SFFQ_{Harvard}, with the exception of flavones ($r_s \leq 0.12$), despite differences in absolute intakes (see online supplementary material, Supplemental Table 1).

The SFFQ demonstrated high reproducibility when comparing total flavonoid intake estimates from two assessments 1 year apart in both men ($ICC_{Harvard} = 0.78$, $ICC_{PE} = 0.80$) and women ($ICC_{Harvard} = 0.71$, $ICC_{PE} = 0.75$) (Table 4). The ICC for SFFQ_{PE} flavonoid subclasses ranged from 0.69 (flavonols) to 0.80 (proanthocyanidins) in men and from 0.68 (anthocyanins) to 0.77 (flavan-3-ol monomers) in women. The ICC ranges were similar for the SFFQ_{Harvard}. As for 7DDR, the ICC was generally lower than SFFQ, ranging from 0.39 (isoflavones) to 0.67 (flavanones) in men and from 0.39 (flavones and isoflavones) to 0.62 (flavonols) in women (see online supplementary material, Supplemental Table 2). These results were similar when not adjusting for total energy intake.

When we identified items in the 7DDR that were assessed on the SFFQ and calculated their total flavonoid intake, we found that these items accounted for 95 and 96% of the total flavonoid intake in men and women, respectively (see online supplementary material,

Supplemental Table 3). SFFQ foods captured the vast majority ($\geq 92\%$) of 7DDR intake for all flavonoid subclasses except for anthocyanins, which accounted for only 75% of intake in men and 76% in women. In *post hoc* analyses, we found that this discrepancy was largely due to the absence of cherries from the SFFQ food list, which contributed 7.4 mg/d (13%) to 7DDR anthocyanin intake in men and 4.9 mg/d (13%) in women. Blackberries, raspberries and cranberries also contributed $>1\%$ of 7DDR anthocyanin intake in both men and women. For flavones, the SFFQ foods accounted for 94% of 7DDR intake in men and 92% in women. Artichokes, which were not listed on the SFFQ, accounted for 0.4 mg/d (4%) and 0.4 mg/d (5%) of the 7DDR flavone intake in men and women, respectively. We also found that the foods represented by the SFFQ captured most of the variance in subclass intake, though anthocyanins and flavones similarly had the least amount of variance explained by SFFQ foods (see online supplementary material, Supplemental Table 4).

Using 7DDR as the reference method, the SFFQ demonstrated moderate to high validity for ranking all major food contributors to flavonoid intake, with similar results for men (mean $r_s = 0.75$) and women (mean $r_s = 0.68$); see Table 5. Among the major food contributors to flavonoid intake, the SFFQ demonstrated the strongest validity for red wine intake and orange juice in both men ($r_s = 0.88$, 95% CI 0.85, 0.91 for red wine; $r_s = 0.88$, 95% CI 0.85, 0.90 for orange juice) and women ($r_s = 0.83$, 95% CI 0.80, 0.86 for red wine; $r_s = 0.82$, 95% CI 0.79, 0.85 for

Table 4 Rank intraclass correlation coefficients comparing flavonoid intake from the first and second semi-quantitative FFQ in Men's Lifestyle Validation Study and Women's Lifestyle Validation Study

	SFFQ _{Harvard}		SFFQ _{PE}	
	Unadjusted	Energy-adjusted*	Unadjusted	Energy-adjusted*
Men				
Total flavonoids	0.81	0.78	0.82	0.80
Flavonols	0.75	0.72	0.71	0.69
Flavan-3-ol monomers	0.79	0.74	0.79	0.77
Proanthocyanidins†	0.77	0.75	0.82	0.80
Flavanones	0.81	0.79	0.81	0.79
Flavones	0.79	0.78	0.74	0.72
Anthocyanins	0.78	0.75	0.77	0.75
Isoflavones	0.68	0.66	0.78	0.75
Women				
Total flavonoids	0.77	0.71	0.78	0.75
Flavonols	0.70	0.67	0.71	0.70
Flavan-3-ol monomers	0.75	0.72	0.78	0.77
Proanthocyanidins†	0.74	0.71	0.78	0.74
Flavanones	0.73	0.69	0.80	0.75
Flavones	0.77	0.70	0.69	0.69
Anthocyanins	0.72	0.70	0.71	0.68
Isoflavones	0.67	0.66	0.73	0.75

SFFQ_{Harvard}, semi-quantitative FFQ derived using the Harvard database; SFFQ_{PE}, semi-quantitative FFQ derived using the Phenol-Explorer database.

*Adjusted for total energy intake using the residual method.

†Includes proanthocyanidin dimers and trimers.

Table 5 Validity and reproducibility estimates by semi-quantitative FFQ (SFFQ) for major food contributors to flavonoid intake in Men's Lifestyle Validation Study and Women's Lifestyle Validation Study (reference methods: 7-d dietary records (7DDR))

	r_s^*	95 % CI	ICC†
Men			
Apple	0.78	0.70, 0.83	0.71
Blueberry	0.77	0.70, 0.82	0.72
Grapefruit	0.79	0.71, 0.85	0.71
Onion	0.43	0.34, 0.51	0.62
Orange	0.81	0.67, 0.90	0.39
Orange juice	0.88	0.85, 0.90	0.40
Red wine	0.88	0.85, 0.91	0.88
Soya milk	0.89	0.84, 0.92	0.74
Strawberry	0.74	0.62, 0.83	0.63
Tea	0.71	0.66, 0.75	0.77
Tofu	0.54	0.45, 0.62	0.62
Women			
Apple	0.63	0.54, 0.70	0.66
Blueberry	0.67	0.61, 0.73	0.70
Grapefruit	0.77	0.59, 0.88	0.67
Onion	0.48	0.37, 0.56	0.62
Orange	0.44	0.39, 0.48‡	0.37
Orange juice	0.82	0.79, 0.85	0.38
Soya milk	0.83	0.80, 0.86	0.66
Red wine	0.83	0.80, 0.86	0.86
Strawberry	0.67	0.52, 0.79	0.59
Tea	0.72	0.67, 0.75	0.75
Tofu	0.59	0.47, 0.68	0.67

ICC, rank intraclass correlation coefficient; r_s , Spearman's rank correlation coefficient.

*Values represent r_s comparing the second SFFQ with the average of 2 weeks of weighed 7DDR.

†Values represent the ICC comparing the first and second SFFQ.

‡The r_s not deattenuated for random measurement error because of high within-person variation in intake (see online supplementary material, Supplemental Table 6).

orange juice). The SFFQ demonstrated the poorest validity for intake of onion in men ($r_s = 0.43$, 95 % CI 0.34, 0.51) and women ($r_s = 0.48$, 95 % CI 0.37, 0.56). The SFFQ estimated food intakes for all major flavonoid contributors were highly reproducible in both men and women, except for oranges (ICC = 0.40 for men and ICC = 0.37 for women) and orange juice (ICC = 0.40 for men and ICC = 0.38 for women). Men reported higher intakes of all major food contributors to flavonoid intake than women, but women consumed more tea in both the SFFQ and 7DDR (see online supplementary material, Supplemental Table 5). When compared with the 7DDR, the SFFQ tended to overestimate intakes of major food contributors to flavonoid intake, particularly apples, blueberries, onions and tofu. However, tea intake was consistently lower for the SFFQ than the 7DDR.

Discussion

The 152-item SFFQ used in the current study generally demonstrated moderate to high validity for measuring intake of total flavonoids and major flavonoid subclasses when compared with the intake from two weighed 7DDR. This was true when using either the PE- or Harvard-based food composition databases, but not for flavones when estimated using the Harvard database. The SFFQ also showed high reproducibility for measuring intake of these compounds when comparing estimates from SFFQ 1 year apart. The SFFQ demonstrated moderate to high validity for measuring intake of all major food



contributors to flavonoid subclass intakes except for onions. The current study highlights that this SFFQ is appropriate for ranking individuals by flavonoid intake, which is often the primary goal of dietary assessment in nutritional epidemiology studies, even when absolute intakes differ from the gold standard. This SFFQ has previously demonstrated moderate to high validity for measuring other major nutrients in WLVS (mean $r_s = 0.64$ among forty-four nutrients)⁽¹²⁾.

Few previous studies have evaluated the validity and reproducibility of SFFQ to measure flavonoid intake. Three studies conducted in China observed similar but slightly higher validity estimates than we did for flavonols and flavones^(16–18); however, these studies compared SFFQ with 24-h recalls, whose errors are expected to be more highly correlated with SFFQ than diet records because both 24-h recalls and SFFQ rely on participant memory⁽¹³⁾. Also, two of these studies were conducted in adolescents or young adults, making it difficult to compare results with the current study. Two other studies, which were conducted in Australia, evaluated the validity of an SFFQ specifically designed to capture flavonoid intake, whereas the current study evaluated an SFFQ designed to capture overall diet. Despite these differences, validity estimates for several flavonoid subclasses were similar or higher for the SFFQ in the current study than for this specialised SFFQ^(19,20). However, these two Australian studies only used 3- or 4-d diet records and had very small sample sizes ($n = 60$ and $n = 42$ participants, respectively). Compared with these previous studies, our study thus had a very large sample size and used two repeated implementations of 7DDR, capturing a total of 14 d of dietary intake, which is well above the minimum number of days of weighed diet records needed for accurately capturing flavonoid intake⁽³¹⁾.

The relatively high validity estimates we observed for most flavonoid subclasses are likely because most of their top food contributors were measured with good accuracy by the SFFQ compared with 7DDR (generally $r_s > 0.65$). The SFFQ demonstrated poorer ability to accurately measure intake of flavones and flavonols. This may be due to the fact that both of these subclasses are found in a variety of foods⁽⁸⁾, some of which may not be measured accurately by the SFFQ. The lower r_s estimates for flavonols, in particular, are likely partially due to the SFFQ's poorer ability to measure onion intake, which is the top contributor to this flavonoid subclass. For flavones, we noted that artichokes and oregano, which contributed significantly to total intake and the variance in intake, were not assessed by the SFFQ. Future investigations that seek to identify the role of flavones in health should consider adding these foods to existing SFFQ. We also noted that the absence of cherries, blackberries, raspberries and cranberries from the SFFQ food list could underestimate estimation of anthocyanin intake because these foods contributed substantially to total intake and variance in intake (except for cranberries)

in the 7DDR. However, the SFFQ demonstrated good ability to measure anthocyanin intake despite the fact that these foods were not assessed.

With the exception of flavones, the estimated validity of the SFFQ to measure flavonoid intakes did not appear to be driven by the choice of which food composition database to apply to the SFFQ; we observed similar results when using both the PE and Harvard databases. Using either database alone has the potential to introduce bias to validity estimates. Comparing SFFQ and 7DDR intake estimates using the same food composition database (i.e., SFFQ_{PE} *v.* 7DDR_{PE}) might introduce the same source of errors in calculating intake and thereby overestimate true validity; conversely, comparing these assessment tools using different databases (i.e. SFFQ_{Harvard} *v.* 7DDR_{PE}) incorporates error that is not solely due to differences in the tools' measurement ability, thereby underestimating true validity⁽¹³⁾. Indeed, when compared against 7DDR_{PE}, nearly all r_s were slightly lower when estimating intake from SFFQ_{Harvard} than from SFFQ_{PE}. However, this discrepancy was small for total flavonoid and flavonoid subclasses (other than flavones), highlighting the robustness of our findings. The larger differences we observed for flavone intake between the two databases may be because absolute flavone intake estimates from SFFQ_{PE} were more than four times greater than from SFFQ_{Harvard}, which was a larger discrepancy than for any other flavonoid subclass. Perhaps more importantly, there was also poor agreement in ranking participants by intake of flavones between the two methods. We have previously shown⁽²⁶⁾ that application of the USDA and PE databases to a different FFQ resulted in participants being ranked differently based on their flavone intake estimates. This lack of agreement was due to differences in the flavone concentration of foods between the two databases as well as analytic differences between the two databases. Primarily, the high proportion of C-glycosides making up this subclass is difficult to hydrolyse, and the data in the USDA database present hydrolysed values only. Thus, the flavone intake estimates from the Harvard database, which is based upon the USDA database, are expected to be lower than estimates from PE. It is worth noting that flavones were minor contributors to total flavonoid intake. Thus, even with these discrepancies in flavone estimates, our total flavonoid estimates were still valid.

To our knowledge, this is the largest validation study of an SFFQ for measuring flavonoid intakes compared with 7DDR, but it has limitations. Participants in the current study were all health professionals, and the vast majority were White. Our results may therefore not be generalisable to other populations, such as those with large proportions of non-White individuals and individuals who are not in healthcare professions. Additionally, diet records have their own sources of errors, including the fact that participants may change their normal eating habits when completing the records⁽¹³⁾. If these errors were uncorrelated



with errors in completing the SFFQ, the validity of the SFFQ could be underestimated. On the other hand, if the errors were correlated, which could occur due to use of the same food composition database, the validity of the SFFQ could be overestimated. However, we observed fairly high validity and reproducibility estimates for total flavonoids and most subclasses when using either of two different databases to derive SFFQ intake, increasing the robustness of our findings. Finally, isoflavone intake was relatively low and very skewed (see online supplementary material, Supplemental Fig. 2) in our study population. Therefore, the validity of SFFQ for measuring isoflavones may not be generalisable to populations with high isoflavone intakes, such as Asian populations.

In conclusion, the 152-item SFFQ demonstrated reasonable validity and reproducibility for measuring intake of total flavonoids and most flavonoid subclasses compared with the mean of two 7DDR assessments, regardless of the food composition database used. Intake of flavonoid-rich foods was also measured with generally high validity and reproducibility. Future investigations that seek to determine the health effects of specific flavonoid subclasses (mainly flavonols and flavones) should consider supplementing questionnaires with additional food items that may be informative for ranking individuals' intake.

Acknowledgements

Acknowledgements: The authors thank the Lifestyle Validation Study staff (Kristie Antonitto, Kirstin Anderson, Stephanie Bostic, Lisa Bowser (deceased), Catherine Clowry, Stefanie Dean, Brenna Murphy, Maria Petkova and Sean Sinnott). **Financial support:** MLVS and WLVS are supported by National Institutes of Health (grants UM1 CA186107, UM1 CA176726, P01 CA055075-18S1, U01CA152904 and U01CA167552). K.L.I. was supported by a National Health and Medical Research Council fellowship. J.P. is supported by T32HL098048 from National Heart, Lung and Blood Institute. **Conflict of interest:** There are no conflicts of interest. **Authorship:** The authors' responsibilities are as follows: K.L.I., J.P., Y.Y. and E.B.R. designed the analysis and had responsibility for the final content; E.B.R., W.C.W., L.S., K.L.I., J.P., S.A.S.-W. and S.R. collected data and performed computations; Y.Y., J.P. and K.L.I. analysed data and performed statistical analysis; Y.Y. and J.P. wrote the final manuscript; W.C.W., SSW, C.Y., S.A.S.-W., S.R., L.S., B.R. and A.C. provided conceptual advice and assisted in interpreting the data and editing the manuscript; all authors read and approved the manuscript. **Ethical standards disclosure:** This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving research study participants were approved by the Harvard T.H. Chan School of Public Health and Brigham

and Women's Hospital. Written informed consent was obtained from all subjects.

Supplementary material

For supplementary material accompanying this paper visit <https://doi.org/10.1017/S1368980020001627>

References

1. Beecher GR (2003) Overview of dietary flavonoids: nomenclature, occurrence and intake. *J Nutr* **133**, 3248s–3254s.
2. Hooper L, Kroon PA, Rimm EB *et al.* (2008) Flavonoids, flavonoid-rich foods, and cardiovascular risk: a meta-analysis of randomized controlled trials. *Am J Clin Nutr* **88**, 38–50.
3. Tang Z, Li M, Zhang X *et al.* (2016) Dietary flavonoid intake and the risk of stroke: a dose-response meta-analysis of prospective cohort studies. *BMJ Open* **6**, e008680.
4. Ivey KL, Lewis JR, Lim WH *et al.* (2013) Associations of proanthocyanidin intake with renal function and clinical outcomes in elderly women. *PLoS One* **8**, e71166.
5. Grosso G, Micek A, Godos J *et al.* (2017) Dietary flavonoid and lignan intake and mortality in prospective cohort studies: systematic review and dose-response meta-analysis. *Am J Epidemiol* **185**, 1304–1316.
6. Ivey KL, Jensen MK, Hodgson JM *et al.* (2017) Association of flavonoid-rich foods and flavonoids with risk of all-cause mortality. *Br J Nutr* **117**, 1470–1477.
7. Ivey KL, Lewis JR, Prince RL *et al.* (2013) Tea and non-tea flavonol intakes in relation to atherosclerotic vascular disease mortality in older women. *Br J Nutr* **110**, 1648–1655.
8. Bhagwat S, Haytowitz DB & Holden JM (2011) *USDA Database for the Flavonoid Content of Selected Foods*. Beltsville, MD: U.S. Department of Agriculture.
9. Ivey KL, Hodgson JM, Croft KD *et al.* (2015) Flavonoid intake and all-cause mortality. *Am J Clin Nutr* **101**, 1012–1020.
10. Raman G, Avendano EE, Chen S *et al.* (2019) Dietary intakes of flavan-3-ols and cardiometabolic health: systematic review and meta-analysis of randomized trials and prospective cohort studies. *Am J Clin Nutr* **110**, 1067–1078.
11. Hui C, Qi X, Qianyong Z *et al.* (2013) Flavonoids, flavonoid subclasses and breast cancer risk: a meta-analysis of epidemiologic studies. *PLoS One* **8**, e54318.
12. Yuan C, Spiegelman D, Rimm EB *et al.* (2017) Validity of a dietary questionnaire assessed by comparison with multiple weighed dietary records or 24-hour recalls. *Am J Epidemiol* **185**, 570–584.
13. Willett W (2013) *Nutritional Epidemiology*, 3rd ed. Oxford: Oxford University Press.
14. Erlund I, Silaste ML, Alftan G *et al.* (2002) Plasma concentrations of the flavonoids hesperetin, naringenin and quercetin in human subjects following their habitual diets, and diets high or low in fruit and vegetables. *Eur J Clin Nutr* **56**, 891–898.
15. Loke WM, Jenner AM, Proudfoot JM *et al.* (2009) A metabolite profiling approach to identify biomarkers of flavonoid intake in humans. *J Nutr* **139**, 2309–2314.
16. Zhang Y, Li Y, Cao C *et al.* (2010) Dietary flavonol and flavone intakes and their major food sources in Chinese adults. *Nutr Cancer* **62**, 1120–1127.
17. Zhang Y, Cao J, Chen W *et al.* (2010) Reproducibility and relative validity of a food frequency questionnaire to assess intake of dietary flavonol and flavone in Chinese university campus population. *Nutr Res (New York, NY)* **30**, 520–526.



18. Sun C, Wang H, Wang D *et al.* (2015) Using an FFQ to assess intakes of dietary flavonols and flavones among female adolescents in the Suihua area of northern China. *Public Health Nutr* **18**, 632–639.
19. Somerset S & Papier K (2014) A food frequency questionnaire validated for estimating dietary flavonoid intake in an Australian population. *Nutr Cancer* **66**, 1200–1210.
20. Kent K & Charlton KE (2018) Development, validation and reproducibility of a food frequency questionnaire to measure flavonoid intake in older Australian adults. *Nutr Dietetics* **75**, 106–116.
21. Grobbee DE, Rimm EB, Giovannucci E *et al.* (1990) Coffee, caffeine, and cardiovascular disease in men. *N Engl J Med* **323**, 1026–1032.
22. Colditz GA, Manson JE & Hankinson SE (1997) The nurses' health study: 20-year contribution to the understanding of health among women. *J Women's health* **6**, 49–62.
23. Cho E, Chen WY, Hunter DJ *et al.* (2006) Red meat intake and risk of breast cancer among premenopausal women. *Arch Intern Med* **166**, 2253–2259.
24. Neveu V, Perez-Jimenez J, Vos F *et al.* (2010) Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. *Database* **2010**, bap024.
25. Lin J, Zhang SM, Wu K *et al.* (2006) Flavonoid intake and colorectal cancer risk in men and women. *Am J Epidemiol* **164**, 644–651.
26. Ivey KL, Croft K, Prince RL *et al.* (2016) Comparison of flavonoid intake assessment methods. *Food Funct* **7**, 3748–3759.
27. Rosner B & Glynn RJ (2007) Interval estimation for rank correlation coefficients based on the probit transformation with extension to measurement error correction of correlated ranked data. *Stat Med* **26**, 633–646.
28. Perisic I & Rosner B (1999) Comparisons of measures of interclass correlations: the general case of unequal group size. *Stat Med* **18**, 1451–1466.
29. Rosner B & Willett WC (1988) Interval estimates for correlation coefficients corrected for within-person variation: implications for study design and hypothesis testing. *Am J Epidemiol* **127**, 377–386.
30. Willett W & Stampfer MJ (1986) Total energy intake: implications for epidemiologic analyses. *Am J Epidemiol* **124**, 17–27.
31. Caldwell K, Charlton KE, Lee S *et al.* (2018) Dietary flavonoid intake in older adults: how many days of dietary assessment are required and what is the impact of seasonality? *Nutr J* **17**, 7.