Original Article

A valid food frequency questionnaire for measuring dietary fish intake

Rosalie K Woods[1] BSc, Grad Dip Diet, MPH, PhD, Rachel M Stoney[2] BSc, M Nutr & Diet, PhD, Paul D Ireland[5] BSc(Hons), Grad Dip Diet, MSc, PhD, Michael J Bailey[1] BSc(Hons), MSc, Joan M Raven[3] RN, Frank CK Thien[4] MD, FRACP, E Haydn Walters[6] MA, DM, FRCP, FRACP, FCCP, and Michael J Abramson[1] MB BS, PhD, FRACP, FAFPHM

Departments of [1]Epidemiology and Preventive Medicine, [2]Nutrition, [3]Respiratory Medicine, [4]Allergy, Asthma and Clinical Immunology, Central and Eastern Clinical School and Alfred Hospital, Prahran, Australia
[5]National Cancer Control Initiative, Melbourne, Australia
[6]Department of Medicine, University of Tasmania, Hobart, Australia

There is considerable interest in the potentially protective effects of high fish consumption on many chronic diseases. Many epidemiological studies use food frequency questionnaires (FFQ) to quantify usual dietary fish intake, so it is important to validate this assessment against objective markers. The objective of this study was to determine the relationship between plasma percentage fatty acids and dietary fish intake as assessed by a FFQ. A semiquantitative FFQ was completed by 174 adults from the community (aged 26–49 years) who also had venous blood analysed for plasma percentage fatty acids. Following linear regression modelling, total non-fried fish intake was a significant predictor of n-3 (regression coefficient, B = 0.94; 95% CI = 0.60–1.28), docosahexaenoic acid (DHA; B = 0.73; 95% CI = 0.47–0.99) and the ratio of n-6 : n-3 fatty acids (B = –1.0; 95% CI = – 1.35– – 0.65). Steamed, grilled or baked fish was a small but significant predictor of eicosapentaenoic acid (EPA) levels (B = 0.13; 95% CI = 0.05–0.21) while total fish intake was a predictor of n-6 fatty acids (B = – 0.88; 95% CI = – 1.41– – 0.36). This semiquantitative FFQ could be useful for ranking subjects according to their likely plasma n-3, DHA, and n-6 fatty acid intake and the ratio of n-6 : n-3 fatty acids, when the available resources may simply not permit biological markers to be used.

Key words: fatty acid patterns, fish intake, food frequency questionnaire, n-3 and n-6 fatty acids, percentage fatty acids, plasma phospholipid, validation.

Introduction

Interest in the beneficial health effects of diets rich in marine fatty acids and fish has occurred following observations that populations with a high dietary intake of fish have a low incidence of atherosclerotic and thrombotic disorders and inflammatory conditions such as rheumatoid arthritis.[1–3] These observations have prompted widespread recognition that long-chain n-3 fatty acids, of which fish is a major source, are an important component of the human diet. The Australian National Health and Medical Research Council (NHMRC) working party on the role of polyunsaturated fats in the Australian diet has recommended that an increase in the intake of n-3 fatty acids from plant foods and fish be encouraged.[4]

Previous research has shown that plasma n-3 fatty acid concentrations are related to the dietary intake of long-chain n-3 fatty acids, as assessed by 24 h dietary recall, and that this relationship does reflect long-term intake.[5] However, only a limited number of studies have validated fish consumption as assessed by semiquantitative food frequency questionnaires (FFQ) with plasma fatty acid concentrations.[6,7]

Given that many epidemiological studies depend upon FFQ to assess usual dietary intake, including fish intake, this relationship needs further investigation. A finding that a clear relationship exists between FFQ-assessed fish intake and plasma long-chain n-3 fatty acid concentrations would provide confirmation that both methods of dietary assessment can be used to obtain useful information on the intake of long-chain n-3 fatty acids. The aim of this study therefore was to measure the fatty acid composition of plasma and compare this with fish consumption as assessed by a semiquantitative FFQ.

Correspondence address: Dr Rosalie Woods, Department of Epidemiology and Preventive Medicine, Central and Eastern Clinical School, Commercial Road, Prahran, Victoria 3181, Australia.
Tel: + 61 39903 0592; Fax: + 61 39903 0576
Email: rosalie.woods@med.monash.edu.au
Accepted 9 August 2001

Subjects and methods

Participants

A community sample of young adults aged 20–44 years was recruited in 1992/1993 to undertake the European Community Respiratory Health Survey (ECRHS) in Melbourne, Australia. Seven hundred and fifty-seven participants attended the laboratory for that particular study. The full methodology of this study has been published elsewhere[8,9] but briefly, 4500 adults aged between 20 and 44 years were randomly selected from the electoral rolls from three federal electorates in the inner south-eastern area of Melbourne, the capital city of Victoria. These subjects were invited to complete a brief postal questionnaire (phase I of the study). A response rate of 72% (n = 3200) was achieved from this phase of the study. A random subsample of 1642 subjects was identified as eligible for follow up in the second phase of the study. A second symptomatic subsample was identified comprising 433 respondents who, in the first phase, had reported being woken by shortness of breath, having had an attack of asthma during the last 12 months or taking medication for asthma. Thus a total of 2075 young adults were invited to our laboratory for testing between November 1992 and March 1994. A total of 757 (553 from random subsample and 204 from the symptomatic subsample) attended our laboratory at that time.

The ECRHS was repeated in 1998 with 463 participants (336 from the random subsample, 127 from the symptomatic subsample) attending the laboratory in Melbourne. As part of this follow up, the first 174 participants who agreed to have an additional 8 mL blood sample taken for analysis of plasma percentage fatty acids were included in the current analysis. We estimated in advance that a sample size of 174 should be adequate to detect a correlation of 0.29 (95% CI from 0.15 to 0.42) with 90% power and a level of significance of 0.01. The Standing Committee on Ethics in Research on Humans at Monash University and The Alfred Hospital Ethics Committee approved this project. Written informed consent was obtained from all participants.

Food frequency questionnaire

Participants attending the 1998 ECRHS also completed a four page optical mark readable semiquantitative food frequency questionnaire, for assessment of dietary intake in the preceding 12 months. This questionnaire was originally designed to derive estimates of macro- and micronutrient intake for a large prospective study of Australian adults from ethnically diverse backgrounds.[10] The questionnaire had 74 food items grouped into four categories: cereal foods, sweets and snacks, dairy products, meats and fish, and fruit and vegetables. Frequency responses were recorded for each food item according to the following scale: never, less than once per month, one to three times per month, once per week, twice per week, three to four times per week, five to six times per week, once per day, twice per day and three or more times per day. In addition, the questionnaire contained a number of questions pertaining to specific food habits, such as the type and frequency of consumption of milk, fruits and vegetables, bread, eggs, cheese, sugar and fat spreads. The questionnaire also included four sets of diagrams relating to portion size of four different but commonly consumed food items (potato, vegetables, steak, casserole), from which a 'portion size factor' was computed for each individual. The standard portion sizes were not age or gender specific. The daily intake of energy and nutrients was derived through multiplication of the food item frequency response options by the standard portion size for each of the food items, and adjusted at the individual level through application of the computed portion size factor. The food database employed utilized nutrient data from McCance and Widdowson[11] for Vitamin E and folate, while all other nutrients were derived from the Australian NUTTAB 95 database.[12]

Specifically, with regard to fish consumption, the questionnaire asked respondents to indicate the frequency with which they consumed fish; as steamed, grilled or baked fish, fried fish (including take-away/take-out) and tinned fish (salmon, tuna, sardines, etc.). Total fish intake was defined as the sum of steamed, grilled or baked fish, fried fish and tinned fish, as reported in the FFQ. Total non-fried fish intake was determined as the sum of steamed, grilled or baked fish and tinned fish, as reported in the FFQ.

Given that the aim of the current study was to assess the validity of the FFQ for assessment of relative long-chain n-3 fatty acid intake, specific questions were added regarding current use or use within the past six months of fish oil/fatty acid supplements. These questions were asked during the respiratory questionnaire. In addition, the test retest reliability of the current FFQ was assessed in a subset of 69 participants, in whom the mean time between administration of the FFQ was 47 days (SD = 23, range = 10–109 days).

Plasma percentage fatty acid analysis

Plasma lipids were extracted with chloroform : methanol as previously described.[13] Plasma phospholipids were separated by thin-layer chromatography and evaporated to dryness under nitrogen. The samples were then methylated in 1% H_2SO_4 in methanol at 70°C for 3 h, and when cooled, the resulting methyl esters were extracted into n-heptane and transferred to vials containing anhydrous Na_2SO_4 as the dehydrating agent.

Fatty acid methyl esters were then separated and quantified using a Hewlett-Packard 6890 gas chromatograph equipped with a 50-m capillary column (0.33 mm internal diameter) coated with BPX-70 (0.25 μm film thickness; SGE, Victoria, Australia). The injector temperature was set at 250°C and the detector (flame ionisation) temperature at 300°C. The initial oven temperature was 140°C and was programmed to rise to 220°C at 5°C per minute. Helium was used as the carrier gas at a velocity of 35 cm/s. Fatty acid methyl esters were then identified by comparison with the retention time of authentic lipid standards obtained from Nucheck Prep (Elysian, MN, USA).

Statistical analysis

Frequency counts, descriptive statistics, Pearson correlations, intraclass correlations and stepwise multiple linear

regressions were performed using the SAS statistical package.[14] For data that were not normally distributed, log transformation was used. Ninety five percent confidence intervals were calculated for the correlation coefficients. The regression model included the following potential predictor variables: age, gender, body mass index, smoking, total energy intake, macronutrient intake (fat, carbohydrate, protein and alcohol), micronutrient intake (beta-carotene, folate, potassium, retinol, sodium, vitamin C, vitamin E), fibre, saturated fat, polyunsaturated fat, monounsaturated fat and cholesterol. In light of multiple comparisons, $P < 0.01$ was regarded as significant.

Results

Subject profile

The mean age of the 174 subjects was 38.3 years (SD = 6.1, range = 26–49). Fifty five percent of subjects were female while 83% were Australian born. Eighty seven percent of respondents were employed, 9.5% performed home duties and 3.5% were not employed. Fifty three percent ($n = 91$) had ever smoked, while 24% ($n = 41$) were current smokers. Table 1 summarises the subject characteristics and energy and macronutrient intake profile for men and women. Twenty one subjects (12%) were currently taking ($n = 13$) or had taken ($n = 20$) fish oil or fatty acid supplements in the past 6 months.

Reliability of the FFQ

The intraclass (within subject) correlations and 95% CI obtained for the dietary measures of fish intake within the subset of individuals to whom the FFQ was readministered were: steamed, grilled or baked fish (0.62, 0.42–0.82); fried fish (0.35, 0.14–0.56); tinned fish (0.63, 0.49–0.77); total fish (0.60, 0.45–0.75); total non-fried fish (0.68, 0.55–0.81).

Plasma percentage fatty acid levels

The largest proportion of fatty acids was saturated (43.6%), followed by polyunsaturated (41.6%) and monounsaturated (13.8%; Table 2). When subjects who were currently or had taken fish oil/fatty acid supplements in the past 6 months were excluded from this analysis, there was no change in the mean plasma saturated or *trans* fatty acid levels. However, the mean proportion of n-6 fatty acids (35.5 ± 2.2%) and n-6:n-3 ratio were slightly higher (6.1 ± 1.4%) while the proportions of n-3 (6.2 ± 1.4%), eicosapentaenoic acid (EPA; 1.1 ± 0.4%) and docosahexaenoic acid (DHA; 3.8 ± 1.0%) were marginally lower than when all subjects were included in the analysis.

Reported fish intake as assessed by the FFQ

The median total daily intake of fish was 40 g/day, which was predominantly from steamed, grilled or baked fish (16 g/day), followed by tinned fish (11 g/day; Table 2). There was no change in the median daily intake of total fish, total non-fried fish or tinned fish when subjects who were currently or had taken fish oil/fatty acid supplements in the past 6 months were excluded from the analysis. However, the median intake of steamed, grilled or baked fish (from 16 g to 15 g/day) and fried fish (from 6.6 g to 6.5 g/day) declined slightly.

Correlations between plasma percentage fatty acids and reported fish intake

Subjects who were currently taking or had taken fish oil/fatty acid supplements in the past 6 months were excluded from this analysis. Plasma n-3 and DHA fatty acids were significantly positively correlated with all types of fish except fried fish (Table 3). EPA levels were significantly positively correlated with the intakes of steamed, grilled or baked fish and total non-fried fish. Similarly, the n-6:n-3 fatty acid ratio was significantly and inversely associated with steamed, grilled or baked fish, tinned fish, total fish and total non-fried fish intakes.

No significant associations were observed between the intake of fried fish and n-3, EPA or DHA fatty acids and the n-6:n-3 fatty acid ratio. Furthermore, no significant correlations were observed between plasma saturated, *trans*, monounsaturated or n-6 fatty acid levels and fish intake (data not shown). When all subjects were included in this analysis the values obtained were not very different to those reported in Table 3.

Multivariate analysis of percentage fatty acids

Table 4 details the significant independent predictors of percentage fatty acids using stepwise multiple linear regression analysis when those who were currently taking fish oil/fatty

Table 1. Subject characteristics and macronutrient intake ($n = 174$)

	Men		Women		All
Characteristic	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)
Age (years)	39.8 (6.1)	27–49	37.2 (5.8)	26–47	38.3 (6.1)
Body mass index (kg/m^2)	26.7 (3.7)	20.9–39.0	25.7 (4.4)	17.2–38.8	26.1 (4.1)
Energy intake (MJ/day)	8.8*	4.1–23.6	6.6*	2.3–13.0	7.48*
Fat, total intake (g/day)	79.7*	27.1–257.2	56.6*	13.1–134.3	66*
Carbohydrate (g/day)	213.3*	91.8–487.8	170.9*	73.0–308.0	188.7*
Protein (g/day)	93.8*	40.0–333.6	70.2*	20.9–148.4	79.8*
Alcohol (g/day)	7.5*	0–79.0	3.7*	0–49.9	5.1*

* These variables were found to follow log-normal distributions and are summarised as geometric means. Due to the nature of the log-normal distribution, standard deviations are not appropriate.

Table 2. Proportion of plasma fatty acids and fish intake in the study population (n = 174)

Item	Mean (SD)	Median	Interquartile range (25–75%)
% plasma fatty acids (% total fatty acid)			
Saturated fatty acid	43.6 (0.9)	43.6	42.9–44.1
Monounsaturated fatty acid	13.8 (1.6)	13.7	12.8–14.6
trans Fatty acid	0.8 (0.2)	0.75	0.6–0.9
n-6 Fatty acid	35.3 (2.25)	35.2	34.2–36.8
n-3 Fatty acid	6.3 (1.4)	5.9	5.2–6.9
n-6:n-3 ratio	5.9 (1.4)	6.0	4.9–6.9
Eicosapentaenoic acid (EPA) [20 : 5n-3]	1.2 (0.5)	1.0	0.8–1.4
Docosahexaenoic acid (DHA) [22 : 6n-3]	3.9 (1.1)	3.7	3.2–4.6
Fish intake as reported via FFQ (g/day)			
Steamed, grilled or baked fish	22.1*	16.2	8.8–26.2
Fried fish	11.5*	6.6	0–15
Tinned fish	20.9*	11.3	7–20
Total fish	54.5*	40.0	26.2–68.8
Total non-fried fish	43.0*	30.0	18.8–50

* These variables were found to follow log-normal distributions and are summarised as geometric means. Due to the nature of the log-normal distribution, standard deviations are not appropriate.

Table 3. Pearson correlation coefficients between percentage plasma fatty acids and reported fish intake excluding those taking fish oil/fatty acid supplements (n = 153)*

Fish intake (g/day)	% n-3 (95% CI)	% n-6:n-3 ratio (95% CI)	% EPA (95% CI)	% DHA (95% CI)
Steamed, grilled or baked fish	0.33 (0.18–0.46)	– 0.33 (– 0.46– – 0.18)	0.20 (0.04–0.35)	0.33 (0.18–0.46)
Fried fish	0.06 (– 0.10–0.22)	– 0.06 (– 0.22– – 0.10)	0.01 (– 0.15–0.17)	0.07 (– 0.09–0.23)
Tinned fish	0.20 (0.04–0.35)	– 0.24 (– 0.38– – 0.08)	0.04 (– 0.12–0.20)	0.27 (0.12–0.41)
Total fish	0.29 (0.14–0.43)	– 0.31 (– 0.45– – 0.16)	0.16 (0.0–0.31)	0.29 (0.14–0.43)
Total non-fried fish	0.33 (0.18–0.46)	– 0.33 (– 0.46– – 0.18)	0.18 (0.02–0.33)	0.34 (0.20–0.48)

* These variables are presented as r-values (95% CI). DHA, docosahexanoic acid; EPA, eicosapentaenoic acid.

Table 4. Predictors of percentage plasma fatty acids in multiple linear regression analyses excluding those taking fish oil/fatty acid supplements (n = 150)*

Dependent variable	Explanatory variable	Regression coefficient (95% CI)	Partial R^2 (%)
Plasma n-6 fatty acid (% total fatty acid)	FFQ polyunsaturated fat intake	2.35 (1.47–3.23)	9.6
	Total fish intake	– 0.88 (– 1.41– – 0.36)	9.4
	Energy intake	– 2.91 (– 4.55– – 1.26)	3.4
Plasma n-3 fatty acid (% total fatty acid)	Total non-fried fish intake	0.94 (0.60–1.28)	10.0
	FFQ polyunsaturated fat intake	– 0.74 (– 1.10– – 0.39)	10.6
Plasma ratio n-6:n-3 fatty acid	Total non-fried fish intake	– 1.00 (– 1.35– – 0.65)	10.2
	FFQ polyunsaturated fat intake	0.91 (0.54–1.28)	12.4
Plasma EPA (% total fatty acid)	Steamed, grilled or baked fish	0.13 (0.05–0.21)	6.4
	FFQ polyunsaturated fat intake	– 0.18 (– 0.30– – 0.05)	4.5
Plasma DHA (% total fatty acid)	Total non-fried fish intake	0.73 (0.47–0.99)	10.8
	FFQ total fat intake	– 0.63 (– 0.98– – 0.27)	9.3

* These variables are presented as r-values (95% CI). DHA, docosahexanoic acid; EPA, eicosapentaenoic acid; FFQ, food frequency questionnaire.

acid supplements and those who had done so within the past 6 months were excluded from the model. Fish intake was not a significant predictor for plasma saturated, monounsaturated or *trans* fatty acids (data not shown). Total non-fried fish intake was a significant predictor of n-3, DHA and the ratio of n-6:n-3 fatty acids. Steamed, grilled or baked fish was a significant predictor of EPA levels while total fish intake was a significant predictor of n-6 fatty acids.

Discussion

This study has shown that an Australian FFQ, originally designed to assess macronutrient and selected micronutrient intake in ethnically diverse populations,[10] provides a valid and reproducible assessment of plasma n-3 and DHA fatty acid concentrations. Indeed, total non-fried fish intake was a significant independent predictor of n-3, DHA and the ratio of n-6:n-3 fatty acids. We also found that total fish intake was a significant predictor of plasma n-6% fatty acid levels while steamed, grilled or baked fish was a small but significant predictor of plasma EPA levels. As would be expected, no such associations were found between reported fish intake and plasma saturated, *trans* or monounsaturated fatty acids.

The median daily intake of fish in this study (40 g/day) was similar to that reported in the 1992 National Seafood Consumption Study,[15] where the per capita intake of finned fish was 25.5 g/day, with other seafood varieties contributing an additional 7.5 g/day. The National Nutrition Survey, which was conducted in 1995, found the median intake of fish and seafood products and dishes to be 100 g/day.[16] The large difference in median intakes of fish between our study and the National Nutrition Survey is largely due to the different definitions of 'fish' used. Our study collected data on the frequency of consumption of steamed, grilled or baked fish, fried fish (including takeaway/takeout) and tinned fish (salmon, tuna, sardines, etc.) while the National Nutrition survey data included the consumption of fin fish, crustacea and molluscs, packed fish and seafood, fish and seafood products and mixed dishes with fish or seafood as the major component.

There is little data available on the n-3 fatty acid intake of Australians. While some estimates have been made,[4,17] the 1995 National Nutrition Survey has not been analysed for n-3 fatty acid content.[18] This is due, at least partly, to the large variability in n-3 fatty acid content between different species of fish[19–21] and incomplete information on the n-3 fatty acid content of foodstuffs other than fish. Furthermore, approximately 20% of fish consumed in Australia are imported species.[22] While fish is considered a significant source of n-3 fatty acids in the diet, it is important to remember that there are also other dietary sources of n-3 fatty acids. Unfortunately, we were unable to estimate the intake of DHA and EPA from the FFQ as no Australian database is currently available for the n-3 fatty acid composition of foodstuffs.

Our data has shown, not surprisingly, that no significant correlation exists between fried fish and plasma percentage fatty acids. Fried fish tends to be of white fleshed varieties, which are characteristically lower in n-3 fatty acids and are generally fried in n-6 containing oils (e.g. sunflower). Of the non-fried fish varieties, a greater correlation would be expected as this would be more representative of the n-3 fatty acid content of these varieties.

This study assessed plasma fatty acid concentrations. Other biological specimens, such as red cell membranes, adipose tissue and cheek cells, have also been used to assess fatty acid levels.[23–27] Katan *et al.* found that EPA levels in plasma esters reflect intake over the past 1–2 weeks, erythrocytes over the past month or two and adipose tissue over a period of years.[28] Thus, it would appear that adipose tissue could potentially reflect dietary n-3 fatty acid intake over longer periods, although the logistic difficulties of biopsying this tissue may be considered disproportionate to the likely improvement in biological signal. Van Houwelingen *et al.* found that the habitual intake of n-6 fatty acids in 61 Dutch men was better reflected by the total serum lipids rather than separate lipid fractions.[23] In a study of 125 Norwegian men aged 20–55 years, Andersen *et al.* found that the relative amount of very-long-chain-3 fatty acids in total serum lipids may be associated with the intake of these fatty acids to the same extent as the relative amounts of these fatty acids in adipose tissue.[26]

However, while plasma fatty acid levels have been shown to reflect dietary fatty acid intake in a reliable fashion[29] there are other factors that can also influence plasma fatty acid levels. These factors include variations in food composition, the method of food preparation, other sources of n-3 fatty acids (such as organ meats, walnuts, soybean, canola and flaxseed oils) and dietary intake of linoleic acid.[23]

Our data are consistent with several other studies[6,25,30,31] which have found that n-3 fatty acid concentrations are increased during, and following, fish oil supplementation. Furthermore, the correlation between FFQ assessed fish intake and plasma percentage fatty acids in the current study were of a similar order of magnitude to that reported previously.[6,7] While Hjartaker *et al.* found that 'fatty' fish was a better predictor of serum n-3 fatty acids than 'lean' fish, the FFQ that was used in the current study did not distinguish between these different varieties of fish.[7] In future studies, it may be prudent to use these classifications when designing FFQ that are to be used for large-scale epidemiological studies.

Using biological markers, such as plasma phospholipid fatty acids, as markers for dietary intake has the advantage of being free of some of the constraints inherent in all dietary intake assessment methods. In particular, dietary intake assessment is largely dependent upon the individual's ability and willingness to report food intakes precisely. Food frequency questionnaires provide a convenient means of estimating usual patterns of dietary intake. They are, however, prone to several sources of error, which can cause varying degrees of misclassification, depending on the food item or nutrient in question. The frequency response options may not provide the most appropriate level of discrimination, the food list may be inadequate and questions regarding usual portion sizes may be ignored or estimated incorrectly.[10] Unfortunately, no indirect dietary assessment methodology has been developed that eliminates this problem. However, while directly measured biological markers are free of this type of error, they do require an invasive procedure, such as venepuncture, to be performed. As a result, additional resources are required in terms of skilled personnel, equipment, correct storage procedures and cold transportation. Even so, Bjerve and coworkers[5] have shown that measurement of plasma phospholipid fatty acids is a better indicator of n-3 fatty acid status than dietary intake data.

Despite this, in large-scale epidemiological studies where field workers are required to collect all of the data required or where data collection methods rely purely on obtaining information via self-administered forms and questionnaires, the available resources may simply not permit biological markers to be used. In these instances it is both useful and desirable to be able to gather dietary information that can be used as a reasonable estimate of dietary intake for the population as a whole, and to rank individuals according to relative intake. Our validation of this FFQ for assessment of plasma percentage fatty acids is therefore timely and useful.

In conclusion, these results provide evidence that fish intake as reported in a semiquantitative FFQ can be used to rank subjects according to their likely n-3 and DHA fatty acid intake and n-6:n-3 ratio in populations. This would make the FFQ a useful tool when trying to determine the fatty acid intake in large-scale epidemiological studies where logistics and resources prevent the use of more objective markers.

Acknowledgements. This study was funded by the National Health and Medical Research Council of Australia (NHMRC). Dr Rosalie Woods holds a NHMRC Postdoctoral Public Health Fellowship (987087). We wish to thank Mark Neumann, Child Nutrition Research Centre, Flinders Medical Centre, South Australia, for performing the plasma phospholipid fatty acid analysis and reviewing this manuscript, Helen Ferrugia and Charmaine Payne of the Anti-Cancer Council of Victoria for FFQ analysis and Ros Bish and Trina Vincent, RN, for assisting with the collection of FFQ and blood samples. We acknowledge that the FFQ was developed by the Anti-Cancer Council of Victoria for use in the Melbourne Collaborative Cohort Study (Chief Investigator, Professor Graham Giles). We would also like to thank Professor Andrew Sinclair of RMIT, Victoria, for guidance with the data collection phase of this study.

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