Original Article

Screening for inadequate dietary vitamin B-12 intake in South Asian women using a nutrient-specific, semi-quantitative food frequency questionnaire

Gael Janine Mearns PhD[1], Elaine Carolyn Rush MNZM, PhD[2]

[1]*School of Clinical Sciences, Faculty of Health and Environmental Sciences, Auckland University of Technology, Auckland, New Zealand*
[2]*Centre for Child Health Research, Faculty of Health and Environmental Sciences, Auckland University of Technology, Auckland, New Zealand*

Background and Objectives: A high prevalence of vitamin B-12 (B-12) deficiency among young women of South Asian origin predisposes to significant health risks for these women and their future offspring. Vegetarian or low-meat based dietary practices contribute to B-12 deficiency. This study validated a nutrient-specific, semi-quantitative food frequency questionnaire (B12FFQ), developed to estimate dietary B-12 intake in South Asian women. **Methods and Study Design:** The B12FFQ was developed, then tested in 60 apparently healthy South Asian women aged 18-50 years, living in Auckland, New Zealand. Participants recalled the frequency and quantity of vitamin B-12-containing foods consumed in the preceding three months. Pearson's correlations measured the associations between dietary B-12 intake and B-12 biomarkers (serum B-12 and holotranscobalamin [holoTC]). Likelihood of B12 insufficiency was calculated for vegetarian and non-vegetarian dietary practices. **Results:** The B12FFQ was a valid measure of dietary B-12 intake - supported by moderate positive associations with serum B-12 (r=0.50, $p<0.001$, 95% CI [0.28, 0.67]) and holoTC (r=0.55, $p<0.001$, 95% CI [0.34, 0.71]). A dietary B-12 intake of less than 2.4 µg/day increased the likelihood of serum B-12 (X^2 (1)=11.79, $p=0.001$) or holoTC (X^2 (1)=6.33, $p=0.012$) insufficiency. A dietary B-12 intake of less than the recommended dietary allowance (2.4 µg/day), occurred in 61% (n=20/33) of participants with vegetarian and 22% (n=6/27) with non-vegetarian dietary practices. **Conclusions:** The B12FFQ provides a valid estimate of dietary B-12 intake. This easily administered food frequency questionnaire has the potential to identify low dietary B-12 intake as a contributor to B-12 depletion or deficiency.

Key Words: food frequency questionnaire, vitamin B-12 deficiency, South Asian women, serum B-12, holotranscobalamin

INTRODUCTION

Vitamin B-12 (B-12) depletion and deficiency are common among populations of South Asian origin due to generations of vegetarian or low meat-eating dietary practices.[1-4] Animal-source (meat, dairy, poultry, seafood) and B-12 fortified are the primary foods for metabolically available B-12.[5] In developing countries, low accessibility and consumption of these foods frequently contributes to B-12 deficiency.[6,7] In developed countries, where these foods are available and consumed, malabsorption or polymorphisms of B-12 metabolism and transport are likely contributors.[8,9] Low meat-eating or vegetarian dietary practices,[4,10] food insecurity[11] and use of medications that inhibit B-12 absorption,[12,13] also contribute.

The recommended dietary (RDA) B-12 allowance is 2.4 µg/day for adults, 2.6 µg/day during pregnancy, and 2.8 µg/day in lactation. The estimated average requirement (EAR) is 2.0 µg/day for adults, 2.2 µg/day during pregnancy and 2.4 µg/day in lactation.[14,15] Insufficient dietary B-12 intake among women of childbearing age, particularly South Asian women, is a significant problem.[2-4] Maternal B-12 deficiency during pregnancy and lactation is associated with neurological and haematological deficits in both mother and child,[3,16] increased risk of foetal neural tube defects,[17,18] and relative adiposity with childhood insulin resistance in offspring.[3,19]

There is the utility for a quick and easily applied screening tool to identify inadequate dietary B-12 intake and the likelihood of B-12 depletion, particularly in populations with low intakes of animal-source food. Early identification of inadequate intake means that tailored dietary advice or low dose oral B-12 supplementation, can be used to manage or prevent B-12 deficiency.[2] Currently, the most common treatment for B-12 deficiency is

Corresponding Author: Dr Gael J Mearns, Faculty of Health and Environmental Sciences, Auckland University of Technology, Private Bag 92006, Auckland, New Zealand.
Tel: +64 9 9219999 ext.7108; Fax: +64 99219796
Email: gael.mearns@aut.ac.nz
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high dose intramuscular injection.[20] An appropriate screen to identify inadequate dietary B-12 intake as the contributor to deficiency may avoid the unnecessary use of these injections. The aim of this study was to develop and validate a nutrient-specific, semi-quantitative food frequency questionnaire (B12FFQ) to estimate dietary B-12 intake in South Asian women.

METHODS

The participants, 60 South Asian women aged 18 to 50 years, living in Auckland, New Zealand, were participants recruited for a larger VitB-12 supplementation study.[2] Recruitment methods included flyers, local media advertisements, and referral through South Asian contacts. A previous publication from the VitB-12 study explained sample size calculations, participant characteristics, sampling and methods for blood testing.[2] Exclusion criteria included major health conditions, food malabsorption, pregnancy or breastfeeding, B-12 supplementation in the previous 18 months, or use of medications known to inhibit B-12 absorption. A University Ethics Committee approved the research (approval number 08/02).

Outcome measurements

Primary outcome measures were the relationships between estimated dietary B-12 intake from the B12FFQ and the B-12 biomarkers serum B-12 and serum holo-transcobalamin (holoTC). Secondary outcome measures were dietary B-12 intake (μg per day) as measured by the B12FFQ, B-12 status as determined by B-12 biomarkers and the contribution of aggregated food groups to total dietary B-12 intake. Previous studies support serum B-12 and holoTC as valid biomarkers against which to compare dietary B-12 intake from a dietary measurement tool.[21,22]

Development of the B-12 food frequency questionnaire

The B12FFQ instrument included 30 questions on consumption of food and beverages containing B-12. The foods and beverages commonly consumed by South Asian women were identified in focus group discussions that preceded development and testing of the B12FFQ. Separate B-12 containing food items such as chicken or yoghurt or eggs were listed, and participants estimated the frequency and quantities of these ingredients in the dishes and beverages consumed over the preceding three months. This process aimed to capture both habitual and infrequent consumption of dietary B-12. The B-12 content of items included in the B12FFQ was calculated from the New Zealand Institute for Plant & Food Research Food Composition Tables[23] and the software program FoodWorks® (Xyris Software, Australia). An accompanying Excel™ spreadsheet contained these B-12 content calculations.

A professor of nutrition critiqued and approved the final version of the B12FFQ and Excel B-12 spreadsheet. Quantities were estimated using standard household measures. Frequencies for consumption of a particular food ranged from 'never', through to 'six or more times per day', with nine options of frequency in total. Five volunteers pre-tested the B12FFQ to check for comprehension, the logic of food order, and the ability to accurately capture the quantities and frequency of foods and beverages consumed from the category options in the B12FFQ.

Procedure

The researcher administered the B12FFQ, which took approximately 10 min to complete. Participants visually estimated food serving size and quantities, using physical examples of standard household plate, bowl, cup and spoon measures. Quantities and frequencies of foods and beverages were entered into the accompanying Excel spreadsheet on completion of the questionnaire, and the mean daily dietary B-12 intake was calculated. Five aggregated categories grouped the foods contributing to B-12 intake; dairy products, eggs, red meat, white meat and seafood, and B-12 fortified foods.

Data analysis

Data (B-12 biomarkers and B-12 dietary intake) were log-transformed to reduce skewness. Descriptive statistics report the medians with 25th/75th percentiles for B-12 biomarkers and estimated dietary B-12. Pearson's correlation coefficients measured the associations between dietary B-12 intakes from the B12FFQ, and baseline serum B-12 and holoTC. Using the correlation and probability values from SPSS 20, 95% confidence intervals were derived using a separate World Wide Web published algorithm.[24]

Dietary preferences of the participants were categorised as 1) lactovegetarian (consume milk, cheese, but no meat or fish), 2) lactoovovegetarian (as for lactovegetarian but also eat eggs), 3) white meat-eating (eat chicken and/or fish but no red meat), and 4) red meat-eating (eat both white and red meats).[25] These were combined into the binary variables of vegetarian (1 & 2 above) and non-vegetarian (3 & 4) dietary practices for further analysis. Descriptive statistics, using a population proportion method, report the contribution of aggregated B-12 food groups to the sum of the estimated dietary B-12 intake for the vegetarian/non-vegetarian dietary practice groups.

Biomarker cut-off values were used to determine 1) B-12 depletion (serum B-12 150-221 pmol/L, holoTC 35-44 pmol/L).[17,26] and 2) B-12 deficiency (serum B-12 <150 pmol/L, holoTC <35 pmol/L).[26,27] For contingency table analysis, biomarker values were combined into B-12 insufficiency (<222 pmol/L) or sufficiency categories (≥222 pmol/L). The relative risks of B-12 insufficiency from vegetarian/non-vegetarian dietary practices and 95% confidence intervals for risk were calculated from the contingency table, with Fisher's exact test applied to determine statistical significance.

Chi-square analysis explored the accuracy of the B12FFQ as a screening tool for B-12 biomarker insufficiency. The latter was based on insufficiency cut-off values of serum B-12 <222 pmol/L and holoTC <45 pmol/L. The sensitivity of the B12FFQ for screening B-12 biomarker insufficiency was derived from the number of true positives (dietary B-12 intake <2.4 μg and B-12 biomarker insufficiency values above). Specificity was calculated from the number of true negatives (dietary B-12 intake ≥2.4 μg and B-12 biomarker sufficiency values above). Unless stated otherwise, data analysis was un-

Table 1. Dietary B-12 intake by dietary practice group

	n	25th†‡	Median†	75th†‡
Lactovegetarian	26	1.0¶	1.8¶	3.1
Lactoovovegetarian	7	1.2¶	1.6¶	2.1
White meat-eating	5	1.1¶	1.6¶	3.7
White and red meat-eating	22	3.1	5.5	7.1

†Measured in µg/day.
‡Tukey Hinges interquartile ranges.
¶Less than both the RDA of 2.4 µg/day and the EAR of 2.0 µg/day

dertaken using SPSS Edition 20 (IBM Corporation).

RESULTS

Sixty South-Asian women (aged 18 to 50 years) completed the FFQ. Participants were of Indian, Fijian Indian, Pakistani and Sri Lankan origin. Of these, 33 reported vegetarian dietary practices (26 lactovegetarian and 7 lactoovovegetarian) and 27 reported non-vegetarian dietary practices (22 ate both red and white meat and 5 ate white meat only). Median B-12 intake was below the RDA of 2.4 µg/day and the EAR of 2 µg/day for the vegetarian and the white meat–eating groups (Table 1).

The B12FFQ estimate of dietary B-12 intake was positively associated with serum B-12 (r =0.50, $p<0.001$, 95% CI [0.28, 0.67]) and holoTC (r=0.55, $p<0.001$, 95% CI [0.34, 0.71]). Insufficient dietary B-12 intake (<2.4 µg/day) was recorded for 44% (n=26) of the participants. Of these, 61% (n=20) reported vegetarian and 22% (n=6) reported non-vegetarian dietary practices. The median dietary B12 intake for the vegetarian group was 1.7 µg/day [1.1, 3.1] and for the non-vegetarian group, 4.3 µg/day (25th/ 75th percentiles [2.5, 6.8]).

A dietary B-12 intake of less than 2.4 µg/day increased the likelihood of serum B-12 (X^2 (1)=11.79, p=0.001) or holoTC (X^2 (1)=6.33, p=0.012) insufficiency. Sensitivity for detecting risk for holoTC insufficiency using the B12FFQ (<45 pmol/L) was 70% (21/30 true positives identified). Specificity was 83% (25/30 true negatives identified) (Figure 1). Sensitivity for detecting serum B-12 insufficiency (B-12 <222 pmol/L) was 69% (20/29 true positives identified). Specificity was 74% (23/31 true negatives identified) (Figure 2). Application of a higher dietary B-12 intake cut-off (4 µg/day), increased the sensitivity to detect serum B-12 insufficiency to 90% (26/29 true positives), but decreased the specificity to 55% (17/31 true negatives).

Relationships between dietary intake of B-12 and B-12 biomarkers

Participants with vegetarian dietary practices had much lower B-12 biomarkers than those with non-vegetarian dietary practices (Table 2). The relative risk of low B-12 biomarkers was compared between the vegetarian and non-vegetarian groups. For the vegetarian group, risk for B-12 insufficiency (≤222 pmol/L) was 2.2 times higher (p=0.02, 95% CI [1.2, 4.4]), and for holoTC insufficiency (≤45 pmol/L) 2.8 times higher (p=0.005, 95% CI [1.4, 5.9]). The median serum B-12 for the vegetarian group was 199 pmol/L (25th/75th percentiles [154, 263]), and for the non-vegetarian group, 313 pmol/L [209, 432]).

Dietary B-12 intake by food groups

The B-12-containing foods contributing to total dietary B-12 intake for the combined vegetarian group were dairy (67%), B-12 fortified foods such as soy or rice milk (28%) and eggs (5%). For the combined non-vegetarian group, it was dairy (30%), red meat (26%), eggs (17%), white meat (17%) and B-12 fortified foods such as yeast extracts (10%).

DISCUSSION

This research supports that this simple and easily applied B12FFQ is a valid measure of dietary B-12 intake in a population with a high prevalence of B-12 depletion and deficiency. Using the RDA cut-off of 2.4 µg of dietary B-12 per day, the 30 question B12FFQ was a sensitive tool with a moderate specificity for screening for risk for B-12 deficiency.

These results for the B12FFQ are important because

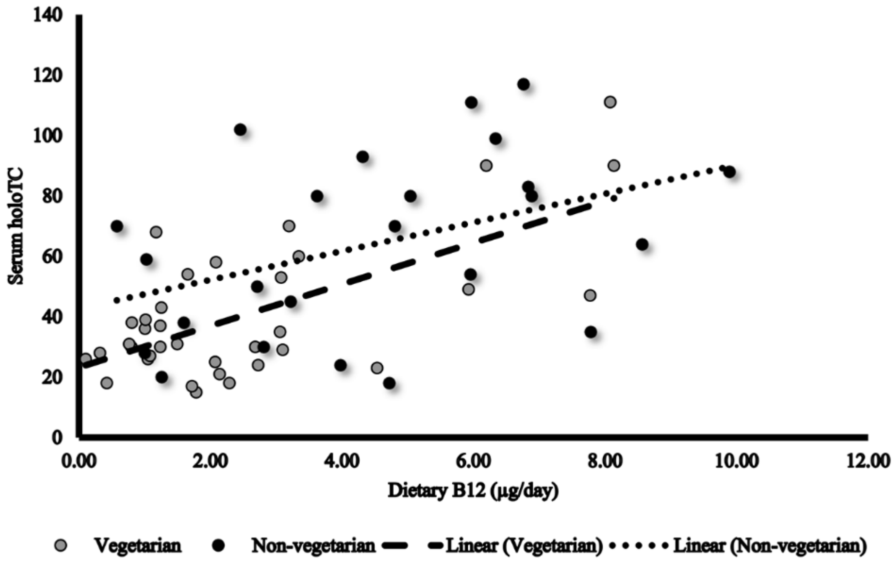

Figure 1. Dietary B-12 intake (B12FFQ) and holoTC by vegetarian/non-vegetarian dietary practices

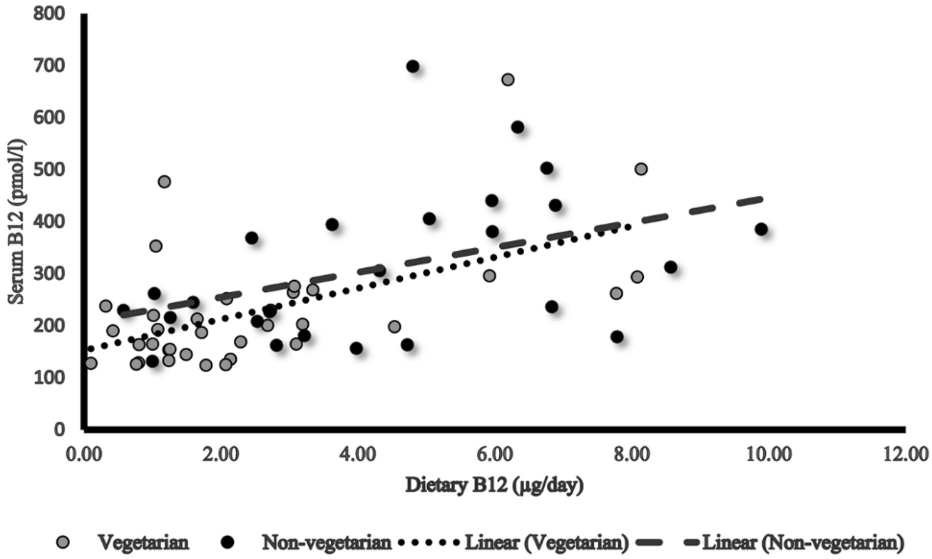

Figure 2. Dietary B-12 intake (B12FFQ) and serum B-12 by vegetarian/non-vegetarian dietary practices.

Table 2. B-12 biomarkers by vegetarian or non-vegetarian dietary practices

Biomarker	Dietary practice	Insufficient†	Sufficient †
Serum B-12	Vegetarian	21 (64)‡,	12 (36)¶
	Non-vegetarian	9 (33)‡,	18 (67)¶
HoloTC	Vegetarian	23 (70)§	10 (30)††
	Non-vegetarian	7 (26)§	20 (74)††

†Data reported as number of participants and percentages of group.
‡Serum B-12 < 222 pmol/L.
¶Serum B-12 ≥222 pmol/L.
§holoT C<45 pmol/L.
††holoTC ≥45 pmol/L.

there currently does not appear to be a valid B-12-nutrient specific FFQ screening tool available to estimate dietary B-12 intake. The B2FFQ demonstrated moderate associations between dietary B-12 intake and B-12 biomarkers (r=0.5 for serum B-12, r=0.55 for holoTC). This value is a higher validity than is usually reported for FFQs, particularly when compared with non-specific FFQs that include a wider variety of foods from which to calculate multiple nutrient intakes.[28,30,31] The B12FFQ findings support previous research on dietary instrument screening for specific nutrient deficiencies. These show that nutrient specific dietary instruments have a high predictive validity for that specific nutrient intake.[29]

A vegetarian diet was a significant contributing factor for inadequate dietary B-12 intake and low B-12 biomarker concentrations in the B12FFQ study. However, eating meat did not exclude B-12 insufficiency, with over one-quarter of women with non-vegetarian dietary practices low in B-12 biomarkers. The most common dietary source for B-12 across both vegetarian and non-vegetarian groups was dairy; for the non-vegetarian group it contributed 30% of the median B-12 daily intake, while for the vegetarian group, it contributed 67%. Studies analysing the relationships between B-12 biomarkers and B-12 dietary intake of different foods found that the B-12 from dairy products and fish,[31] dairy and fortified cereals,[32] appear to be more closely associated with B-12 biomarkers than the B-12 from eggs or meat. Although dairy was the biggest contributor to B-12 intake for participants from the vegetarian group, the quantity of dairy consumed was not enough to prevent serum B-12 and holoTC biomarker insufficiency in approximately two-thirds of participants

Research suggests that the RDA for B-12 may be set too low at 2.4 µg per day and that a daily intake of 4 µg per day is more appropriate to prevent B-12 deficiency.[21] A daily B-12 intake cut-off of 4 µg per day was applied to the B12FFQ in this study to ascertain if this would increase the sensitivity of the B12FFQ for identifying risk for B-12 insufficiency. While it did improve the sensitivity of the B12FFQ to detect B-12 insufficiency, this was offset by a loss of specificity, thus reducing the usefulness of the 4 µg cut -off.

The reliability of the B12FFQ has not been formally tested so further work is required to measure the test-retest reliability. The reliability and validity of the B12FFQ also needs testing in other populations, across different ethnic and age groups, and in populations with a variety of dietary practices. Once validated in different populations, the B12FFQ could be developed into an electronic, non-invasive tool or application to aid diagnostic decision making on the contributors to B-12 deficiency.

Conclusion

The B12FFQ is an easily administered, non-invasive tool with a moderate predictive ability to screen for inadequate dietary B-12 intake in South Asian women. By identifying women of childbearing age at risk of low B-12 status from inadequate dietary B-12 intake, this provides the opportunity to offer dietary advice to prevent B-12 deficiency, or where appropriate, to intervene with low dose oral B-12 supplements to treat depletion or early B-12 deficiency. This is an important public health step to reducing the maternal and offspring health risks associated with maternal B-12 deficiency during pregnancy and breastfeeding, particularly among South Asian populations.

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AUTHOR DISCLOSURES

The authors declare that they have no conflict of interest.

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