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Estimating nitrate intake in the Australian diet: Design and validation of a food frequency questionnaire

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Abstract

Background: Dietary nitrates may play a role in mediating several key physiological processes impacting health and/or exercise performance. However, current methods for assessing dietary nitrate (NO_3^-) consumption are inadequate. The present study aimed to examine the dietary nitrate intake in a sample of 50 healthy adults, as well as test the validity of a purposefully developed food frequency questionnaire (FFQ).

Methods: Dietary nitrate intake was estimated over a week using (i) three 24-h dietary recalls; (ii) a short-term (7-day) FFQ; and (iii) a biomarker (urinary nitrate), in conjunction with a nitrate reference database.

Results: Daily dietary nitrate intake estimates were 130.94 mg (average of three 24-h recalls) and 180.62 mg (FFQ). The mean urinary NO_3^- excretion was 1974.79 µmol day^{-1} (or 917.9 µmol L^{-1}). Despite the difference between the two dietary assessment methods, there was a moderate positive correlation ($r = 0.736$, $\rho < 0.001$) between the two tools. There was also a positive correlation between urinary NO_3^- and 24-h recall data ($r = 0.632$, $\rho < 0.001$), as well as between urinary NO_3^- and FFQ ($r = 0.579$, $\rho < 0.001$).

Conclusions: The ability to accurately estimate nitrate intakes depends on having suitable reference methods to estimate the concentrations of nitrate in the food supply, coupled with valid and reliable dietary assessment tools. Based on the findings from the present study, at an individual level, dietary recalls or records may be more accurate in estimating intakes of NO_3^-. However, given the lower cost and time needed for administration relative to recalls, the FFQ has merit for estimating NO_3^- intakes in health interventions, dietary surveys and surveillance programs.

KEYWORDS
database, dietary assessment, dietary nitrate intake, food frequency questionnaire, urinary nitrate

Key points
- Nitrate occurs naturally in plant foods and water, and are also commonly used as food additives in cured products. Dietary nitrates may be beneficial for health and exercise performance; however, current methods to assess dietary nitrate consumption are inadequate.
- Estimates of nitrate intake from our purposefully designed food frequency questionnaire showed moderate associations with estimates from 24-h food recalls, as well as from a urinary biomarker.

- At an individual level, dietary recalls or records may be more accurate in estimating dietary nitrate intake. However, the food frequency questionnaire developed in the present study may be useful to estimate nitrate intake in health interventions, dietary surveys and surveillance programs.

INTRODUCTION

Nitrates (NO_3^-) occur naturally in plant foods (e.g., vegetables) and water, and are also commonly used as food additives in cured (animal-derived) products, primarily to prevent bacterial growth and improve the microbiological safety of these foods.[1–3] Traditionally, dietary NO_3^- has been considered to be biologically unnecessary or a potentially harmful component of the food and water supply, if consumed in excess.[4] Consequently, guidelines for the maximum acceptable value in drinking water,[5] food additive permissions, and acceptable daily intake values[4] have been developed.

Dietary assessment involves the collection of information on foods and beverages consumed over a specified time, which are then coded and processed to compute intakes of energy, nutrients and other dietary constituents using food composition (i.e., 'reference') tables.[6] However, values for NO_3^- compounds in foodstuffs are not included in national food composition tables.[7] Several NO_3^- intake studies have relied upon smaller databases or have only included dietary NO_3^- intake estimates from food sources (i.e., excluding beverages) or vegetables only. In 2018, Babateen et al.[8] performed a systematic review of the available literature to provide an evidence-based evaluation of the methods used to assess NO_3^- intake, and to assess usual NO_3^- intake in both healthy and clinical human populations. The review included data from > 3 million participants across 15 countries and reported estimates of the median daily nitrate consumption from individual studies. Babateen et al.[8] observed high heterogeneity in both the types of dietary assessment methods used to record dietary intakes, as well as in the food composition tables/databases used for converting the dietary intake measures to an estimate of nitrate intake.

There is also a lack of consensus regarding a reference method for assessing dietary NO_3^-, which may explain differences in NO_3^- intake reported between studies.[9] Most studies in the review by Babateen et al.[8] used food frequency questionnaires (FFQs) ($n = 43$) to assess dietary intake, one study adopted the use of 24-h food recalls ($n = 1$) and the others used a combination of both ($n = 2$). Dietary records ($n = 3$) and diet history ($n = 3$) were also used, and one study used a 48-h recall.

There is a growing body of evidence suggesting that high intakes of nitrate-rich food sources, particularly green leafy vegetables, have been associated with improved cardiovascular and metabolic health,[10-17] and improved exercise performance.[14,15,18] However, the ability to measure the association of dietary NO_3^- intake on health and exercise performance depends on having reliable reference methods and dietary assessment tools to assess dietary NO_3^- intake.[19] The concentration of NO_3^- in foodstuffs is influenced by a number of factors such as: geographical location, season, growing method, processing factors and the analytical method used, and thus varies considerably both within and between plant species.[20] For example, washing, peeling, cooking (e.g., boiling) and/or pickling have been shown to reduce the nitrate content of vegetables by up to 75% (61%–64%) which is often not considered in dietary assessment. van den Brandt et al.[21] demonstrated that a FFQ could be a useful assessment tool for estimating NO_3^- intake, used in conjunction with a reference database. Of the 55 studies included in the review by Babateen et al.,[8] only six reported the use of an objective measurement (i.e., plasma, urine or saliva) to estimate dietary nitrate intakes while other studies used various food composition databases. Consequently, the accuracy of published estimates of nitrate intake remains uncertain.

Subsequent to the review by Babateen et al.[8] in 2018, a comprehensive NO_3^- reference database has been developed [McMahon NF, Brooker PG, Pavey TG and Leveritt MD, unpublished data]. Therefore, the present study aimed to: (1) develop a short-term (7-day) FFQ to estimate dietary NO_3^- intake; (2) estimate the average daily dietary nitrate intake in a sample of healthy adults, using three dietary assessment tools (i) three 24-h dietary recalls; (ii) a short-term (7-day) FFQ; and (iii) a biomarker (urinary nitrate), in conjunction with the newly developed NO_3^- reference database; and (3) test the validity of a purposefully developed FFQ to estimate NO_3^- intake.

METHODS

Ethics approval was obtained from the University of Queensland's Human Research Ethics Committee (HMS16/1210R1).

Participants and recruitment

Healthy, active (≥150 min week^{-1} of moderate to vigorous physical activity) adults between the ages of 18 and 54 years were recruited from the local community and a large metropolitan university via electronic media, print advertising and snowball sampling. Individuals were

excluded from the study based on the following criteria: (1) history of clinical illness or a disease such as cancer; diabetes; a history of symptomatic cardiovascular or peripheral vascular disease; chronic kidney disease; recent history of psychiatric illness; (2) currently prescribed a diuretic; (3) have smoked cigarettes or quit smoking in the last 3 months; (4) body mass index $< 18.5 \, \text{kg m}^{-2}$ or $> 34.9 \, \text{kg m}^{-2}$; (5) insufficiently active ($<150 \, \text{min week}^{-1}$ of moderate to vigorous physical activity); (6) significant weight loss or gain within the previous 6 months (>6% of body weight); (7) use of antihypertensive medication; (8) unable to read or speak English; and (9) pregnant or lactating.

Procedures

An overview of the trial design is shown in Figure 1. Prior to enrolment, potential participants were asked to complete a modified version of the Active Australia Survey,[22] along with a series of questions relating to the inclusion and exclusion criteria to confirm eligibility.
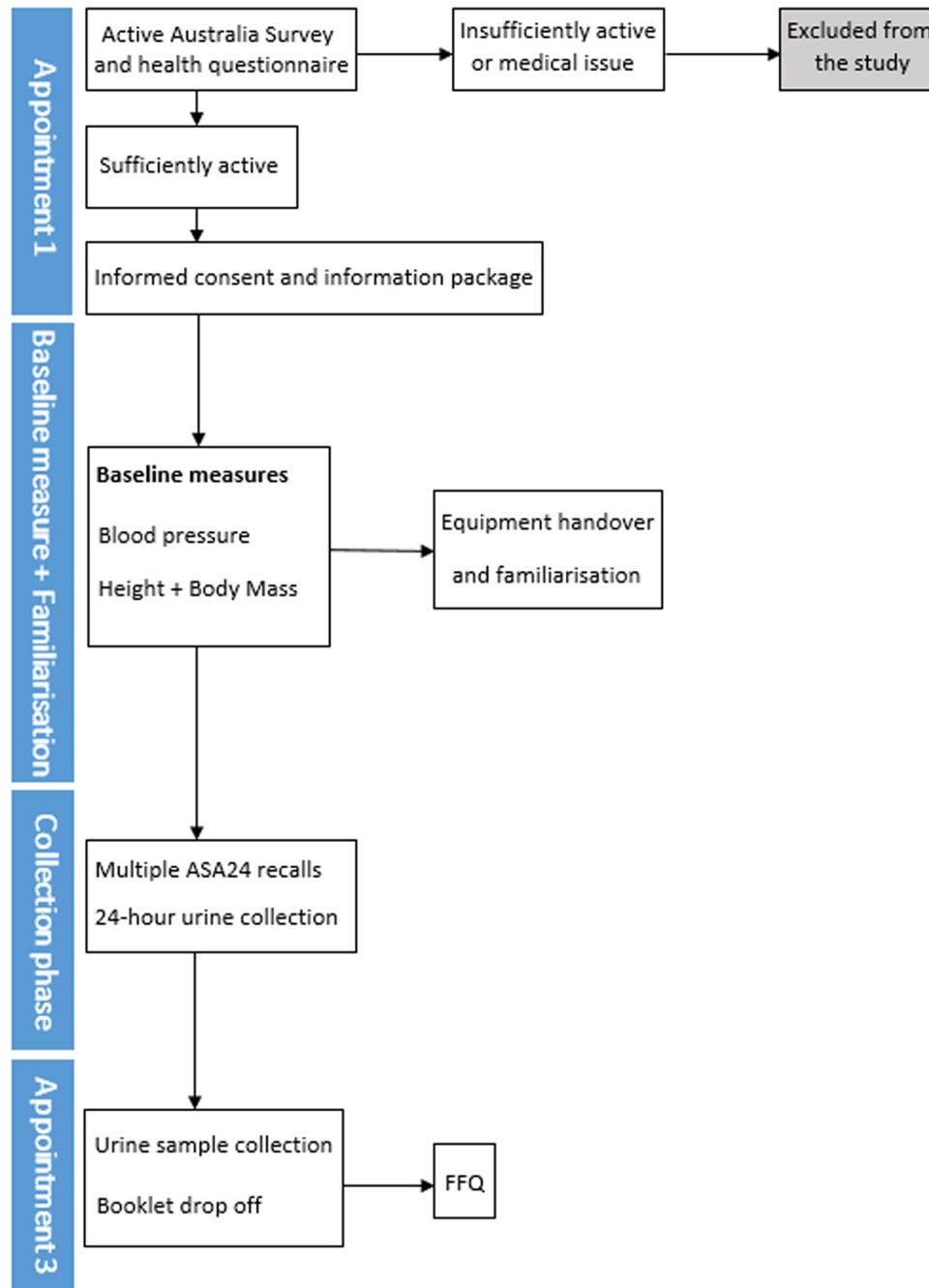

FIGURE 1 Participant trial schedule. ASA24, Automated Self-Administered 24-h; FFQ, food frequency questionnaire

The Active Australia Survey has demonstrated moderate agreement with other self-report measures of physical activity (Cohen's κ of 0.52).[23] Eligible participants were invited to attend the laboratory for an initial face-to-face session. Participants were instructed to avoid caffeine, alcohol, exercise and smoking in the 12 h prior to their visit to the laboratory at the University of Queensland. During this session, participants were provided with an overview of the study, had an opportunity to ask questions about the study protocol and procedures, and provide informed consent. Enrolled participants were familiarised with the Automated Self-Administered 24-h (ASA24) Dietary Assessment Tool and 24-h urine collection procedure (described below). Participants were asked to maintain their usual level of physical activity and to avoid the use of antibacterial mouthwash throughout the study period.

Anthropometry

Standing height was measured using a wall-mounted stadiometer (model 217-172-1009; Seca) and body mass was measured using a set of electronic stand-on scales (A&D Mercury Load Cell Digitizer; A&D Weighing), in accordance with the International Society for the Advancement of Kinanthropometry method[24] and recorded to the nearest 0.1 cm and 0.01 kg, respectively.

Blood pressure

Resting blood pressure (BP) was measured using an aneroid sphygmomanometer (single-hand dual-tube 883; Prestige Medical) after participants rested in a quiet room for 10 min. Measurements were taken twice with a 10-min break between each measurement. Mean arterial pressure (MAP) was calculated using[25]:

$$MAP = \text{diastolic BP} + (0.333\,[\text{systolic BP} - \text{diastolic BP}]).$$

Dietary assessment

24-h dietary recalls
Participants were asked to recall all foods and beverages consumed during the previous 24-h (midnight–midnight) using the ASA24 system. Participants were also prompted to provide a brief description of time, cooking/preparation methods, brand names, and recipes. To account for day-to-day variation, participants completed three 24-h dietary recalls (two weekdays and one weekend day) over a 1-week period. A trained nutritionist reviewed the records for completeness; follow-up phone calls were made if

clarification was needed or if the information was inadequate. The ASA24 system requires a certain level of skill; therefor, participants were provided with an instruction booklet of how to record their food and beverage intake using the ASA24 system during their first appointment. Compared to interviewer-assisted recalls, the ASA24 method is less time and resource intensive and demonstrates similar levels of completeness (80% vs. 83%, respectively).[26]

FFQ
The FFQ (see Supporting information, Doc. S1) was purposefully designed to capture participant's habitual NO_3^- food and beverage intake over the previous 7 days. Participants reported their frequency of consumption of particular foods across a period of 1 week via a FFQ (online). Sample responses ranged from 'never' to '6 per day.' Portion sizes were set to '1 serve = 1 metric cup' to avoid confusion. Participants were provided with verbal instructions regarding how to complete the FFQ, and written instructions at the start of the questionnaire. The quantitative FFQ was made up of food and drink items gathered into the following categories: (1) vegetables; (2) legumes; (3) herbs; (4) wild plants/herbs; (5) fruits; (6) processed meats; (7) other; (8) juice; (9) supplements; and (10) drinking water (tap and bottled). Participants were also instructed to report if the food item was thermally processed (steamed, boiled, microwaved, fried or cooked – non-specific) because of the resultant variation in NO_3^- content.[27] The FFQ was completed online during or directly after the final lab-visit to avoid influencing participant's habitual dietary intake, by educating participants about dietary sources of NO_3^-. Seven days was chosen in favour over a longer-term FFQ because this has previously been shown to be highly correlated with the daily mean intake taken from three 24-h recalls.[28] Additionally, a 1-week washout period is the most common duration for dietary NO_3^- studies measure performance and health outcomes.[29]

Prior to the present study, feedback was sought from a sample of 10 allied health professionals. Face and content validity, feedback on content, length, and language incorporated in the questionnaire and comments were integrated into the design process. After incorporating relevant feedback, the final version of the FFQ was assessed for its test-retest reliability, separated by 24-h ($n = 20$). The level of agreement between reviewers evaluating the reliability of the questionnaire was assessed using Cohen's κ statistics using The κ values were interpreted using the ranges suggested by Landis and Koch[30] and was considered 'almost perfect' (0.89).

Estimating dietary nitrate intakes

Values for NO_3^- compounds in foodstuffs are not included in national food composition tables, therefore intakes were estimated from a recentlyy developed NO_3^-

reference database, [McMahon NF, Brooker PG, Pavey TG and Leveritt MD, unpublished data] which is currently the most comprehensive NO_3^- database available for use. The database contains 5024 records for NO_3^- values (mg per 100 g) of food and beverages spanning 64 countries, established from a systematic literature search including data from original research studies and previously developed databases. Therefore, the most appropriate values were chosen for each item based on a ranking system, giving preference to: (1) Australia from 1990 to present; (2) Australia from 1960 to 1989; (3) countries with predominately Western Diets (US, Canada, UK and other European countries, and New Zealand from 1990 to present; 4) countries with predominately Western Diets (US, Canada, UK and other European countries, and New Zealand from 1960 to 1989; (5) countries with predominately non-Western diets from 1990 to present; and (6) countries with predominately non-Western diets from 1960 to 1989. The cut-off dates were chosen to account for changes in laboratory methods, food preservation techniques and manufacturing technologies [i.e., the addition of ascorbate during meat processing (added to reduce the formation of nitrosamines)], and legislation regulating the amounts of NO_3^- used in the curing process created significant reductions in food and beverages.[31,32]

Nitrate values were available for most commonly consumed food and beverages. Table 1 shows the number of items included in the database, sorted into 12 subcategories. Nitrate intake was estimated by multiplying the food or beverage item consumed (g day^{-1}) by the weighted mean value of that item identified using the multistep process described above. If a NO_3^- value was unattainable a value of 0 mg g^{-1} was assigned to the food

TABLE 1 The number of items included in the nitrate reference database, grouped by food and beverage subcategories

Subcategory	*n*
Alcohols	20
Baby	288
Dairy	223
Fats, oils, nuts, spices and sugars	143
Fruit products	185
Grain products	77
Legumes (beans, peas, lentils)	229
Other	3
Processed meats	213
Protein foods (of animal origin)	311
Vegetables and herbs	3191
Water and other beverages	141
Total	5024

or beverage item. The database includes both raw and thermally-processed samples; where participants reported they had consumed cooked foods (e.g., boiled broccoli), the most appropriate value from the database was chosen. Weighted mean was selected as some mean values were adversely affected by extreme values.

24-h recall

To estimate dietary NO_3^- intake across the three recalled days, the NO_3^- content of all individual foods reported in the ASA24 system was calculated, using values from the database.[33] In the case of multicomponent foods (e.g., pizza, juices, salad mix, soup and baby-food mixtures) nutrient values were determined by calculating the NO_3^- of all ingredients contained in the recipe list or, in the case of commercial products, by looking and the ingredients list or using the recipe reproduction. Total NO_3^- (mg day^{-1}) were determined by calculating the sum of daily NO_3^- values and the average was calculated across the 3 days.

FFQ

To estimate dietary NO_3^- intake from the FFQ, first, food and beverage intake data for each participant (measured in number of servings, where 1 serve = 1 metric cup) were copied into Excel, version 2018 (Microsoft Corp.) and converted to intakes in grams. Cup weights were calculated for each food using values from the United States Department of Agriculture Food Data Central Database,[34] based on a best match (including whether the food was cooked or raw). For example, one cup of broccoli (raw) was recorded as 76 g and one cup of broccoli (boiled, microwaved or streamed) was recorded as 156 g. Next, the NO_3^- content was calculated for each item, using values from the reference database (described above), and all items were summed to give a total weekly intake. Finally, the weekly intake of dietary NO_3^- was divided by seven to calculate an average daily intake for comparability to the estimates from the 24-h recall data.

Urinary nitrate

Urinary NO_3^- was chosen as a NO_3^- biomarker because 65%–70% of ingested NO_3^- is excreted in the urine after a period of 24 h.[35] The urine collection was undertaken following standard procedures.[36] The collection began at the participant's second urine sample of the morning and concluded after their first urine sample collection on the following morning.

Participants were provided with a urine specimen container (hat), designed to be placed under the toilet seat to collect and measure whole urine samples. For each void over a 24-h period, participants were asked to record the total volume of each void (in millilitres) and collect a small sample (poured from the urinal hat) into a 70-ml sterile urine container without preservative, after the first urine void of the morning. Participants were asked to record the exact time of each collection (hh:mm)

in a small booklet provided at the commencement of the study. To preserve the sample for future analyses, participants were given clear instructions regarding storage and transport processes; samples were to be stored in a refrigerator (2–8°C) immediately after collection, and were not to be placed in direct sunlight. Participants were provided with deionised water to clean the urinal hat after each collection and left to air dry.

The 24-h urine collection was completed on the same day as the final ASA24 recall. Participants were asked to report whether the 24-h collection period was completed as planned, whether samples were missed/spilt (and if so, how many) and whether their 24-h collection period mirrored the amount/frequency of a typical day. If participants did not comply to the collection protocol, or reported missing samples, the 24-h urine sample was not included in the analysis. Participants returned their urine samples to the laboratory where they were aliquoted into 1-ml portions and stored at −80°C for subsequent analysis.

The chemiluminescence method has shown to offer the highest analytical consistency in terms of sensitivity and precision and is the generally accepted 'gold standard,[37] and was used to measure urinary NO_3^- concentrations in the present study. To prepare the samples for analysis, the frozen urine aliquots were heated at approximately 90°C in an acidic environment (1 M hydrochloric acid) in the presence of vanadium (III) chloride to catalyse the reduction of NO_3^- to nitrite and then to nitric oxide. Nitric oxide levels were measured with a chemiluminescent analyser in accordance with the manufacturer's recommendations (Sievers NOA 280i; GE Analytical Instruments). Samples were analysed in duplicate and compared with a standard curve created using dilutions of NO_3^-. No samples were above or below the limit of detection for the NO analyser. Duplicate urine measurements had a mean coefficient of variation of 1.17%.

Statistical analysis

A sample size of approximately 50 to 100 is recommended for FFQ validation studies[38] and was the target for the present study. Normality of distribution of data was assessed by the Kolmogorov–Smirnov test and by visual inspection of histograms for skew and kurtosis. The raw data for the urinary NO_3^- totals, 24-h recall and FFQ were not normally distributed but were positively skewed, as generally found with nutrient intake data.[39] Logarithmic transformations were used, when appropriate, to normalise the data. Pearson correlation coefficients were used to evaluate the consistency of dietary patterns derived from dietary data collected with the three 24-h recalls. Spearman's rho correlation coefficients were used on the unadjusted data to assess the linear relationship between urinary NO_3^- excretion and NO_3^-

intake calculated from the 24-h recall and FFQ ($n = 50$). Bland–Altman analyses of the raw NO_3^- intake data for the 24-h recall and the FFQ were used to evaluate the limits of agreement and the presence or absence of systematic bias.[40] A paired t test was used to evaluate the presence of fixed bias between methods. The values of the correlation coefficient were interpreted using the ranges suggested by Rowntree[41] of <0.10–0.20 = very weak, 0.20–0.40 = low, 0.40–0.70 = moderate, 0.70–0.90 = high and 0.90–1.00 = very high. Statistical analyses were performed using SPSS, version 25 (IBM Corp.). $p < 0.05$ (two-sided) was considered statistically significant.

RESULTS

Fifty-nine participants were enrolled in the study, of whom 50 ($n = 32$ male) completed the study. Three individuals withdrew after the first study visit, and six failed to return urine samples. The baseline characteristics of the sample of participants included in the final analyses are presented in Table 2.

Estimates of nitrate intakes from the 24-h recalls ranged between 4.4 and 667.0 mg day^{-1}. Pearson correlations comparing daily intakes of nitrate estimated from the three 24-h recalls are listed in Table 3. Pearson correlation coefficients were statistically significant between the three recalled days (day 1 vs. day 2: $r = 0.394$, $p = 0.005$; day 1 vs. day 3: $r = 0.573$, $p < 0.001$; and day 2 vs. day 3: $r = 0.578$, $p < 0.001$), suggesting that estimates of dietary nitrate were similar between the three recalled days.

The mean NO_3^- intake estimated from the three 24-h recalls was 130.94 ± 99.44 mg day^{-1} and 180.62 ± 214.95 mg day^{-1} from the FFQ (Table 4). There was a moderate positive correlation between the FFQ and 24-h recall data ($r = 0.736$, $\rho < 0.001$) (Figure 2). Bland–Altman analysis and the paired sample t test revealed a significant fixed bias with the FFQ measuring 49.68 mg higher mean NO_3^- intake (mg day^{-1}) (95% confidence interval = 5.08–94.28 mg day^{-1}, $\rho < 0.015$) than the 24-h recall.

TABLE 2 Baseline characteristics of study participants ($n = 50$)

	Mean (SD)	Interquartile range
Age (years)	25 (8.2)	20–28
Height (cm)	174.33 (8.1)	170.1–179.1
Weight (kg)	69.82 (10.4)	63.7–76.8
Body mass index (kg m^{-2})	22.88 (2.3)	21.4–24.2
Systolic blood pressure	117.98 (10.7)	110–123
Diastolic blood pressure	77.26 (7.9)	73–80.1
Mean arterial pressure	90.83 (8.3)	85.8–94.6

TABLE 3 Daily nitrate (mg day^{-1}) estimates from multiple 24-h recalls

| | Nitrate estimates (mg day^{-1}) | | | Pearson correlations | | |
| | | | | Day 1 vs. day 2 | Day 1 vs. day 3 | Day 2 vs. day 3 |
	Day 1	Day 2	Day 3			
Mean	136.13	114.50	141.51	0.394	0.573	0.578
Range	4.4–667.0	5.9–444.5	8.5–444.4			

The overall difference between the two dietary assessment tools was 49.68 mg day^{-1} of NO_3^- (higher average intake estimated from the FFQ). In terms of relative contribution, the greatest discrepancy between the 24-h recall and FFQ came from cooked herbs (0.4% vs. 7.7%) and uncooked herbs (0.2% vs. 4.3%) and other cooked vegetables (0.4% vs. 7.7%), respectively. A further discrepancy of 43.17 mg day^{-1} of NO_3^- was recorded between the tools for food and beverage items not listed in the FFQ, but reportedly consumed during the 24-h recall. These items included high NO_3^- sources such as vegetable combinations (soup, salads and other mixed vegetable items) and low NO_3^- sources such as coffee, beef, strawberries, rice and bread that, when consumed in large quantities, contributed to the difference in the total NO_3^- intake. If these had been captured in the FFQ, the discrepancy between the two tools would have been close to 100 mg day^{-1} (24-h recall, 130.94 mg day^{-1} vs. FFQ, 223.79 mg day^{-1}; 180.62 + 43.17 mg day^{-1}).

The mean total urinary NO_3^- excretion was 1974.79 ± 1168.92 µmol day^{-1} (917.9 ± 691 µmol L^{-1}). There was a moderate positive correlation between urinary NO_3^- excretion and NO_3^- estimated from the FFQ ($r = 0.579$, $\rho < 0.001$) and a strong positive correlation between urinary NO_3^- excretion and NO_3^- estimated from the 24-h recall ($r = 0.632$, $\rho < 0.001$).

DISCUSSION

The purpose of the present study was two-fold: (1) to estimate the average daily dietary nitrate intake in a sample of healthy adults, using three dietary assessment tools, in conjunction with a nitrate reference database, and (2) to test the validity of a purposefully developed FFQ to estimate nitrate intake. Daily dietary nitrate intake estimates were 130.94 mg (average of three 24-h recalls) and 180.62 mg (FFQ). Despite the variation between the two dietary assessment methods, there was a moderate positive correlation ($r = 0.736$, $\rho < 0.001$), suggesting reasonable comparability between the 24-h diet recalls and FFQ in estimating dietary nitrate intake. The mean urinary NO_3^- excretion was 1974.79 µmol day^{-1} (or 917.9 µmol L^{-1}). There was a moderate positive correlation between urinary NO_3^- excretion and NO_3^- estimated from the FFQ ($r = 0.579$, $\rho < 0.001$) and a

strong positive correlation between urinary NO_3^- excretion and NO_3^- estimated from the 24-h recall ($r = 0.632$, $\rho < 0.001$).

Irrespective of the dietary assessment method used, estimated average nitrate intakes were below the acceptable daily intake of 3.7 mg NO_3^- per kg body weight (approximately 250 mg day^{-1} for an adult weighing 70 kg) established by the World Health Organization.[42] The reported nitrate values in this study are similar to other published estimates.[8,43] For example, Jackson et al.[43] estimated dietary nitrate intakes were, on average, 65–70 mg day^{-1} in a sample of 8161 representative Australians from the Australian Longitudinal Study on Women's Health from 2001 and 2013. From their systematic review that included data from > 3 million participants across 15 countries, Babateen et al.[8] estimated median daily nitrate intake was 108 and 110 mg/day for healthy and clinical populations, respectively.[8]

Vegetables and herbs were the largest contributor to NO_3^- in the diet. Based on the average of the three 24-h recalls, vegetables and herbs accounted for 62% of daily NO_3^- intake (approximately 80 mg day^{-1}). Estimates from the FFQ showed 93% of daily NO_3^- intake from vegetables and herbs (approximately 169 mg day^{-1}). These findings are similar to other estimates in the literature which report approximately 60%–80% of the total daily NO_3^- intake comes from vegetables.[2,8,31,43–45] Similar to consumption patterns published in other studies, there was a higher proportion of vegetables consumed in their cooked form (24-h recall, 61%; FFQ, 57%) versus in their raw form (24-h recall, 39%; FFQ, 43%).

There was a moderate positive correlation between the 24-h recalls and FFQ in this study, but estimates of NO_3^- intake differed by approximately 100 mg day^{-1}. There was a stronger association between estimates from the 24-h recalls and urinary NO_3^- excretion than the FFQ, suggesting intakes of NO_3^--rich foods may have been overestimated in the FFQ. All dietary assessment tools are susceptible to bias and have the potential for error. Misreporting is a common challenge in dietary assessment, regardless of the assessment tool, likely because of a variety of factors. For example, systematic bias may be introduced where the assessment tool does not capture specific aspects of the local diet (such as foods indigenous to certain population groups)[26]. Under-reporting of habitual dietary intake is also well-documented in research studies, which may be a result of social desirability, opportunistic bias or memory.[6,46] However, over-reporting is more common in FFQs, dependent on the number of food categories.[6,47,48]

The customised FFQ used in the present study only included foods and beverages with a moderate to high NO_3^- level, whereas the 24-h recall included NO_3^- values from every food and beverage consumed. Additionally, multiple 24-h recall administrations were used to account for day-to-day variation, however this is only an average

TABLE 4 Comparison between food and beverage items contributing to total dietary nitrate intake from the food frequency questionnaire (FFQ) and the 24-h recall

Food items	FFQ		24-h recall	
	mg day^{-1}	Percent of daily intake	mg day^{-1}	Percent of daily intake
Uncooked dark green vegetables (such as broccoli, rocket, silverbeet, spinach, kale or pak choy)?	28.21	15.6	24.18	18.5
Cooked dark green vegetables (such as broccoli, rocket, silverbeet, spinach, kale or pak choy)?	29.12	16.1	23.64	18.1
Uncooked cabbage (such as brussels sprouts, Chinese cabbage, coleslaw, savoy or sauerkraut)?	0.49	0.3	0.80	0.6
Cooked cabbage (such as brussels sprouts, Chinese cabbage, coleslaw, savoy or sauerkraut)?	1.54	0.8	1.55	1.2
Uncooked lettuce (such as cos, iceberg, oak-leaf or mixed)?	12.95	7.2	8.42	6.4
Uncooked red and/or orange vegetable (such as carrot, pumpkin, chili pepper, capsicum, sweet potato or tomato)?	3.85	2.1	0.80	0.6
Cooked red and/or orange vegetable (such as carrot, pumpkin, chili pepper, capsicum, sweet potato or tomato)?	2.52	1.4	1.71	1.3
Uncooked radish (such as black, Japanese, radish sprouts, red, sweet or white)?	2.52	1.4	0.00	0.0
Cooked radish (such as black, Japanese, radish sprouts, red, sweet or white)?	0.77	0.4	0.43	0.3
Other uncooked root or tuber vegetables (such as artichoke, beetroot, celeriac, potato, radish, swede or turnip)?	17.29	9.6	2.60	2.0
Other cooked root or tuber vegetables (such as artichoke, beetroot, celeriac, potato, radish, swede or turnip)?	11.76	6.5	8.86	6.8
Uncooked stem vegetable (such as bamboo shoots, celery, fennel, rhubarb or leek)?	2.10	1.1	1.02	0.8
Cooked stem vegetable (such as bamboo shoots, celery, fennel, rhubarb or leek)?	5.60	3.1	0.87	0.7
Other uncooked vegetable (such as cauliflower, cucumber, eggplant, garlic, mushroom, onion, squash or zucchini)?	5.18	2.9	2.38	1.8
Other cooked vegetable (such as cauliflower, cucumber, eggplant, garlic, mushroom, onion, squash or zucchini)?	13.93	7.7	0.49	0.4
Uncooked legumes (such as broad, French, and green/string beans, and bean sprouts)?	4.41	2.4	0.18	0.1
Cooked legumes (such as broad, French, and green/string beans, and bean sprouts)?	7.28	4.0	1.39	1.1
Uncooked herb (such as basil, chives, coriander, dill, ginger or parsley)?	7.84	4.3	0.20	0.2
Cooked herb (such as basil, chives, coriander, dill, ginger or parsley)?	11.48	6.4	0.00	0.0
Uncooked bananas?	4.13	2.3	2.86	2.2
Cooked bananas?	0.07	0.1	0.00	0.0
Uncooked exotic fruit (such as jackfruit, towel, bitter, round or wax gourd [winter melon])?	0.21	0.1	0.00	0.0
Cooked exotic fruit (such as jackfruit, towel, bitter, round or wax gourd [winter melon])?	0.28	0.2	0.00	0.0
Processed meat products (bacon, sausages, pâté or luncheon meats)?	3.01	1.7	0.84	0.6
Potato crisps, gyoza or quiche (vegetable)?	3.85	2.1	4.54	3.5
Vegetable juice (homemade or commercial)?	0.14	0.1	0.00	0.0
Nitrate supplements	0.00	0.0	0.00	0.0

TABLE 4   (Continued)

| Food items	FFQ mg day^{-1}	Percent of daily intake	24-h recall mg day^{-1}	Percent of daily intake
Alcohol			1.52	1.2
Other leafy			1.60	1.2
Other–combination foods such as sushi, vegetable soups (canned), pizza, spaghetti, rice dishes, mixed sauces etc. (may include nitrate-rich foods, but are not captured in the FFQ)			21.80	16.6
Water			1.89	1.4
Other–low NO_3^- sources such as coffee, beef, strawberries, rice, and bread (not captured in the FFQ)			16.36	12.5
Total	180.62	100	130.94	100
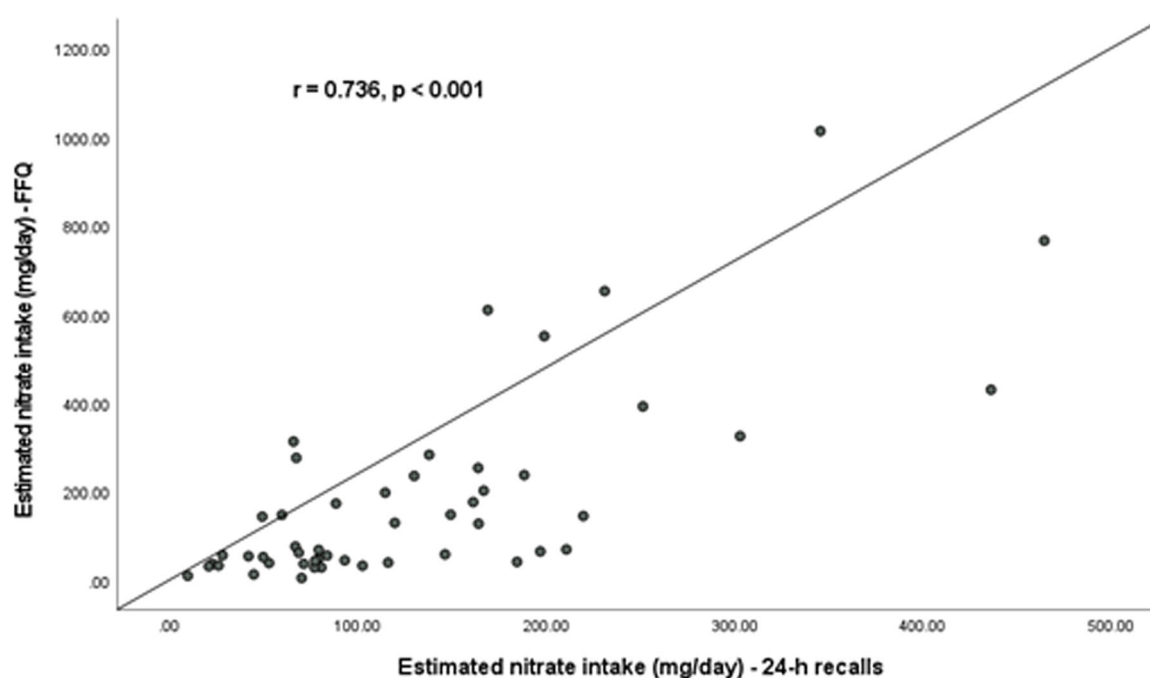
FIGURE 2   Scatter plot of mean nitrate intake (mg day^{-1}) measured by a food frequency questionnaire (FFQ) and 24-h recall showing Spearman's correlation coefficient ($n = 50$)

and days with large NO_3^- intake amounts may have contributed to the discrepancy between dietary assessment methods. Despite the current FFQ being a useful ranking tool because of the quickness, ease of administration and cost-effectiveness, for greater dietary NO_3^- intake accuracy, the findings from the present study suggest multiple administrations of 24-h recall to be the more reliable method. Blekkenhorst et al.[20] reported similar findings in their study which aimed to assess the correlations of vegetable NO_3^- intake between 24-h food recalls and a short-term (4-week) and long-term (12-month) FFQ. In their sample of 41 adults, the mean NO_3^- intake estimated from the 24-h recall and 12-month FFQ was 89.3 ± 64.7 mg day^{-1} and

71.9 ± 33.9 mg day^{-1}, respectively. The researchers reported a moderate positive correlation between NO_3^- intakes estimated from the 24-h recalls and 12-month FFQ.

Regardless of the tool used, accurate assessment of dietary intake is always challenging and complex due to intra-individual variability (i.e., the day-to-day variance in foods people eat) and inter-individual variability (such as differences between populations and the types of foods consumed).[47,49] This research adds a valuable contribution to the existing literature. Dietary NO_3^- was estimated using the most comprehensive and up-to-date reference database available, [McMahon NF, Brooker PG, Pavey TG and Leveritt MD, unpublished data] and is one of only

seven studies to have assessed dietary NO_3^- intake in humans using a biomarker, and the only study that has collected samples continuously for more than 12 h.[8]

Despite the strengths of this research, a number of limitations must be acknowledged. The daily NO_3^- intakes estimated from the FFQ and 24-h recalls differed by approximately 100 mg. By design, the customised FFQ used in this study only included foods and beverages with a NO_3^- moderate to high level (> 10 mg per 100 g) whereas the 24-h recalls included NO_3^- values from every food and beverage reportedly consumed. There is currently no acceptable biological indicator to assess nitrate intake. Urinary nitrate may provide an indication of dietary nitrate intake, however, urinary nitrate levels are affected by the L-arginine-NO synthase pathway, and the high nitrate excretion rate (approximately 75%) and therefore do not provide a robust indicator of longer-term nitrate intake.[20,50] The discrepancy in the measurement of nitrate intake between the FFQ and 24-h recalls should also be acknowledged in the context of health outcomes. This is roughly the equivalent of more than one cup of leafy green vegetables. In recognition of the dose-response relationship between fruit and vegetable intake and mortality, the FFQ should not be used to draw conclusions about absolute intakes.[51]

CONCLUSIONS

There are a number of studies which highlight the benefit of consuming a nitrate-rich diet.[4,43,52] Despite this, optimal NO_3^- intake strategies are unclear. Habitual NO_3^- intake is a factor that has the potential for discovery and development of health interventions designed to positively impact cardiovascular, metabolic and cognitive health across the lifespan. However, the ability to assess nitrate intakes depends on having suitable reference methods to estimate the concentrations of nitrate in the food supply, coupled with valid and reliable dietary assessment tools. There was a moderate positive correlation between the 24-h recalls and FFQ in this study, but there was a sizeable difference in NO_3^- intake (105.14 mg). There was a stronger association between estimates from the 24-h recalls and urinary NO_3^- excretion than the FFQ, suggesting intakes of NO_3^--rich foods may be underestimated in the FFQ. This discrepancy is likely because of limitations in the dietary assessment methods. Based on these findings, at an individual level, dietary recalls or records may be more accurate in estimating intake of NO_3^-. Conclusions cannot be drawn about absolute nitrate intakes measured from the FFQ. However, given the lower cost and time needed for administration relative to recalls, the FFQ has merit for estimating NO_3^- intakes in health interventions, dietary surveys and surveillance programs such as large cohort studies. Future research should consider methods to improve the FFQ so it may be used as a tool to provide more reliable estimates about absolute nitrate intakes and aid researchers in drawing conclusions about diet–disease relationships.

AUTHOR CONTRIBUTIONS

Nicholas F. McMahon conceptualised the study. Nicholas F. McMahon and Michael D. Leveritt developed the study design. Nicholas F. McMahon was responsible for data collection and analysis, and contributed to preparing the manuscript. Paige G. Brooker assisted with data collection and analysis, and was responsible for drafting and preparing the manuscript. Stefan Kadach contributed to data analysis. Toby G. Pavey provided statistical advice. All authors contributed to the interpretation of the results and critically reviewed the manuscript.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ETHICAL STATEMENT

Ethics approval was obtained from the University of Queensland's Human Research Ethics Committee (HMS16/1210R1).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.