## ORIGINAL RESEARCH

# Development, validation and reproducibility of a food frequency questionnaire to measure flavonoid intake in older Australian adults

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### Abstract

**Aim:** To develop and assess the validity and reproducibility of a food frequency questionnaire (FFQ) to measure total flavonoid intake, and individual flavonoid subclasses, in older adults.

**Methods:** Retrospective analysis of flavonoid intake in older adults informed the development of a FFQ to measure flavonoid intake and determine the flavonoid subclasses consumed (anthocyanins, flavan-3-ols, flavones, flavonols and flavanones). Older adults (n = 42, mean age 75.3 ± 8.6 years) attended two interviews 1 month apart where anthropometrics (height and weight), blood pressure (BP), demographic data and a 93-item self-administered FFQ were collected. A 4-day food record (FR) was randomly administered between the two interview dates, and each food item was assigned a flavonoid and flavonoid subclass content using the United States Department of Agriculture flavonoid database. The criterion validity and reproducibility of the FFQ was assessed against a 4-day FR using the Wilcoxon signed-rank sum test, Spearman's correlation coefficient (r), Bland-Altman Plots and Cohen's kappa.

**Results:** Total flavonoid intake was determined (median intake FFQ = 919.3 mg/day, FR = 781.4 mg/day). Tests of validity indicated that the FFQ consistently overestimated total flavonoid intake compared with the 4-day FR. There was a significant difference in estimates between the FFQ and the 4-day FR for total flavonoid intake (Wilcoxon signed-rank sum $P < 0.001$; Bland-Altman plots indicated large bias and wide limits of agreement), but they were well correlated (Spearman's r 0.93, $P < 0.001$; Cohen's kappa $\kappa = 0.619$, $P < 0.001$). For individual flavonoid subclasses, the tests of validity indicated greater discrepancy compared with 4-day FR. The FFQ showed high reproducibility for estimating total flavonoid intake (FFQ1vsFFQ2: Wilcoxon signed-rank sum test, $P > 0.05$; Spearman's r 0.91, $P < 0.001$; Bland-Altman plots visually showed small, non-significant bias and wide limits of agreement; and Cohen's kappa $\kappa = 0.619$, $P < 0.001$), with a small mean percentage difference (6.7%). For individual flavonoid subclasses, the tests of reproducibility between FFQ1 and FFQ2 showed similarly high reproducibility.

**Conclusions:** The developed FFQ appears suitable for satisfactorily ranking individuals according to total flavonoid intake. The FFQ shows limitations for estimating absolute total flavonoid intake and intake of flavonoid subclasses in comparison to a 4-day FR in terms of overestimating intake. Refinement and further validation of this tool may be required.

**Key words:** dietary assessment, flavonoids, food frequency questionnaire, older adults.

## Introduction

Flavonoids are a class of dietary phytochemicals, which are further divided into six major classes: anthocyanins, flavanols, flavanones, flavones, flavonols and isoflavones.[1] A diet rich in the flavonoids has been linked to a reduced risk of conditions associated with ageing, including cancers, cardiovascular and neurodegenerative diseases.[2] Despite a large body of evidence that links flavonoid consumption with improved health outcomes in older adults, information on the consumption patterns of flavonoids in this population

is sparse, with only a few Australian estimations available in the literature.[3]

The dietary assessment instruments currently used to measure flavonoid intake are limited in their ability to accurately assess habitual dietary intake. The limitations associated with current methods hinder the interpretation of observational research outcomes that associate dietary flavonoid intake and specific health outcomes.[4] Inconsistent methodologies have been used to quantify flavonoid intake, and few survey tools have been validated specifically for this purpose.[5] Previous studies have most commonly collected dietary information using food recall or food record (FR) methods in order to determine intake of flavonoids. These dietary data are then cross-referenced with flavonoid-specific food composition databases to assign foods a flavonoid content value. However, the burden of these methods can be large on both participants and researchers.

Previously published flavonoid-specific food frequency questionnaires (FFQ) have focused on measuring individual flavonoids or flavonoid subclasses, rather than measurement of the comprehensive range of total flavonoid intake. For example, a FFQ[6] was validated to assess intake of tea-related polyphenols, compared with a standard FFQ and 4-day FRs. The same research group developed a more focused FFQ to measure D-limonene intake provided from a range of citrus sources[7] as the basis for the assessment of intake of this individual flavonoid. Although accurate, if these types of instruments were expanded to provide information on the full range of flavonoids, the time burden on the respondent would be excessive. Research has also utilised *post hoc* analysis of pre-existing FFQs that were not developed or validated for the purpose of specifically measuring flavonoid intakes.[8,9] This method has demonstrated large limitations in accurately assessing flavonoid consumption because of food groupings that may have similar nutritional profiles (i.e. pears and apples) that may appear sensible to the respondent to consider together when reporting their fruit intake, but which may have vastly different flavonoid contents, leading to inaccurate assessment of flavonoid intake.

Recent Australian research has validated a flavonoid-specific FFQ in young adults against 3-day FRs.[10] Additionally, total flavonoid consumption[3] and the main sources of flavonoids[5] have been estimated in an Australian population using 24-hour recall data. The different eating patterns of older Australians was reflected in different dietary sources of flavonoids compared to younger adults, whereby contributions from tea and wine flavonoids increased markedly with age.[3] Given the differences in total flavonoid intake, as well as the different major dietary contributions thereof in young and older adults,[3] it is important to develop and validate a FFQ to measure the intake in older adults specifically. A flavonoid-specific FFQ, which includes a list of both flavonoid-rich foods and the most commonly consumed food items that contribute to total overall intake, would provide a useful contribution to research that investigates flavonoid consumption in older Australian cohorts. The advantages of both FFQ and diet history methods to assess long-term intake patterns of nutrients, and to a lesser extent, foods and food groups, is well established in comparison to recall or diary methods, which typically determine intakes over a shorter time period.[11] In addition, a FFQ tends to be cheaper and considerably less burdensome to the participant.[12]

A focused retrospective analysis of dietary flavonoid intake in older adults was necessary to inform initial development of the novel dietary assessment tool. We recently estimated the mean intake of total flavonoids, and the major dietary sources thereof, in an older Australian population using comprehensive dietary data that included 12 days of weighed FRs,[13] collected as a part of the Blue Mountains Eye Study (BMES).[14] These findings[13] were utilised as the basis for the development of a FFQ for use in older adults. The validation and reproducibility of this tool is the objective of the current study. Validity, in this context, is the degree to which the FFQ measures specific dietary attributes, and the validation process involved the appraisal of the FFQ against a reference method to determine agreement in measuring the same dietary attributes.[15] The reproducibility of the FFQ relates to the ability of the tool to accurately and equally measure the same dietary attributes on more than one occasion.[15]

The aim of the present study was to assess the validity and reproducibility of a FFQ developed to measure total flavonoid intake, and flavonoid subclasses, in older adults against a 4-day FR.

## Methods

A FFQ was developed (by KK and KC) to quantitatively assess individual intake of dietary flavonoids over 12 months and was structurally based on the National Health and Nutrition Examination Survey (NHANES) FFQ.[16] All points of recommendation as outlined by Cade *et al.*[11] were addressed in the development of the instrument.

An overview of the process and criteria for selecting foods for the development of the FFQ is provided in Figure 1. As reported elsewhere,[13] 12 days of weighed FR data from the BMES were used as the main basis for the development of the FFQ. The BMES dietary data were cross-referenced with the USDA Database for the Flavonoid Content of Selected Foods (Release 3.1, May 2014)[17] to identify flavonoid-containing foods (including the subclasses anthocyanins, flavan-3-ols, flavones, flavonols and flavanones). To ensure that all rich sources of flavonoids and the top contributors to flavonoid intake were captured, foods were selected for inclusion in the FFQ if they met any of the following criteria:

1 Any food with >30 mg/100 g total flavonoids and/or >30 mg/average portion size (as determined by the median portion size as reported in the BMES weighed FR; in the absence of a published definition of a 'flavonoid-rich food', the cut-off of 30 mg/100 g of total flavonoids was utilised by the present study);
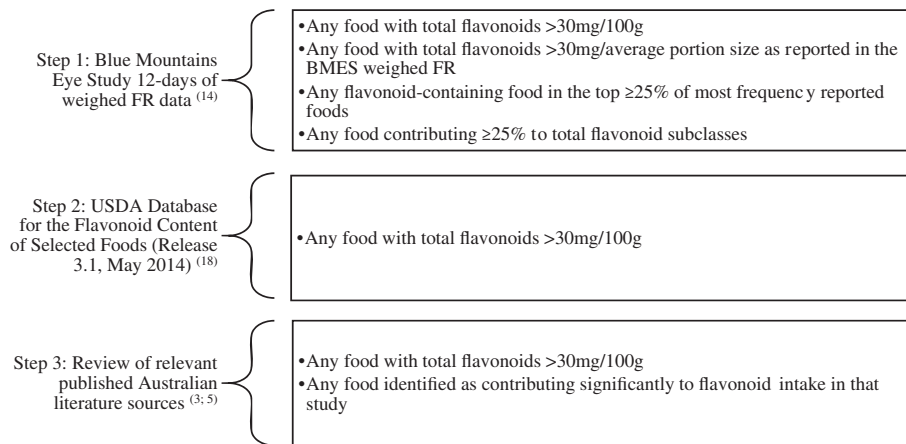2 Any flavonoid-containing food in the top 25% of all foods;

| Step 1: Blue Mountains Eye Study 12-days of weighed FR data [14] | • Any food with total flavonoids >30mg/100g<br>• Any food with total flavonoids >30mg/average portion size as reported in the BMES weighed FR<br>• Any flavonoid-containing food in the top ≥25% of most frequency reported foods<br>• Any food contributing ≥25% to total flavonoid subclasses |
| Step 2: USDA Database for the Flavonoid Content of Selected Foods (Release 3.1, May 2014) [18] | • Any food with total flavonoids >30mg/100g |
| Step 3: Review of relevant published Australian literature sources [3; 5] | • Any food with total flavonoids >30mg/100g<br>• Any food identified as contributing significantly to flavonoid intake in that study |

**Figure 1** Overview of criteria for selecting foods for the development of the FFQ.

3 A top 25% contributor in each flavonoid subclass according to the average portion size (g) of that food.

Of the total 96 foods included in the FFQ, 73 foods were identified from the BMES weighed FR data. An additional 23 foods were included from rich sources (>30 mg/100 g) identified from the USDA Database for the Flavonoid Content of Selected Foods (Release 3.1, May 2014) (n = 12) and from other published Australian literature sources[3,5] (n = 11). These foods were mainly herbs (parsley, dill, thyme, mint, coriander and oregano); some vegetables, including eggplant, red onion, and rocket; and various fruits (lime and blackcurrant juice). Additionally, any foods highlighted as top contributors of flavonoid intake by previous national Australian literature[3,5] were also included and consisted of fruits (cherries, mango, rhubarb, lemon) and vegetables (silverbeet, endive, beans, spring onion, kale, olives (green and black)).

Grouping of food items was decided *a priori* to the analysis. Once a food item was identified for inclusion in the FFQ, it was coded into a relevant food category: non-alcoholic beverages, alcoholic beverages, fruit, vegetables, herbs and other foods. The 'other foods' category included foods such as pickles, nuts, dried fruit and fruit spreads, which were separated from other food categories because of their vastly different flavonoid profiles. With the aim of accurately quantifying flavonoid intake, portion sizes derived from the BMES WFR data were used.[13] The portion sizes for each of these additional foods were determined by matching them to a nutritionally similar food (e.g. kale to spinach or red onion to brown onion). Median portion size (g) and the interquartile range informed the basis for four portion size options, specific to each food. The portion sizes were allocated according to: (i) first quartile or less, (ii) median, (iii) second quartile or (iv) more than second quartile. A time period of 12 months was selected to ensure that habitual intake and the influence of seasonal dietary change was captured in the FFQ.[11]

A total of 42 community dwelling older adults aged 60 years and over from the Illawarra region of New South Wales, Australia, were recruited to the study between June and September 2014. Healthy older adults were recruited via advertising material distributed at local community groups. The study sample excluded individuals with major food intolerances or a condition that impacts usual dietary choices (e.g. Crohn's disease). Ethics approval was obtained from the University of Wollongong Human Research Ethics Committee, and all subjects provided written informed consent. An independent statistician (MB) calculated an adequate sample size based on a previous study,[18] which assessed the validity of a FFQ against multiple 24-hour recalls in older Australians and determined that a minimum of 42 subjects would be required to obtain a correlation coefficient of 0.377.

Participants attended two identical interviews, held 1 month apart and based on their preference for either a home visit or attendance at the university testing facility (Illawarra Health and Medical Research Institute). A single interviewer (KK) conducted the interview, during which body weight (kg) and height (m) were measured and body mass index (BMI; weight (kg)/ height (m)$^2$) was calculated. Body weight was measured with participants standing in an upright position without shoes, in minimal clothing, using digital scales (Tanita TBF-622; W.W. Wedderburn Pty Ltd, Ingleburn, NSW, Australia). A stadiometer was used to measure height of participants without shoes on. Resting BP and heart rate (HR) were measured while seated, in triplicate and were averaged using an Omron HEM7200 (Deluxe Automatic Monitor HEM-7200-E-Omron Healthcare, Kyoto, Japan).[19] Personal and demographic information was collected using a survey developed for the purpose of the present study, which included questions related to age, smoking and drinking habits and years of education.

The self-administered FFQ was completed by the participant at home on the day before the interview. The booklet was checked during the interview for inconsistencies and missing data (KK). If data were missing, participants were asked to independently fill in the missing questions in the booklet to ensure completeness of the data and to reduce interviewer bias. Questions that had arisen from the participants about any foods listed in the FFQ were also clarified.

The paper-based FFQ data were entered into a template created for this purpose in Microsoft Excel (Microsoft Office version 14.1.2).[20] The template was developed using nutritional information for each food or beverage based on each portion size option provided in the FFQ, and was based on the FoodWorks dietary analysis package (Xyris software, version 5, 2007, Highgate Hill, QLD, Australia), with AUS-NUT 2011–2013 as the food composition database[21] and the USDA database selected for the flavonoid content of various foods (Release 3.1, May 2014).[17] The flavonoid and flavonoid subclass content was assigned for each item in the FFQ based on the most similar and appropriate food/beverage available in the reference USDA flavonoid database. The USDA Database for the Flavonoid Content of Selected Foods (Release 3.1, May 2014) is a comprehensive FCDB but contains limited information pertaining to some cooked foods. Therefore, if a raw food's flavonoid content was available for a food that was usually consumed as cooked, the raw value was still applied in the absence of a cooked value. Pre-quantified portion sizes of foods were converted to daily intakes by multiplying a frequency factor based on the NHANES FFQ[16] as follows: 6+ per day = 7, 4–5 per day = 4.5, 2–3 per day = 2.5, 1 per day = 1, 5–6 per week 0.8, 3–4 per week = 0.5, 1–2 per week = 0.2, 2–3 per month = 0.08, 1 per month or less = 0.03 and Never = 0.

Participants were trained by a nutritionist (KK) and instructed to complete a 4-day FR on any four consecutive days between the two interview dates, including three week days and one weekend day. Following recommendations by Cade et al.,[11] the FFQ1 was administered before the training, and completion of the 4-day FR to mediate the learning effects was associated with completing a more comprehensive dietary assessment method. Participants were instructed to record in detail all the foods and drinks that they had consumed during that period and were encouraged to estimate their portion sizes using cup measures, weights or other household measurements. Dietary data from the 4-day FR were screened for missing data, and clarification was sought from participants during the final interview (KK). The 4-day FRs were entered and analysed using the FoodWorks dietary analysis package.[21] As dietary data relating to flavonoid content of foods are not integrated into FoodWorks, each food item reported in the 4-day FR was manually assigned a value for total flavonoid content as well as each flavonoid subclass according to the USDA database for the flavonoid content of selected foods.[17] This allowed calculation of flavonoid intake and intake of flavonoid subclasses per person per day.

Statistical analysis was performed (KK and KC) using the Statistical Package for Social Sciences (SPSS) software (V15.0.0 SPSS Inc., Chicago, IL, USA).[22] Descriptive statistics were performed on demographic data. For each participant, mean (±SD), median and range for flavonoid intake and of each flavonoid subclass from FFQ1, FFQ2 and 4-day FR were calculated. Major dietary sources of flavonoids and each flavonoid subclass were determined. The percentage contribution that each food made to total flavonoid

**Table 1** Top 10 foods contributing to total flavonoid and flavonoid subclass intake ranked in order of contribution to total consumption according to the developed FFQ

| | Flavonoid Total | | Anthocyanin | | Flavan-3-ol | | Flavanone | | Flavone | | Flavonol | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Food | % | Food | % | Food | % | Food | % | Food | % | Food | % |
| 1 | Tea, black | 56.9 | Wine, red | 31.1 | Tea, black | 75.2 | Orange juice | 40.8 | Parsley | 35.5 | Tea, black | 29.0 |
| 2 | Wine, red | 7.6 | Grapes, red | 13.3 | Tea, green | 7.4 | Mandarin | 19.2 | Wine, red | 17.1 | Onion, brown | 9.8 |
| 3 | Tea, green | 6.1 | Cherry | 10.3 | Broccoli | 4.8 | Oranges | 17.6 | Pumpkin | 11.1 | Beans | 8.9 |
| 4 | Orange | 3.3 | Blueberry | 7.9 | Apple, red | 2.5 | Wine, red | 6.8 | Grapes, red | 7.4 | Peas | 5.0 |
| 5 | Broccoli | 2.6 | Banana | 7.0 | Peach | 1.5 | Wine, white | 3.6 | Tea, green | 4.2 | Onion, red | 4.4 |
| 6 | Grapes, red | 2.1 | Pear | 5.9 | Wine, red | 1.4 | Brussels sprout | 3.6 | Celery | 4.0 | Wine, red | 4.3 |
| 7 | Mandarin | 2.1 | Strawberry | 5.5 | Banana | 1.1 | Grapefruit | 2.5 | Spinach | 2.3 | Tea, green | 3.8 |
| 8 | Cherry | 1.9 | Apple, red | 3.8 | Nectarine | 1.0 | Lemon, flesh | 2.3 | Orange | 2.3 | Apple | 3.4 |
| 9 | Apple, red | 1.9 | Plum | 2.7 | Cherry | 0.9 | Tomato | 1.6 | Kiwi fruit | 1.9 | Kale | 2.7 |
| 10 | Banana | 1.1 | Peach | 1.5 | Grapes, green | 0.7 | Lemon juice | 0.7 | Rockmelon | 1.8 | Spinach | 2.6 |

intake and intake of each flavonoid subclass was calculated for each person (FFQ1) and averaged to indicate the major (top 10) foods contributing to intake in the total group (Table 1). Mean daily flavonoid intakes for the FFQ1, FFQ2 and 4-day FR were assessed for normality using histograms and the Shapiro Wilk test for normality and were found to be non-normally distributed for FFQ1, FFQ2 and 4-day FR.

A combination of tests was applied to assess several different facets of validity (criterion validity) and reproducibility because this has been recommended as providing a more superior assessment of validity than the use of a single measure.[23] Mean percentage difference was calculated between the FFQ1 and 4-day FR and between FFQ1 and FFQ2. A Wilcoxon signed-rank sum test was used to compare flavonoid and flavonoid subclass intakes obtained from the two methods (FFQ1 *vs* 4-day FR) and two time points (FFQ1 *vs* FFQ2). Spearman's correlation coefficients were calculated to determine the strength of association between the two dietary assessment methods (FFQ1 *vs* FR, and FFQ1 *vs* FFQ2) for total flavonoid intake and each dietary flavonoid subclass. The FFQ1 and 4-day FR were assessed for level of agreement using Bland-Altman plots,[24] where the difference between the FFQ1 and 4-day FR (FFQ1 – 4-day FR) was plotted against the mean of the FFQ1 and 4-day FR (FFQ1 + 4-day FR)/2. Bland-Altman graphs were constructed to examine the difference between FFQs (FFQ1 – FFQ2) plotted against the mean of the FFQs ((FFQ1 + FFQ2)/2). Limits of agreement (LOA; the mean difference ± 1.96SD) for the difference between the two measures were calculated to evaluate whether they were acceptable. A regression line was fitted to detect proportional differences and to indicate the direction and magnitude of the bias. Lastly, Cohen's kappa ($\kappa$) test was used to determine the ability of the FFQ to rank individuals according to quartiles of intake (FFQ1 *vs* 4-day FR and FFQ1 *vs* FFQ2) for total flavonoid intake and flavonoid subclasses.[25,26] Values ≤0 indicated no agreement; 0.01–0.20 as none to slight; 0.21–0.40 as fair; 0.41–0.60 as moderate; 0.61–0.80 as substantial; and 0.81–1.00 as almost perfect agreement.

## Results

The developed flavonoid FFQ included 93 questions pertaining to 9 non-alcoholic beverages, 5 alcoholic beverages, 25 fruit, 33 vegetables, 8 herbs, 13 other foods and an additional 3 summary questions about usual alcohol intake and fruit and vegetable consumption (Supplemental File 1). The characteristics of the study participants are presented in Table 2.

The mean and median intake, standard deviation and range of total flavonoid intake and intake of each flavonoid subclass (mg/day) for each method were determined (Table 3). For both the FFQ[1,2] and the 4-day FR, a large variability in dietary flavonoid intake and intake of flavonoid subclasses is highlighted by the wide range (Table 3).

The mean percentage difference between FFQ1 and FR showed that the FFQ estimated total flavonoid intake to be higher by 21.4% and higher intakes for individual flavonoid

**Table 2** Summary statistics of subject characteristics (n = 42)

| | | |
|---|---|---|
| Gender | Male | 14 (33.3%) |
| | Female | 28 (66.7%) |
| Age (y) | Mean ±SD | 75.3 ± 8.6 |
| BMI (kg/m$^2$) | Mean ± SD | 27.7 ± 4.3 |
| Primary language | English | 40 (95.2%) |
| | Other | 2 (5.8%) |
| Smoking status | Non-smoker | 28 (66.7%) |
| | Current smoker | 1 (2.4%) |
| | Ex-smoker | 13 (30.9%) |
| Supplement use | Any supplement | 24 (57.2 %) |
| | No supplement | 18 (42.8%) |
| Education | Tertiary | 27 (64.3%) |
| (highest level | Secondary (complete) | 15 (35.7%) |
| completed) | Secondary (incomplete) | 0 |
| | Primary | 0 |
| Blood pressure | Systolic mean ± SD | 138.1 ± 18.4 |
| | Diastolic mean ± SD | 83.7 ± 9.3 |
| | Heart rate: mean ±SD | 67.2 ±10.6 |

subclasses flavan-3-ols (9.4%), flavones (54.3%), flavonols (70.5%), flavanones (78.4%) and anthocyanins (90.7%). The mean percentage difference between FFQ1 and FFQ2 showed small variations for total flavonoid intake (6.7%) and flavonols (–1.1%), with slightly higher variation for intake of flavanones (10.7%), anthocyanins (13.8%), flavan-3-ols (14.1%) and flavones (22.3%).

The Wilcoxon signed-rank sum test showed significant differences in total flavonoid ($P < 0.001$), anthocyanin ($P < 0.001$), flavanone ($P < 0.001$), flavone ($P < 0.004$) and flavonol ($P < 0.001$) intakes as measured by the FFQ1 and 4-day FR, but no significant difference for flavan-3-ol measurements (Table 4). The strength of the associations, as tested by Spearman's correlation coefficients between variables assessed using the FFQ1 and 4-day FR, are shown in Table 4. The Wilcoxon signed-rank sum test showed no significant differences in the measurement of total flavonoid or any flavonoid subclass intakes (mg/day) between FFQ1 and FFQ2 (Table 4). Total flavonoid intake showed substantial reliability (>0.9), and each flavonoid subclass showed between moderate (>0.7) and substantial reliability (Table 4).

The Bland-Altman plot of the bias (average of the differences between methods) against the mean value for the two methods is shown in Figure 2a. Visually, this demonstrates that the bias and LOA are large for total flavonoid intake (+203.0 mg) and intake of anthocyanins (+64.5 mg), flavan-3-ols (+74.8 mg), flavanones (+23.9 mg) and flavonols (+35.8 mg). The bias is small, with narrow LOA, for flavone intake (+2.77 mg). The linear regression identified in the Bland-Altman plots (Figure 2a) indicates a strong positive correlation for total flavonoid intake and intake of anthocyanins, flavan3ols and flavonols, whereby the greater an individual estimates their flavonoid intake, the greater the systematic bias (or overestimation by the FFQ). This bias is less for flavone and flavanone intakes, as shown visually in Figure 2a. The Bland-Altman plots visually showed

**Table 3** Description of mean flavonoid intake (mg/day) according to the FFQ1, FFQ1 and 4-day FR (n = 42)

|  | (mg/day) | Mean | Std. Deviation | Median | Minimum | Maximum | % contribution |
|---|---|---|---|---|---|---|---|
| FFQ 1 | Total flavonoids | 1050.5 | 725.5 | 919.3 | 80.0 | 3253.5 | |
| | Anthocyanins | 103.4 | 104.7 | 52.7 | 11.4 | 368.3 | 9.8 |
| | Flavan-3-ols | 829.6 | 690.6 | 778.5 | 32.3 | 2953.1 | 78.9 |
| | Flavanones | 42.5 | 28.5 | 39.7 | 2.4 | 105.4 | 4.0 |
| | Flavones | 6.5 | 8.0 | 3.4 | 0.3 | 37.7 | 0.6 |
| | Flavonols | 68.5 | 34.9 | 58.1 | 19.7 | 147.2 | 6.5 |
| FFQ 2 | Total flavonoids | 1047.0 | 724.9 | 911.3 | 140.1 | 2488.9 | |
| | Anthocyanins | 103.4 | 99.0 | 69.0 | 14.8 | 367.8 | 9.9 |
| | Flavan-3-ols | 829.8 | 686.6 | 755.7 | 60.9 | 2312.2 | 79.3 |
| | Flavanones | 40.4 | 29.4 | 28.2 | 4.9 | 103.6 | 3.9 |
| | Flavones | 7.4 | 9.2 | 4.8 | 0.46 | 37.7 | 0.7 |
| | Flavonols | 66.0 | 32.8 | 55.5 | 16.4 | 131.8 | 6.3 |
| 4-day FR | Total flavonoids | 847.5 | 534.8 | 781.4 | 22.1 | 2490.5 | |
| | Anthocyanins | 38.9 | 58.9 | 7.2 | 0.00 | 224.7 | 4.6 |
| | Flavan-3-ols | 754.7 | 502.2 | 706.9 | 6.9 | 2366.5 | 89.1 |
| | Flavanones | 18.5 | 20.9 | 9.6 | 0.00 | 69.8 | 2.2 |
| | Flavones | 3.7 | 5.2 | 1.7 | 0.00 | 27.4 | 0.4 |
| | Flavonols | 32.8 | 18.9 | 29.5 | 1.2 | 99.4 | 3.9 |

small, non-significant bias (Figure 2b) but wide LOA. Linear trends indicate that the bias is small between FFQ1 and FFQ2 (Figure 2b).

Cohen's kappa ($\kappa$) indicates that there was substantial agreement[25] between the FFQ1 and 4-day FR for total flavonoid intake and flavonol intake; moderate agreement for flavan-3-ol intake; fair agreement for flavone intake; and none to slight agreement for anthocyanin and flavanone intake. Cohen's kappa ($\kappa$) indicated substantial agreement[25] between the FFQ1 and FFQ2 for total flavonoid intake, anthocyanin intake, flavan-3-ol intake, flavone intake and flavonol intake, and moderate agreement[25] for flavanone intake.

## Discussion

A flavonoid-specific FFQ was developed for use in older Australians by applying a systematic approach using data obtained from 12 days of weighed FRs and the most appropriate flavonoid food composition data that were presently available.[17] Total flavonoid intake as measured by the FFQ (1050 mg/day) was significantly higher than that reported in other Australian studies,[3,5,27] which may relate to the use of 24-hour diet recall data in previous studies. However, it is also significantly higher than the analysis of 12 days of weighed FRs in the population of older adults, which was used to inform the development of this tool[13] (683 mg/day). The 4-day FR similarly measured flavonoid intake (847 mg/day) and intake of flavonoid subclasses as being higher than previous Australian estimations; however, the percentage contributions of each flavonoid subclass to total flavonoid intake were closely related to previously reported estimates for the total Australian population.[3] The major sources of total flavonoids, as estimated by the FFQ, are black tea (57.0%), red wine (7.6%) and green tea (6.0%), which have

**Table 4** Comparison of the total flavonoid intake and intake of flavonoid subclasses (mg/day) for FFQ-1 versus FR (validity) and FFQ-1 versus FFQ-2 (reliability)

| (mg/day) | | Wilcoxon signed-rank sum test sig. (P-value) | Spearman's correlation coefficient sig. (P-value) | Cohen's Kappa ($\kappa$) sig. (P-value) |
|---|---|---|---|---|
| FFQ-1 versus 4-day FR | Total flavonoids | <0.001 | 0.93 (<0.001) | 0.619 (<0.001) |
| | Anthocyanins | <0.001 | 0.32 (0.042) | 0.142 (0.111) |
| | Flavan-3-ols | 0.413 | 0.87 (<0.001) | 0.492 (<0.001) |
| | Flavanones | <0.001 | −0.17 (0.27) | 0.048 (0.587) |
| | Flavones | 0.004 | 0.18 (0.25) | 0.238 (0.008) |
| | Flavonols | <0.001 | 0.75 (<0.001) | 0.619 (<0.001) |
| FFQ-1 versus FFQ −2 | Total flavonoids | 0.912 | 0.91 (<0.001) | 0.619 (<0.001) |
| | Anthocyanins | 0.955 | 0.92 (<0.001) | 0.651 (<0.001) |
| | Flavan-3-ols | 0.957 | 0.92 (<0.001) | 0.746 (<0.001) |
| | Flavanones | 0.350 | 0.73 (<0.001) | 0.492 (<0.001) |
| | Flavones | 0.119 | 0.85 (<0.001) | 0.714 (<0.001) |
| | Flavonols | 0.328 | 0.90 | 0.619 (<0.001) |
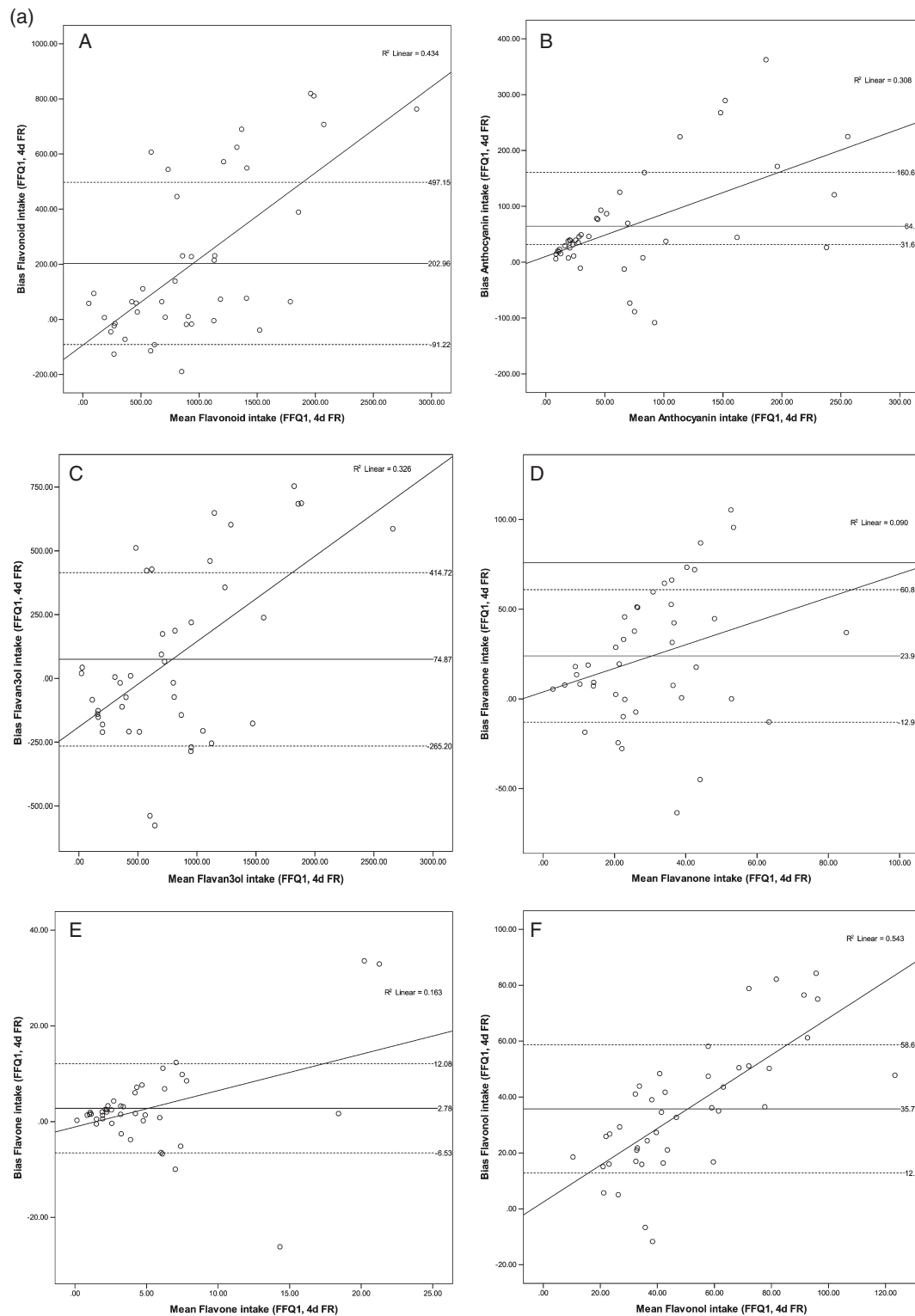
**Figure 2** (a) Bland-Altman plots (difference in intake (mg/day) (FFQ1 – 4-day FR) against the mean intake of flavonoids and subclasses (mg/day) [FFQ1 + 4-day FR)/2]) showing the relative validity of the FFQ1 versus the 4-day FR for total (A) flavonoids, (B) anthocyanin, (C) flavan-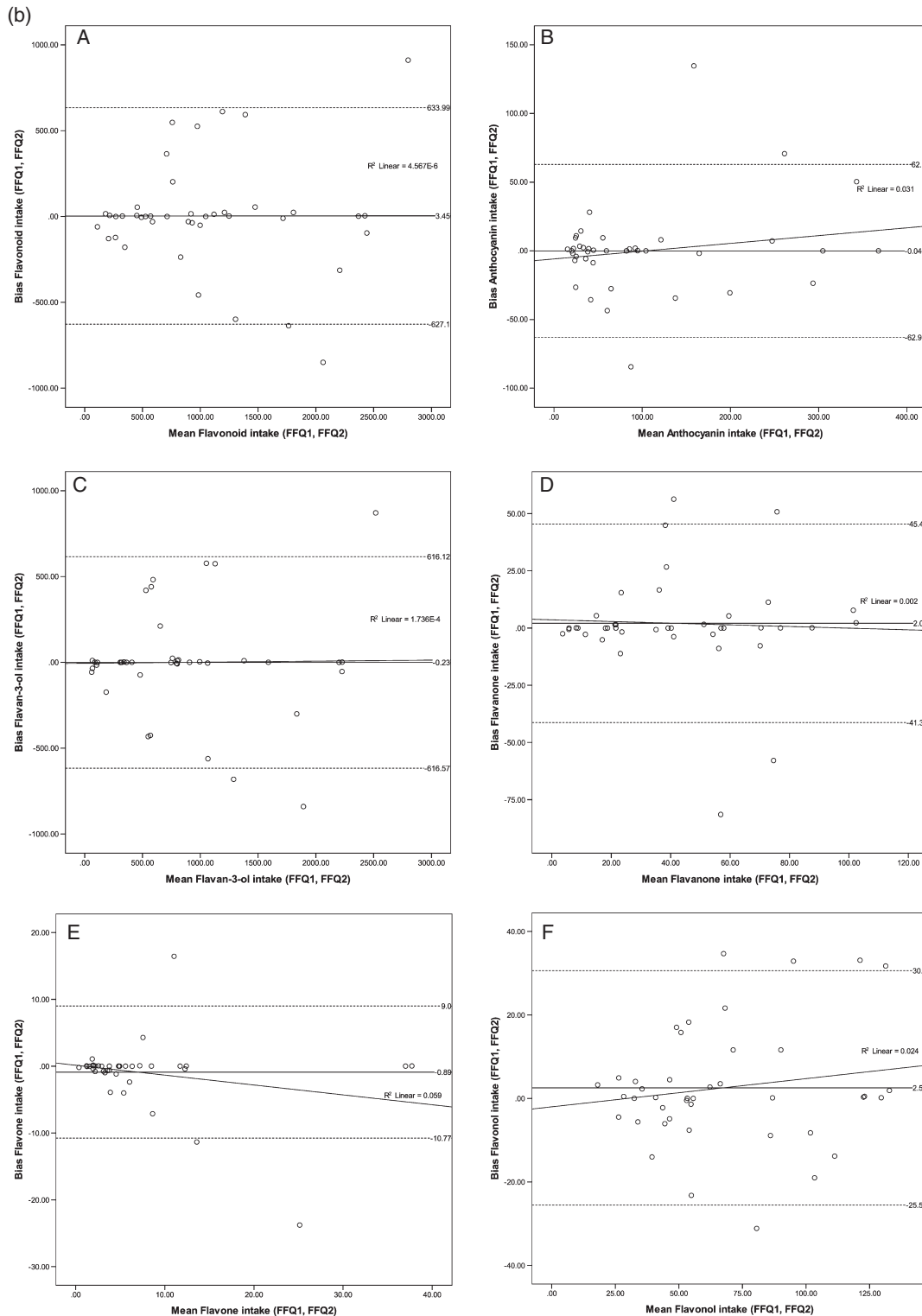3-ol, (D) flavanone, (E) flavone and (F) flavonol intake. (b) Bland-Altman plots (difference in intake (mg/day) (FFQ1 – FFQ2) against the mean intake of flavonoids and subclasses (mg/day) [FFQ1 + FFQ2)/2]) showing the relative validity of the FFQ 1 versus the FFQ2 for total (A) flavonoids, (B) anthocyanin, (C) flavan-3-ol, (D) flavanone, (E) flavone and (F) flavonol intake.

**Figure 2** Continued

previously been described as significant contributors to flavo-noid intake in this age group.[3] Inclusion of the most commonly consumed flavonoid-containing foods, in addition to the richest dietary sources according to the food composition database in the FFQ, provided a sound basis that enabled the most accurate estimation of habitual intake for all flavonoids. For example, although consumed sporadically and in small amounts, parsley was identified as the major source of

flavone intake. Previous dietary questionnaires that have not incorporated both considerations[28–30] have shown limitations in the assessment of total flavonoid intake.

The validity for estimating absolute intakes for flavonoids appears poor, with systematic (positive) bias observed. An overestimation of flavonoid intake was similarly reported in a validation study of a FFQ developed to measure flavonoid intake in younger Australian adults,[10] and this appears to be a typical finding when validating a FFQ against FRs.[31] Despite the FFQ1 and 4-day FR being strongly correlated, except for flavanone and flavone intake, there were significant differences in the estimation of the total flavonoid intake and intake of flavonoid subclasses (except for flavan-3-ols) between the two measures. The mean percentage difference indicates that the FFQ1 systematically overestimated intakes, compared to the 4-day FR, ranging from 9% for flavan-3-ols to 90% for anthocyanins. This finding was confirmed by the Bland-Altman analysis, which indicates a case of proportional error (strong positive correlation) (Figure 2a) and systematic bias (overestimation) for both total flavonoid intake and intake of flavonoid subclasses. Because correlation coefficients quantify the degree to which two variables are related but do not necessarily imply good agreement between two methods,[24] it is not uncommon to demonstrate acceptable correlation in the presence of bias. The Bland-Altman analysis identified that for a number of flavonoids assessed (notably for anthocyanins and flavonol), more than 5% (i.e. >2 values of n = 42) of the data points fell outside the LOA. This clearly indicates a lack of equivalence between the two methods. Formal equivalence testing was not conducted in the present study, although this has recently been recommended by Batterham et al.[32] and may be a consideration in future studies.

The agreement between the FFQ and the 4-day FR in assigning individuals to quartiles of intake (Kappa coefficients) suggests that the FFQ may be an appropriate method for assessing total flavonoid intake but exhibits differing levels of agreement for individual subclasses of flavonoids, particularly for flavanone intake, which is commonly provided by citrus fruits. The misclassification of individual flavonoid subclasses may also relate to the influence of sporadically consumed foods or seasonal foods being underreported in the 4-day FR in comparison with the longer duration FFQ. This is of particular relevance for anthocyanins and flavanones that showed the poorest agreement between the two methods. Berries and cherries (summer fruits) and mandarins (winter fruit) are highly seasonal fruits that are available for short periods of time in Australia but are rich in anthocyanins and flavanones, respectively. The period of reporting for the FFQ was the previous 12 months, whereas the 4-day FR measured intake over a short period between June and September, which corresponded to winter months and early spring in Australia. Thus, the FFQ could be expected to overestimate the flavonoid intake related to seasonal fruit consumption. Alternatively, the 4-day FR, which was considered a robust reference method in the present study, could be considered inadequate at estimating

overall habitual flavonoid intake, and therefore, repeated recalls may be needed to overcome this issue. These considerations are important when deciding on the purpose of the instrument, whether to measure absolute intakes in order to assess acute changes as would be needed in a short-term intervention study or clinical trial, or whether the instrument is required for ranking individuals according to usual intake, as is required in epidemiological studies.[12] The present study has demonstrated the latter, as is the desirable strength of a FFQ, and highlights its potential application for use in large cohort studies.

Our findings are consistent with a Flemish study in which an 86-item FFQ was validated against a 4-day non-consecutive food diary in a sample of dietitians.[33] That FFQ was similarly able to assign subjects to correct quartiles of flavonoid intake and to identify high flavonoid-containing foods and regular sources of flavonoid intake, but was less suitable for estimating total intake (mg/day). The FFQ also showed only weak or no correlation to the 4-day food diary in relation to specific flavonoid subclasses, for example, anthocyanins.[33] In that study, the poor correlation was related to the sporadic consumption of high flavonoid-containing foods, such as red wine, that were not captured in the 4-day food diary, a result that appears to have been replicated in the present study.

Using various statistical tests, our novel FFQ showed a substantial level of reproducibility. The repeated FFQs were highly correlated, displayed a small percentage of disagreement[25] and showed no difference between repeated measures of total flavonoid intake or their subclasses. These findings were confirmed in the Bland-Altman analyses that visually indicated a low level of bias and Cohen's kappa (κ) results that indicated moderate (κ = 0.41–0.60) to substantial agreement (κ = 0.61–0.80)[25] between the two time points. The FFQs were administered 1 month apart; therefore, it is unlikely that any seasonal variation in diet would have impacted its reproducibility.

The main limitation of the present study was the small sample size (n = 42), which limits the interpretation of the study findings. Previous authors have suggested that a sample size of at least 50 is desirable,[11] and ideally, a sample of between 100 and 200 should be used, particularly if the FFQ is designed to provide information on nutrient intakes.[34] The present study experienced recruitment issues given the study burden associated with the 4-day FR, which further highlighted a need for a simple, rapid method to determine flavonoid intake in this age group.

There are other limitations to the present study, which partially relate to the general limitations associated with using a FFQ to measure dietary components. While FFQs are relatively cheap and simple to administer, they may be associated with large measurement error due to inaccuracies in estimating frequencies over the longer term and determination of pre-quantified food portion sizes.[12] FFQs that have a prolonged reference period have been shown to overestimate fruit and vegetable intake,[12] which could have been a contributor to measurement error in the present study.

Our FFQ was developed based on secondary analysis of 12 days weighed FR data, which were not collected for the primary purpose of estimating flavonoid intake, and this may have further influenced the study findings. In addition, the reference method (4-day FR) is not generally considered a gold-standard reference method. The 4-day FR method was chosen for the present study in order to minimise study burden and to retain participants. The 4-day FR method is advantageous in its ability to capture food intake without the reliance of memory and has the fewest correlated errors with a FFQ.[11] However, FRs are often associated with underreporting,[35] and in the present study, food underestimation in the FR may have occurred due to forgetfulness and/or limitations of food knowledge in this population. Future research might consider using weighed FRs or repeated 24-hour diet recalls as the reference method, and could consider adjusting for underreporting to improve the validity of the chosen reference method. Another limitation relates to the utilisation of the USDA database to determine the flavonoid content of selected foods as a reference database. The flavonoid content of foods in the database may not have accurately reflected that of Australian produce as flavonoid content is heavily influenced by cultivar and growth and processing conditions, and the USDA recognises this variability.[36] However, given the lack of Australian-specific data, and the fact that the USDA database is one of the most comprehensive and most commonly applied databases, it was an appropriate choice for the present study. However, this process highlights a need for Australian flavonoid food composition data to be collated in an FCDB, ideally integrated into a dietary assessment software package, such as FoodWorks.[37] This concept has been recently reviewed using an Australian anthocyanin food composition database as an example.[37] Preliminary studies are investigating the difference in flavonoid intake estimates when different FCDBs are applied to the same dietary data, with one study showing significant differences[38] and another showing minimal variation.[39] Another well-established limitation relates to the inability of a reference database to account for storage and cooking methods,[36] which is a potential source of bias in validation studies of this nature. As the FFQ is unable to make provisions for information on the cooking processes associated with each food, a 'raw food' value was attributed for each food, which may have over-inflated flavonoid intake values. However, in the case of the 4-day FR method, cooking-related flavonoid losses were able to be accounted for as cooking methods were reported. Future work should attempt to address differences in flavonoids according to cooked or raw variants of individual foods. The potential to add a retention factor to the raw food values and address the degradation of flavonoids and a lowered yield associated with cooking should be investigated in the further refinement of this instrument.

The strength of the present study lies in the design of the novel FFQ, which is relatively short and practical in comparison to other dietary assessment methods for estimating flavonoid intake. This characteristic may be useful in time-limited surveys where detailed dietary assessment is not possible. To improve the validity of a FFQ, design issues, including the length, closed versus open-ended responses, seasonality, time frame and portion sizes, can be manipulated.[12] Future research may focus on adjusting the time frame of the recall if short-term intake is required rather than habitual consumption. Additionally, a shorter version of the FFQ could be trialled, excluding foods that did not significantly contribute to flavonoid intake in the present study, such as some herbs). However, due to the highly specific flavonoid content of foods, grouping of food items that are similar nutritionally (e.g. citrus fruits) into a single question[12] is not recommended. As diet is highly influenced by season, future studies may consider confirming these findings across all seasons in a year. Presently, there is little consensus regarding the most appropriate biomarker to reflect flavonoid intake due to methodological difficulties in assessing intact flavonoids and their metabolites. Undoubtedly, this is an area of active research, and in future, objective biomarkers may be elucidated for use in validation studies.

In conclusion, a novel FFQ developed to estimate flavonoid intake for use in older Australians appears satisfactory for ranking individuals according to total flavonoid intake, but shows limitations for estimating absolute total flavonoid intake. The FFQ especially shows limited validity for estimating intakes of flavonoid subclasses. With further validation, the FFQ could allow for an easier estimation of flavonoid intake and intake of flavonoid subclasses in older adults, especially when ranking individuals according to total flavonoid intake in epidemiological research. Future studies may attempt to validate this tool against repeated FRs collected across several seasons in order to assess the instrument's ability to more accurately capture seasonal food intake and potentially improve the validity of the tool for measuring flavonoid subclasses.

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## Conflict of interest

The authors have no conflicts of interest to declare.

## Authorship

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## Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Supplemental File 1** Kent & Charlton Flavonoid FFQ.