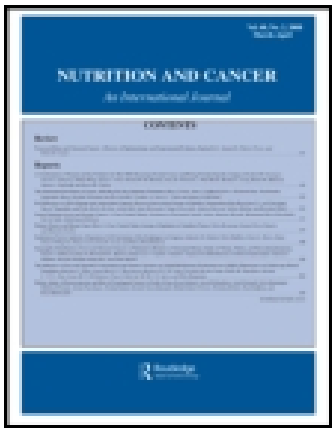

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A Food Frequency Questionnaire Validated for Estimating Dietary Flavonoid Intake in an Australian Population

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Flavonoids, a broad category of nonnutrient food components, are potential protective dietary factors in the etiology of some cancers. However, previous epidemiological studies showing associations between flavonoid intake and cancer risk have used unvalidated intake assessment methods. A 62-item food frequency questionnaire (FFQ) based on usual intake of a representative Australian adult population sample was validated against a 3-day diet diary method in 60 young adults. Spearman's rank correlations showed 17 of 25 individual flavonoids, 3 of 5 flavonoid subgroups, and total flavonoids having strong/moderate correlation coefficients (0.40–0.70), and 8 of 25 individual flavonoids and 2 of 5 flavonoid subgroups having weak/insignificant correlations (0.01–0.39) between the 2 methods. Bland-Altman plots showed most subjects within ±1.96 SD for intakes of flavonoid subgroups and total flavonoids. The FFQ classified 73–90% of participants for all flavonoids except isorhamnetin, cyanidin, delphinidin, peonidin, and pelargonidin; 73.3–85.0% for all flavonoid subgroups except Anthocyanidins; and 86.7% for total flavonoid intake in the same/adjacent quartile determined by the 3-day diary. Weighted kappa values ranged from 0.00 (Isorhamnetin, Pelargonidin) to 0.60 (Myricetin) and were statistically significant for 18 of 25 individual flavonoids, 3 of 5 subgroups, and total flavonoids. This FFQ provides a simple and inexpensive means to estimate total flavonoid and flavonoid subgroup intake.

INTRODUCTION

Flavonoids are a group of nonnutrient food components found extensively and exclusively in plant-based foods. However, substantial variations in the distribution of flavonoid classes can occur, with certain individual flavonoids being restricted to only a small number of foods (1). Over the past 2 decades, an expanding evidence base has emerged for the diverse range of potential health benefits and protective effects provided by this phytochemical category (2).

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There is mounting evidence that specific dietary flavonoid groups are associated with reduced cancer risk. In particular, flavonones associate with lower risk of esophageal squamous cell carcinoma (3), flavones and flavonols against renal cell carcinoma (4), and increased intakes of anthocyanadins, flavones, and flavonols may lower risk of colorectal cancer (5). More generally, flavonoid intake is associated with lower risk of esophageal adenocarcinoma (6,7) and breast cancer (8). These epidemiological patterns have been guided by in vitro mechanistic studies on immune function, cell proliferation and apoptosis (9,10). In addiiton, flavonoid intake can affect lipid storage and oxidation processes, possibly influencing cancer risk indirectly through obesity risk (11). Further, flavonoids can interfere with the bioavailability of various food-borne toxins (12), providing another preventive pathway.

The myriad of preventive and therapeutic possibilities associated with flavonoid intake has seen the development of numerous flavonoid analogues as candidates for new anticancer pharmaceuticals (10). An integral part of understanding the epidemiology associated with flavonoid intake, and indeed the testing of new flavonoid analogues, is the development of straightforward, cost-effective methods to assess flavonoid intake in individuals and populations. This need has been identified through previous review of flavonoid intake methods (13).

The literature on direct assessment of dietary flavonoid intake for epidemiological investigation is relatively sparse. Some key studies have investigated associations between flavonoid intake and disease risk using data and methods that were not designed specifically for that purpose. For example, dietary data from the Nurses Health Study and the Health Professionals Follow-Up Study were analyzed for associations between flavonol (14) and flavonoid (15) intakes with cancer risk using a food frequency questionnaire (FFQ) validated for measuring individual flavonoid sources, but not total dietary intake of these compounds. Similarly, a Finish study (16) assessed flavonol and flavone intake with a FFQ that was not

designed or validated for this purpose. For example, in that study red and white wine were not separated in their FFQ, despite having substantially different flavonoid contents. Fink et al. (8) included 50 items from the Block FFQ on the basis of having measurable flavonoid contents. However, rich sources such as blueberries and raspberries were not included.

More recently, Cassidy et al. (17) have published a post-hoc analysis linking flavonoid intake to hypertension incidence, using data sources comprising two large prospective cohort studies (Nurses Health Study and Health Professionals Follow-up Study). The FFQ was reported to be validated for whole food intake (apple, tea, wine) (17), but validation for flavonoid intake was not reported. Further, the original FFQ grouped some foods together inappropriately and missed some important flavonoid sources (18).

Previously, the intake profiles of flavonoid groups according to age were determined in a representative sample of the Australian population (19). This and another previous study (20) reported variations in flavonoid intake according to age. These previous findings were used to develop a FFQ for use in adults, the validation of which was the objective of the present study, to facilitate collection of longitudinal data on flavonoid consumption, enable direct comparisons of flavonoid intake, enable more precise description of epidemiological links between flavonoid intake and cancer risk, and inform the development and refinement of recommendations to enhance vegetable and fruit intake.

METHODS AND MATERIALS

Participants

Study participants comprised 60 adult undergraduate university (health science) students aged between 18 and 55 years. All participants gave informed consent for their information to be accessed and used. Participants were asked to record their dietary intake on three separate occasions. Firstly, an initial FFQ (FFQ1) was administered during the last week of February (summer). Subjects also recorded a 7-consecutive day diet diary, commencing 16 days post-FFQ1, of which 3 consecutive days (including 1 weekend day) were selected for analysis. Finally, a repeat of the first FFQ (FFQ2) was administered during the first week of June (winter).

FFQ

A self-administered semi-quantitative FFQ was specifically developed to assess dietary flavonoid consumption. The 62 foods selected for the FFQ are representative of the major flavonoid sources for the Australian population (19,21). The FFQ format was modeled on the Harvard adult questionnaire, in terms of frequency categories and serving sizes (22). Reported intake frequencies of each food item were entered into Excel (Microsoft version 14.1.2) and converted to weekly intake frequencies of each food as follows: never or < once/mo = 0; 1–3 times/mo = 0.7; once/wk = 0.43; 2–4 times/wk = 1.7; once/day = 5; 2–3 times/day = 15; 4–5 times/day = 31.5; and 6> times/day = 48. Gram weights of all serving sizes were derived from the food composition database AUSNUT 2007, using the diet analysis software Food Works (Professional 7, Xyrus Software). The flavonoid content of each food was calculated using the 2011 USDA flavonoid content data, which was multiplied by intake frequency to derive intake of each flavonoid (23).

Diet Diary

Subsequent to a training program on competent completion of a dietary record, participants were requested to record a 7-consecutive day diet diary. To enhance accuracy, participants were supplied visual resources to help assess food-serving sizes (24). Participants were asked to weigh and/or measure foods (grams and ml) where possible and include packaged brand names if available. Daily flavonoid intakes from the diet diaries were calculated using AUSNUT 2007 food composition data using the diet analysis software Food Works (professional 7, Xyrus Software) and the USDA flavonoid content data (23). After the first 3 days (Monday, Tuesday, Wednesday), a review session was held to address questions and clarify data quality requirements to participants. Completed diet diaries were collected after completion and three consecutive days comprising (Thursday, Friday, and Saturday) were selected from each participant's diet diary. Diet diaries were used to derive specific gram intakes of all flavonoid rich foods for each subject. The USDA SNAP-ed Education Recipe Finder (25) was used to translate ambiguous recipes including salad, fruit salad, and stirfry for participants who had not specified gram intakes of individual foods. Gram intakes of foods were multiplied by the flavonoid content for each flavonoid rich food using the USDA flavonoid content data (23).

Statistical Analysis

All flavonoid intake data were analyzed using SPSS version 20 and the MedCalc version 12.3.0 program. The relative validity of the FFQ was assessed using a combination of various statistical strategies. The daily mean and median flavonoid intakes (and standard deviations) of all 25 flavonoids recorded by the FFQ1 and 3-day diet diaries were calculated. The daily mean intake frequencies of all 62 foods from the FFQ2 and FFQ1 were also computed. Means from the 3-day diet diaries, the FFQ1 and the FFQ2 were tested for normality using the Kolmogorov-Smirnov test and histograms. A food-based approach to assess the reliability of the FFQ has been used previously for studies on flavonoid intake (26,27) and was applied using a correlation coefficient analysis. Because the FFQ1 and FFQ2 data did not follow a normal distribution, reliability of the FFQ was tested using Spearman correlation. The validity

of the FFQ1 against the 3-day diet diaries was assessed using the correlation coefficient (28), quartiles classification (29), Cohen's Kappa coefficient (30), and the Bland and Altman plot (28) methods.

Because data were not distributed normally, FFQ1 and 3-day mean flavonoid intakes were compared using the Wilcoxon signed rank sum. The strength of association between the FFQ1 and the reference 3-day diet data was assessed using a Spearman rank correlation suitable for assessing non-normally distributed data (31). The FFQ1 and 3-day diet data were tested for level of agreement using Bland and Altman plots, by plotting the difference between the FFQ1 and 3-day diet diaries (FFQ1 vs. 3-day diets) against the average of the FFQ1 and 3-day diets (FFQ1 + 3-day diets)/2 (28). Cohen's Kappa coefficient (K_W) was calculated to assess concordance between the FFQ1 and the 3-day diet diaries, using counts allocated to either high or low daily mg intake categories for all flavonoids and flavonoid subgroups. The Kappa values were interpreted as follows: <0 (poor concordance), 0.00–0.20 (weak concordance), 0.21–0.40 (fair concordance), and 0.41–0.60 (moderate concordance) (32). To determine the ability of the FFQ to rank individuals by intake, individual flavonoids, flavonoid subgroups, and total flavonoid intakes from the 3-day diets and the FFQ1 were each ranked into quartiles and then compared according to whether they occurred in the same, adjacent, or nonadjacent quartiles (29).

RESULTS

In the validation process of comparing the FFQ1 with the 3-day diet diary, mean intakes of each of the 25 flavonoids were consistently higher in the FFQ (Wilcoxon $P < 0.05$). The Spearman's rank correlations for the FFQ1 and the 3-day diet diary showed 17 of 25 individual flavonoids, 3 of 5 of the flavonoid subgroups, and total flavonoids having strong to moderate correlation coefficients (0.40–0.70), and 8 of 25 individual flavonoids along with 2 of 5 flavonoid subgroups having weak or insignificant correlations (0.01–0.39). The Bland and Altman plots indicated that the 2 tools had either a good or fair agreement for the majority of the participants' intakes. These plots also displayed that the 2 tools shared a higher level of agreement at lower intake levels than at higher intake levels (Fig. 1). The allocation of quartiles according to flavonoid intake classified between 73.0–90.0% of all 60 participants for 20 of the 25 flavonoids in the same or adjacent quartile and between 8.3–26.7% in a nonadjacent quartile for the same 20 flavonoids. The 2 tools classified between 56.7–68.4% of all 60 participants for 5 out of the 25 flavonoids in the same or adjacent quartile and between 31.6–43.3% in a nonadjacent quartile for the same 5 flavonoids (see Table 1). The allocation of quartiles according to flavonoid subgroup intake classified between 73.3–85.0% of all 60 participants for 4 of the 5 flavonoid subgroups in the same or adjacent quartile and between 15.0–25.0% in a nonadjacent quartile for the same 4 flavonoid subgroups. The 2 tools classified 63.3% of all 60 participants for 1 of the 5 flavonoid subgroups in the same or adjacent quartile and 36.7% in a nonadjacent quartile for the same 1 flavonoid subgroup. The allocation of quartiles according to total flavonoid intake classified 86.7% of all 60 participants in the same or adjacent quartile and 13.3% in a nonadjacent quartile for total flavonoid intake.

The weighted kappa values ranged from 0.00 for Isorhamnetin and Pelargonidin to 0.60 for Myricetin and were statistically significant for 18 of the 25 individual flavonoids, for 3 of the 5 subgroups and for total flavonoids. Among the flavonoid subgroups, there was moderate concordance (kappa values 0.41–0.60) for both flavon-3-ols and for total flavonoids. There was fair concordance (kappa values 0.21–0.40) for the flavonols and flavanones subgroups. There was weak concordance (kappa values 0.00–0.20) for the flavones and anthocyanidins subgroups. Among the individual flavonoids, there was moderate concordance (kappa values 0.41–0.60) for kaempferol, myricetin, epicatechin, catechin, epicatechin 3-gallate, epigallocatechin, gallocatechin, theaflavin, theaflavin-3, 3′-digallate, theaflavin-3′-gallate, and thearubigins. There was fair concordance (kappa values 0.21–0.40) for the following flavonoids: apigenin, luteolin, eriodictyol, hesperetin, naringenin, malvidin, and petunidin. There was weak concordance (kappa values 0.00–0.20) for the following flavonoids: isorhamnetin, quercetin, peonidin, pelargonidin, cyaniding, and delphinidin (Table 1).

The test-retest reliability for the FFQ (FFQ1 vs. FFQ2, Spearman's rank) found that 60 of the 62 foods ranged between 0.43 and 0.93. Two foods (kale and common lettuce) showed weak correlation coefficients (0.37–0.38). All 62 foods had statistically significant correlation coefficients (see Table 2).

DISCUSSION

The validity of a FFQ is the degree to which it measures a specific (set of) dietary attribute(s), and the validation process involves comparison of the FFQ with another (reference) method to assess the same dietary attributes (33). The FFQ developed for the present study showed a good level of validity for dietary total flavonoid and flavonoid subgroup intake, using a diet diary as the reference method in this specific population. The FFQ1 mean intake levels were consistently significantly higher than the 3-day diet diary means, which is a typical finding when comparing these 2 methods (28,34,35). The Spearman's correlation found that 17 of 25 flavonoids, 3 of 5 of the flavonoid subgroups and total flavonoids shared moderate to strong correlation coefficients. The 8 of 25 flavonoids and 2 of 5 flavonoid subgroups with weak or insignificant associations comprised cyanidin, delphinidin, peonidin, malvidin and pelargonidin (anthocyanidins), quercetin and isorhamnetin (flavonols), and apigenin. Only a limited number of food sources contain high amounts of anthocyanidins (36). One such food is blueberries, the availability of which is

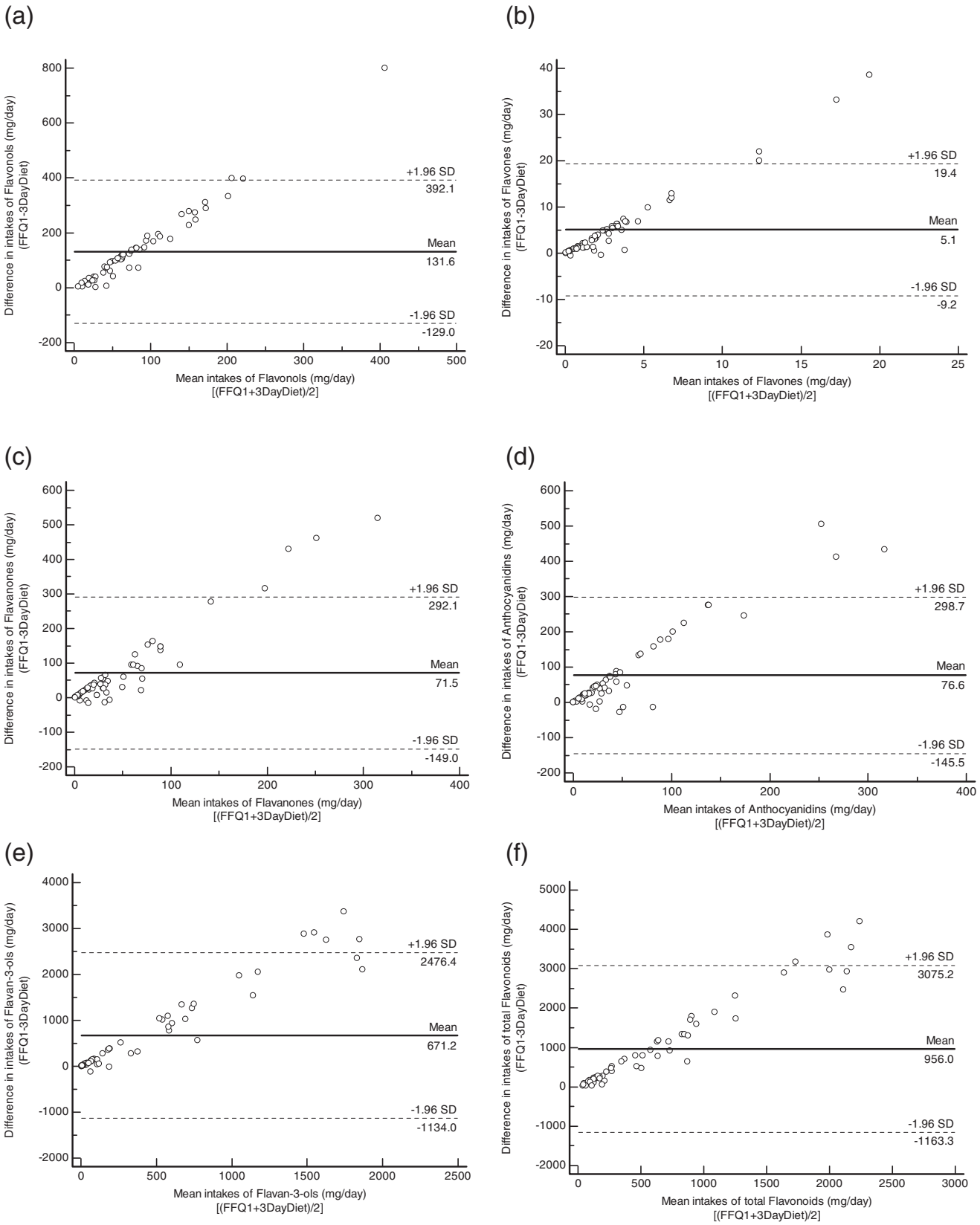

FIG. 1. Bland-Altman plots showing the relative validity of the initial food frequency questionnaire (FFQ1) vs. the 3-day diet diaries for the following flavonoid subgroups: flavonols (a), flavones (b), flavanones (c), anthocyanidins (d), flavon-3-ols (e), and for total flavonoids (f).

TABLE 1
Comparison of within subject allocation to quartiles of intake (mg/day) for each flavonoid between initial food frequency questionnaire (FFQ1) and 3-day food record methods

	3-day diet		FFQ1						
N25 flavonoid	Median (min, max)	IQR	Median (min, max)	IQR	Spearman *r*	Same quartile	Adjacent quartile	Nonadjacent quartile	K_W
Isorhamnetin	0.0 (0.0, 8.8)	0.0	0.1 (0.0, 24.1)	0.9	0.10	18	23	19	0.00
Kaempferol	0.8 (0.0, 17.6)	1.8	6.3 (0.2, 86.2)	17.7	0.63*	26	27	7	0.53***
Myricetin	0.3 (0.0, 3.6)	0.9	3.0 (0.0, 35.0)	8.4	0.67*	36	15	9	0.60***
Quercetin	7.4 (1.0, 40.6)	8.3	104.5 (5.6, 788.5)	115.0	0.26*	20	24	16	0.20
Flavonols subgroup	9.4 (1.5, 48.6)	12.6	114.1 (6.7, 806.1)	143.8	0.39**	22	23	15	0.27*
Apigenin	0.0 (0.0, 3.3)	0.2	1.6 (0.0, 33.8)	3.6	0.34*	20	30	10	0.40**
Luteolin	0.2 (0.0, 2.1)	0.3	1.4 (0.0, 27.4)	1.9	0.54*	23	27	10	0.40**
Flavones subgroup	0.2 (0.0, 3.4)	0.5	3.53 (0.0, 38.6)	4.8	0.41**	21	24	15	0.20
Eriodictyol	0.0 (0.0, 0.8)	0.0	0.2 (0.0, 28.4)	1.3	0.40*	19	31	10	0.37**
Hesperetin	1.2 (0.0, 54.0)	13.5	26.4 (0.0, 471.4)	52.5	0.42*	17	28	15	0.27*
Naringenin	1.3 (0.0, 14.6)	4.2	11.0 (0.3, 221.4)	17.7	0.40*	22	23	15	0.40**
Flavanones subgroup	4.7 (0.0, 61.6)	19.9	41.1 (0.5, 574.7)	89.3	0.40**	19	25	16	0.40**
Epicatechin	8.7 (0.0, 37.6)	14.2	54.1 (0.4, 568.8)	85.4	0.50*	21	30	9	0.47***
Catechin	2.6 (0.0, 18.9)	4.7	22.1 (0.0, 195.8)	44.0	0.57*	25	22	13	0.47***
Epicatechin 3-gallate	0.0 (0.0, 88.7)	8.9	8.8 (0.0, 409.9)	84.4	0.67*	26	24	10	0.47***
Epigallocatechin	0.4 (0.0, 61.5)	13.0	14.0 (0.0, 566.8)	121.1	0.70*	31	21	8	0.53***
Epigallocatechin 3 gallate	0.1 (0.0, 370.5)	9.7	23.8 (0.0, 1595.0)	219.5	0.41*	23	22	15	0.23
Gallocatechin	0.0 (0.0, 8.6)	0.1	0.3 (0.0, 31.0)	3.5	0.40*	26	25	9	0.41**
Theaflavin	0.0 (0.0, 10.9)	0.1	0.3 (0.0, 39.2)	4.5	0.59*	24	31	5	0.53***
Theaflavin-3, 3'-digallate	0.0 (0.0, 12.0)	0.0	0.2 (0.0, 43.3)	5.0	0.55*	23	31	6	0.46***
Theaflavin-3'-gallate	0.0 (0.0, 19.0)	0.0	0.3 (0.0, 68.3)	7.8	0.55*	23	31	6	0.46***
Thearubigins	0.0 (0.0, 558.9)	2.2	9.1 (0.0, 2015.2)	230.4	0.55*	23	31	6	0.46***
Flavan-3-ols subgroup	15.2 (0.0, 813.0)	115.0	175.7 (0.8, 3428.0)	1038.4	0.60***	27	24	9	0.53***
Malvidin	0.0 (0.0, 52.6)	0.1	4.2 (0.0, 231.4)	16.9	0.30*	20	28	12	0.24*
Peonidin	0.0 (0.0, 11.4)	0.1	1.4 (0.0, 58.4)	4.6	0.17	15	24	21	0.10
Petunidin	0.0 (0.0, 8.7)	0.0	0.7 (0.0, 45.3)	3.4	0.44*	18	32	10	0.25**
Pelargonidin	0.0 (0.0, 0.1)	0.0	0.1 (0.0, 1.4)	0.1	0.01	18	16	26	0.00
Cyanidin	1.9 (0.0, 20.2)	3.7	14.3 (0.0, 212.7)	32.4	0.10	12	29	19	0.07
Delphinidin	0.0 (0.0, 22.8)	0.0	3.2 (0.0, 146.4)	8.7	0.15	13	27	20	0.17
Anthocyanidins subgroup	2.0 (0.0, 99.8)	8.0	43.5 (0.0, 533.5)	66.0	0.10	14	24	22	0.07
Total flavonoids	60.3 (1.8, 874.3)	130.9	597.3 (53.6, 4343.8)	1292.6	0.64***	24	28	8	0.53***

IQR = interquartile range; Kw = weighted Kappa.
* Significant at $P < 0.05$.
** Significant at $P < 0.01$.
*** Significant at $P < 0.001$.

TABLE 2
Reliability of the food frequency questionnaire (FFQ; Spearman's rank correlation between FFQ1 and FFQ2 for each of the 62 food items)

Foods	Spearman *r*	Foods	Spearman *r*	Foods	Spearman *r*
Avocado	0.75***	Onion	0.64***	Grapefruit juice	0.52***
Broad bean	0.68***	Onion cooked	0.52***	Lemon	0.62***
Green beans	0.72***	Parsley	0.43**	Lemon juice	0.66***
Beets	0.78***	Parsley salad	0.59***	Mandarin	0.62***
Broccoli	0.63***	Peas	0.74***	Mango	0.67***
Brussels sprouts	0.43**	Rhubarb	0.57***	Nectarine	0.69***
Green cabbage	0.71***	Silver beet	0.48***	Orange	0.73***
Red cabbage	0.60***	Chinese spinach	0.70***	Orange juice	0.72***
Capsicum	0.65***	English spinach	0.63***	Pear	0.72***
Carrot	0.71***	Sweet potato	0.62***	Dark chocolate	0.76***
Carrot juice	0.62***	Tomato	0.80***	Milk chocolate	0.69***
Cauliflower	0.56***	Tomato sauce	0.67***	Cocoa	0.67***
Celery	0.63***	Zucchini	0.73***	Brewed coffee	0.93***
Eggplant	0.72***	Apple	0.75***	Instant coffee	0.72***
Endive	0.47***	Apple juice	0.69***	Black tea	0.69***
Kale	0.37*	Apricot	0.58***	Green tea	0.85***
Lettuce common	0.38*	Blueberries	0.80***	Tomato juice	0.47***
Lettuce iceberg	0.48***	Cherries	0.59***	Vegetable juice	080***
Lettuce cos leaf	0.71***	Grapes	0.74***	Red wine	0.77***
Mushroom	0.73***	Grape juice	0.66***	White wine	0.70***
Olive	0.56***	Grapefruit	0.80***		

*Significant at $P < 0.05$.
** Significant at $P < 0.01$.
*** Significant at $P < 0.001$.

highly seasonal, and the 3-day diet diary was recorded during late March (autumn) contrasting with blueberry season (December). Intakes for the flavonoids cyanidin, delphinidin, peonidin, malvidin, and pelargonidin in the 3-day diet diaries were on average 35% lower than for the FFQ1 for the same flavonoids. Multiple recordings of the 3-day diet diaries taken across all seasons would likely increase reported consumption of these flavonoids. Previous research also found weak correlation coefficients for the flavonoids quercetin, isorhamnetin, and apigenin (36). Although these flavonoids are available in a broader range of food sources than the anthocyanidins, they are still limited to a small variety of foods (quercetin—tea, onions, apples, cocoa; isorhamnetin—parsley, almonds, onions; apigenin—parsley, celery, capsicum) (36).

The Bland and Altman analysis extends the process of validation beyond simple correlation assessment. It tests agreement between a (test) method of interest and a reference method by plotting the differences between the two dietary methods against the average of the two methods (29). The limits of agreement demonstrate how well the two tools agree and is set within (+ 2SD) from the overall mean difference of the two measurement tools (28). Differences between the paired means within + 1 SD of the mean difference are generally considered to have good agreement, differences between the paired means equal to the LOA (+ 2SD) are considered to have fair agreement and differences between the paired equal to + 3SD are considered poor (37). The Bland and Altman plots in the present study found a systematic bias where differences between the two methods increased as intake increased. This bias does not compromise the ability of the FFQ to rank subjects according to intake and reflects the semiquantitative nature of FFQs (18).

In relation to variations between individual subjects, both the FFQ and dietary diary classified the majority of all 60 participants for 20 of 25 flavonoids, for 4 of 5 flavonoid subgroups and for total flavonoid intake in the same or adjacent quartile. These two tools classified over half of all 60 participants for the 5 flavonoids cyanidin, delphinidin, peonidin, malvidin, and pelargonidin and the anthocyanidin subgroup in the same or adjacent quartile and as high as 43.3% in a nonadjacent quartile for the same 5 flavonoids. The 20 flavonoids and the total flavonoids displayed a very small frequency of misclassification and very good agreement between the 2 tools. The frequency of misclassification was higher for the same 5 flavonoids (above) and the anthocyanadin subgroup found to have the weakest correlation coefficients earlier. However as over half of participants were classified into the same or

adjacent quartiles for these 5 flavonoids and the anthocyanidin subgroup the tools still showed good agreement. In general, the FFQ classified flavonoid intake into the same quartile of intake as the diet diaries. Generally, misclassification occurred for anthocyanidin intake, likely due to these flavonoids being derived from highly seasonal food sources. However, a small proportion of participants showed a higher consistency between the methods for these flavonoids. Fourteen of 25 flavonoids yielded zero median intakes from the diet diary, thus making comparisons of quartile classification by individual flavonoid intakes difficult. To account for this, quartile classification by flavonoid subgroups and total flavonoids were included. Likewise, the assessment of concordance between the FFQ1 and the 3-day diet diaries was assessed using flavonoid intake data that were dichotomized into high and low intakes rather than by quartile classification. The findings from this analysis together with the findings from the quartile classification of flavonoid intakes by subgroups and total flavonoids revealed a similar level of agreement to the individual flavonoid intake quartile comparison.

The FFQ and 3-day diet diaries displayed a moderate concordance in their assessment of total flavonoid intake. Similarly, the flavan-3-ols subgroup and the majority of its associated flavonoids also displayed a moderate concordance between the two tools. The majority of all of the individual flavonoids and their subgroups displayed a fair concordance at the minimum. The flavonoid myricetin had the highest kappa value (representative of a moderate concordance). This finding was consistent with the high correlation and quartile classification findings for this flavonoid (Table 1). This may reflect the ubiquitous occurrence of myricetin across vegetables and fruits (1). Few of the individual flavonoids and their subgroups displayed a weak concordance. Conversely, when the weighted Kappa coefficient was calculated based on counts allocated by quartiles, the majority of the flavonoids and their subgroups displayed weak concordance (not shown).

In general, these data show that the FFQ is useful at assessing intakes at this level, but more specific tools may be required to assess intake of the individual flavonoids for which median intakes were zero (Table 1).

Reliability assessment of the FFQ was conducted to assess its stability over time. Thus, responses to individual food items within the FFQ were assessed. Reliability estimates varied for vegetables, 0.37 (kale) to 0.78 (beets); fruit, 0.58 (apricots) to 0.80 (tomato, blueberries, grapefruit); and beverages, 0.47 (tomato juice) to 0.93 (brewed coffee), with most falling into the generally acceptable range of >0.5 (33). Previously reported reliability figures for FFQs measuring vegetable and fruit intake vary according to factors such as age group, consumption environment, and specific food items tested. One early study (38) found correlations of 0.41 for both vegetables and fruits. More recently, Knett et al. (39) showed short-term reliabilities of 0.63 and 0.64 for vegetables and fruits, respectively. Landais et al. (40) found 0.47 for vegetables and 0.54 for fruit, with combined reliability for vegetables and fruit of 0.56. However, Macedo-Ojeda et al. (41) found lower values of 0.5 and 0.59 for vegetables and fruit, respectively. In contrast to the present study, Jarvinen et al. found that specific foods such as berries have lower levels (0.39) (42). Reliability estimates seem higher in children, with common vegetables (including green salad, peas, green beans, corn) and fruits (such as banana, apple, grapes) of 0.83 and 0.88, respectively (43).

The literature on direct assessment of dietary flavonoid intake, either for empirical measurement, or for epidemiological investigation is relatively sparse. A range of post-hoc analyses of large case-control and cohort studies have explored potential links between FFQ-derived flavonoid intake and risk of various forms of cancer. A case-control study by Christensen et al. (44) found an association between low flavonoid intake and increased lung cancer risk, with odds ratios (ORs) consistently lower for higher intakes of anthocyanidins, flavon-3-ols, flavones, flavonols, and flavonones (0.82, 0.67, 0.68, 0.62, and 0.67, respectively), as well as 0.63 for total flavonoids. In another case-control study, Theodoratou et al. (45) showed lower risk of colorectal cancer for highest quartiles of intake for flavonols, quercetin, catechin, epicatechin, and procyanidins (ORs = 0.73 0.68, 0.68, 0.74, and 0.78, respectively). Post-hoc analysis of the EPIC study, a major European prospective study on nutrition-related determinants of cancer risk, revealed associations between total flavonoid intake and decreased risk of gastric adenocarcinoma (46) in the general population, and eosophageal cancer in smokers (47). Flavonol intake was associated with decreased risk of hepatocellular carcinoma (48). However, no association between flavonoid intake and breast cancer risk was identified (49), although a recent meta-analysis concluded that flavanol and flavone intake may inversely associate with breast cancer risk (50).

All of these studies assume a FFQ measure of flavonoid intake which is valid (i.e., the questionnaire actually measures the aspect of diet that it was designed to measure) (33). There have been various approaches to the use of FFQ to assess flavonoid consumption, with many centering on the distinction between using a validated FFQ and a FFQ validated specifically for flavonoid intake. Emphasis often is placed on the quality of food composition data and size of the study, rather than the ability of FFQ to measure flavonoid intake. Although the FFQ used in the study by Christensen et al. (44) was validated against a diet diary method (51), validation was conducted only on energy, macronutrient, vitamin, and mineral intake and not flavonoid intake. The EPIC FFQ was indeed validated in terms of foods and food groups. However, validation was reported only for vegetables and fruit collectively rather than the individual vegetables and fruits (52). Further, the design of the FFQ is problematic in terms of estimating the intake of total flavonoids and flavonoid subgroups, because the Norfolk version of the EPIC FFQ did not contain rich sources of anthocyanidins such as aubergine and blueberries, did not distinguish red and white wine nor black and green tea, yet

grouped each of "peaches, plums, apricots;" "dried fruits;" "tinned fruit;" "green salad, lettuce, cucumber, celery;" and "strawberries, raspberries, kiwi fruit" as single items. Bingham et al. (53) noted that the EPIC FFQ was based on the US Nurses' Health Study, which also has been shown to have limited validity in terms of assessing flavonoid intake (18).

In contrast, the FFQ of Theodoratou et al. (45) was validated for flavonoid intake using a 4-day diet diary as a reference using correlation coefficients, rather than Bland-Altman anlaysis (54). Data were collected using the Scottish Collaborative Group FFQ, samples of which indicate that food items may present food grouping issues similar to the EPIC FFQ (e.g., "fruit juice" that does not distinguish between apple and orange, having 2 very distinct flavonoid profiles). In addition, the study by Theodoratou et al. (45) omitted apigenin, luteolin and gallates from their analysis on the basis of an absence of compositional information, which may reflect the design of the original FFQ.

Fink et al. (8) selected foods with measureable amounts of at least 1 flavonoid from the Block questionnaire, which has been validated for multiple nutrients but not flavonoids. Bosetti et al. (55) reported using a validated FFQ for their study on flavonoids and breast cancer. However, whilst the FFQ was validated for a range of macronutrients, minerals and vitamins, it was not validated for flavonoid intake. Similarly, Peterson et al. (56) used a FFQ validated for a broad range of nutrients, but not flavonoids (57), to establish a link between flavonoid intake and breast cancer risk. The study by Adebamaowo et al. (26) conducted a food-based validation of their FFQ, which is an important step in the validation process. However, the NHS FFQ groups together some foods with dissimilar flavonoid profiles, which compromises validity for flavonoid intake assessment. The study by Arts et al. (27) explicitly states that their FFQ was not validated for catechin intake, despite being the major independent variable of the study. This study did however conduct a food-based validation. The study by Wang et al. (58) only determined intake of apple, broccoli, onion, tofu, and tea (nominating these as major sources of flavonoids in the US diet). That study specified that intake of flavonols, flavones and flavonoid-rich foods as independent variables. This list is remarkably restricted.

Because the primary dietary data collection methods in many of these post-hoc studies were not specifically designed to measure flavonoid intake, the aforementioned associations need to be interpreted with care. Within the emerging literature on associations between flavonoid intake and cancer risk, there seems to be some uncertainty about the process of validation of FFQs for the purpose of the estimation of flavonoid intake. The FFQs for major studies such as EPIC and the U.S. Nurses' Health Study have indeed been validated for energy, macronutrient, vitamin and mineral intake, but not for flavonoid intake. The use of FFQs out of context increases the likelihood of both type I and/or type 2 errors.

A single Belgian study has focused on the specific methodological issues related to assessing flavonoid intake using an FFQ methodology. The subject group in that study comprised dietitians and tested the validity of a FFQ developed for assessing flavonoid intake using the previous 2003 USDA flavonoid content table. The authors concluded that their FFQ was a reliable tool for estimating total flavonoid intake when assessing relationships between flavonoid intake and health outcomes but conceded that their FFQ was only validated for certain flavonoids (36). Further, the authors reported that their FFQ was developed using flavonoid rich foods, however did not include a reference to which foods were included in the FFQ and where the food source information was derived from, which makes specific comparison with the present study difficult. A noteworthy flavonoid excluded in their study was epigallocatechin gallate, which is the most abundant of all the catechins and is usually consumed through green tea (59). Its antioxidant activity is 25 to 100 times more powerful than vitamin C and E (59) and has also shown to be beneficial for helping to treat the HIV virus, blocking the Hepatitis C virus and also for helping to treat various types of cancers (60). Such exclusions are an important consideration in the choice of FFQs for assessing flavonoid intake and their potential associations with disease outcomes.

Many validation studies calculate the correlation coefficient between a new tool and the standard reference tool (29,37,61,62) despite criticism by Bland and Altman who suggested that even tools with poor agreement could still be highly correlated (63). The present study calculated the correlation coefficient between a new tool (FFQ) and a reference method (weighed diet diary) in conjunction with other statistical methods recommended for assessing a FFQ's validity (29, 64). Although there is no gold standard reference tool (36), weighed dietary records have shown to have the least amount of correlated errors in previous validation studies (62).

The ideal duration of a diet diary is a continuing polemic. It is generally accepted that it takes up to 1 mo of recording dietary intake to adequately reflect an individual's diet. However, there is some evidence that the validity of a diet record decreases over such a long period because of respondent fatigue (62), and there may be little advantage, in terms of dietary data quality, in extending dietary diary data beyond 3 days (65).

The best method for assessing repeatability of a measurement tool is by repeated measurements in a group of participants (63). The FFQ was administered on 2 separate occasions, 3 mo apart to reduce the likelihood of a memory effect, an approach that has been used effectively elsewhere (62). The FFQ showed high reliability, as demonstrated by moderate to high correlations for 60 of 62 foods and weak but significant correlations for only 2 of 62 foods.

The flavonoids that showed misclassification of intake quartile between FFQ and diet diary can globally be described as either having highly seasonal food sources or having particularly low levels of intake in general. In the case of eriodictyol, the major dietary source in the present study was citrus,

which have very low concentrations. Small variations in reported intake of these foods may correspond to potentially large variations in the proportion of intake, and a separate specific dietary assessment tool may be necessary to estimate intake for such flavonoids

In the validation of FFQs, the choice of reference (comparison) method for intake is critical. The present study indicated the validation of the FFQ against a 3-day diet diary. A further step in relating FFQ intake to biological context would be to compare intake data to biochemical/clinical assay measures such as a serum indicator of intake. Because there is no general clinical indicator of total flavonoid intake, this would need to be done by assaying for individual flavonoids and/or their metabolites—a complex undertaking, given variations in bioavailability, chemistry, persistence, and potential destination within the body according to flavonoid.

The present study also has limitations that need to be considered. A relatively homogenous, purposive sample comprised the study population, which, whilst enhancing internal validity, presents limitations in generalizability (66). Further studies in other settings and populations are required to extend the validity of this FFQ more generally. In addition, a repeated measure study on the FFQ covering a full 12-mo cycle, will provide better insight into reliability and potential bias due to seasonality (67). This FFQ does not measure energy intake or other attributes used to control for bias or confounding. The FFQ was designed as a stand-alone module, to provide versatility in terms of other data collection tools it is combined with. For example, to control for isoflavone intake, this FFQ could be combined with a well-established tool such as that developed by Frankenfeld et al. (68). The present study assumes a relatively consistent bioavailability of flavonoids. The content of this FFQ may require modification as more specific information on variations in flavonoid bioavailability according to food preparation factors emerges. Further, the issue of how to deal with mixed foods or recipes in FFQs has been identified previously as a continuing challenge (67).

There are several critical development features of the FFQ in the present study that support its validation which are important to note, and have, to varying degrees, been absent in previous studies. Firstly, the FFQ comprised individual food items that are either flavonoid-rich, or known to be major flavonoid sources in the Australian population. Further, foods that have important variations in flavonoid profiles (e.g., tea, wine) were subcategorized to reflect such variations. A food-based test-retest reliability analysis was conducted to assess the reliability of FFQ and individual questions therein. Finally, the validity of the FFQ to assess an extensive list of flavonoids was conducted using a 3-day diet diary as a reference method. The aforementioned 4 steps may provide a useful guide for FFQ development in general.

In the absence of the complexity of clinical testing, the FFQ developed in the present study provides a general means by which total and flavonoid subgroup intake can be measured simply and inexpensively. As such, it may be a useful adjunct to studies which explore the role of flavonoid intake across human populations. Validation against biomarkers of intake of specific flavonoids would enhance its utility further.

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