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Validation of an FFQ to estimate the intake of fatty acids using erythrocyte membrane fatty acids and multiple 3 d dietary records

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Abstract

Objective: The estimation of dietary intake in population-based studies is often assessed by the FFQ. The objective of our study is to evaluate the validity of an FFQ used to assess dietary fatty acid intake among middle-aged Chinese adults in Southern China.

Design: The method of triads was applied to obtain the validity coefficients (VC) of the FFQ for specific fatty acids. A subsample was randomly selected from an earlier cross-sectional study. The FFQ and 3 d dietary records were used for dietary assessment, and the fatty acid composition of erythrocyte membranes was determined as the biomarker.

Results: The Spearman correlation coefficients between the FFQ and 3 d dietary records were moderate to good ($r = 0{\cdot}28$–$0{\cdot}66$). The VC of the FFQ estimated by the method of triads were 0·72, 0·61, 0·65, 0·75 and 0·67 for MUFA, total *n*-6 fatty acids, α-linolenic acid, EPA and DHA, respectively. The VC could not be calculated for SFA, PUFA and total *n*-3 fatty acids because of negative correlations among the three measurements. But, the correlations between the FFQ and the dietary records were moderate for these fatty acids.

Conclusions: Our FFQ applied in Southern Chinese adults was valid to estimate their dietary fatty acid intake and was thus suitable for use in a large cohort study.

Keywords
Method of triads
FFQ
Fatty acids

Earlier epidemiological studies and clinical trials suggest a protective effect of unsaturated fatty acids, especially *n*-3 fatty acids, against CVD[1,2]. Proposed mechanisms include TAG-lowering effect, reduced platelet aggregation, plague stabilisation, antiarrhythmic effect and decreased blood pressure[3]. Assessment of long-term dietary fatty acids intake becomes increasingly necessary for population studies.

Traditional methods for dietary assessment include the quantitative FFQ and 24 h dietary records[4]. The use of biochemical indicators of dietary intake (i.e. biomarkers) is a recent addition to these techniques[5]. Each approach has advantages and limitations that must be weighed in selecting the method which best meets the needs of a particular study[4]. Although diet records (DR) obtained from free-living individuals provide the most accurate measurement of dietary intake on a particular day, their utilisation as a usual measurement of long-term dietary intake is tempered by considerable day-to-day variation of each individual's food intake[6]. FFQ are easy to administer and relatively inexpensive to use in large populations, and could collect information on usual or average diet over an extended period rather than a period of just a few days[4]. Inaccuracy of absolute nutrient value is one of the major limitations of FFQ. However, for most epidemiological investigations of dietary intake and disease, relative rankings of food and nutrient intakes are adequate for determining correlations. Biomarkers are objective but may not be better than traditional methods, because the levels of a nutrient in blood or tissue can be affected by genetic influences, lifestyle factors, such as smoking, physical activity, or the intake of other nutrients[4,7].

FFQ have been the primary method for dietary assessment in most epidemiological studies. However, FFQ are very sensitive to cultural and dietary practices[8]. An FFQ suitable for one population and for intake estimation of certain nutrients might be invalid when

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applied to another. Therefore, a validity study is necessary when using an FFQ to make dietary assessment. Traditionally, the FFQ was validated by comparing it with the reference method, dietary records, and then calculating the correlation coefficients between the two measurements[9]. The problem for this approach is that these two measurement instruments always have the same source of random errors[10]. On the other hand, random errors in the two methods are not independent but correlate with each other, which may lead to an overestimation or underestimation for the validity of the FFQ[11].

To attenuate the possible bias in FFQ validation studies, biomarkers have been applied as an additional measurement[4,12]. Biomarkers are generally considered to be objective, and measurement errors in these studies are truly independent of self-report methods (e.g. FFQ and dietary records). However, biomarker measurement might not perfectly reflect the dietary intakes in consideration of the effects of metabolism and/or environmental factors. Therefore, when the information of biomarkers is available, the method of triads has been recommended for the validity study[12]. This technique estimates the correlation between the dietary assessment and a person's true intakes from three pairs of correlation among the FFQ, the dietary records and the biomarkers.

The present study was aiming to assess the validity of an FFQ used in an earlier study[13] to estimate the dietary intake of some specific fatty acids, especially *n*-3 fatty acids, by the method of triads based on the triangular comparison of the FFQ, the 3 d dietary records and the fatty acid composition of erythrocyte membranes.

Methods

Subjects and study design

The subjects in the present validation study were a randomly selected subset from a cross-sectional study during 2005 and 2006 in Guangzhou, China[13]. Briefly, this study comprised 406 Guangzhou residents aged from 40 to 65 years. They had no history of chronic diseases, such as diabetes, hypertension and cancers, before enrolling in the study, and did not take any medications in the past 3 months. The study was approved by the Medical Ethics Committee of the Sun Yat-sen University, and written informed consent was obtained from all subjects. According to the only study evaluating the within- and between-individual variation of dietary intake in Chinese, the intraperson variations were the major contributor to the dietary intake variation and a validation study could be adequately carried out with 12 d of dietary records for a study of 100 or more participants[14]. On completion of the above-mentioned study, we randomly selected 200 participants for this validation study. In the following year, a total of 12 d of dietary records were administered. To account for seasonal variations in nutrient intake, the dietary record assessments for each subject were distributed across 12 months. At months 0 (baseline), 3, 6, 12, the subjects were asked to complete a 24 h dietary record on three consecutive days including two weekdays and one weekend, which yield a total of 12 d of dietary record. At the end of the validation study, they were required to complete an FFQ and gave blood to analyse fatty acid composition in erythrocyte membranes. A total of 148 subjects agreed and 125 subjects completed the validation study.

FFQ

The quantitative FFQ was a comprehensive instrument consisting of 119 items of food or food groups. The food items were grouped into the following categories: cereals, legumes and legume products, pork, beef, lamb, chicken, fish, shrimp and crab, eggs, dairy, nuts, mushrooms, vegetables, fruits, beverages, alcoholic beverages, supplements and cooking oils. The frequency section consisted of four categories: times per day, week, month or year. Seasonal foods were weighted by the proportion of the year that the food was available. The questionnaires were completed through a face-to-face interview by trained staff. For each food item of the FFQ, participants were asked to recall how often and how much, on average, they had consumed over the preceding year. Food models and standard portion size were used during the interview as aids to quantify the food they consumed.

Three-day dietary records

Subjects were asked to record detailed information on all foods, beverages, supplements and cooking oil they consumed in 24 h on three consecutive days, which included two weekdays and a weekend day. We gave examples on the cover page as a reference record and provided each subject with a measuring glass to quantify the cooking oil. If subjects had regularly consumed fish oil supplements for more than 3 months, the brand, intake dosage and frequency were required to record. Laboratory analysis of fatty acid composition of fish oil supplements with different brands was conducted at the end of the study together with erythrocyte membrane fatty acids composition. Trained staff reviewed every 3 d dietary record for the completeness, and follow-up telephone calls were made to clarify any incomplete or ambiguous information, when necessary.

Erythrocyte membrane fatty acids

Venous blood sample from study subjects, collected in Vacutainer tubes containing EDTA-K3 (≤2 mg/ml of blood), was centrifuged at 900 rpm for 15 min at room temperature. After isolation of plasma, white blood cells, platelets and erythrocytes were washed three times with an equal volume of saline solution to eliminate the residual plasma and buffy coat. After another centrifugation at 3000 rpm for 10 min (haematocrit = 98 %), several aliquots of 500 μl of packed red blood cells (RBC) with 2 %

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butylated hydroxytoluene (BHT) in methanol (0·1 mg/ml of packed RBC) and N_2 were immediately frozen in 2-ml plastic microtubes at −80°C until assay[15].

RBC were thawed and haemolysed in hypotonic Tris–HCL buffer, 10 mmol/l, pH = 7·4, at 4°C for 2 h. A membrane pellet was obtained by ultracentrifugation (12 000 rpm for 30 min at 4°C) from which lipids were extracted with chloroform/methanol (2:1, v/v) added with 0·005 % BHT, and the extract was dried in N_2[16]. Fatty acids methyl esters (FAME) were obtained by incubation with 14 % boron trifluoride ether/methanol (1:3, v/v) solution at 100°C for 5 min. FAME were extracted into 1 ml hexane, vaporised to dryness and redissolved in hexane for gas chromatography analysis[17].

FAME were analysed using an Agilent 6890 gas chromatograph (Agilent, Palo Alto, CA, USA) fitted with flame ionisation and a WCOT-fused silica CP-SIL 88 fame column (50 m × 0·25 mm inside diameter, film thickness of 0·2 μm; Varian, Bergen op Zoom, Netherlands). The carrier gas was nitrogen and the split–splitless injector was used with a split:splitless ratio of 50:1. The injection temperature was 250°C and detection temperature was 300°C. The starting temperature of the column was 80°C. After 2 min, the temperature was programmed from 80°C to 120°C at a rate of 10°C/min and then from 120°C to 180°C at a rate of 5°C/min; 2 min later, the temperature was continuously increased 2°C/min up to 206°C and finally 5°C/min up to 230°C.

Individual fatty acids were identified by comparison with known standards (Sigma-Aldrich Inc., St Louis, MO, USA), and expressed as a percentage of total fatty acids quantified from peak areas.

Of thirty-two fatty acids identified in erythrocyte membranes, nineteen fatty acids that have meaningful concentrations (mean concentration >0·10 %) are reported here, which together account for 96·9 % of total identified fatty acids. For the current analysis, MUFA and PUFA include *cis* isomers only. Blinded (indistinguishable from other samples) duplicate samples (n 5) were analysed throughout the study. The range of CV for these samples was 5·9 %–42·8 %. The CV for the most abundant fatty acids (>10 %) were 5·9 % for palmitic acid (16 : 0), 10·5 % for stearic acid (18 : 0), 8·6 % for oleic acid (18 : 1*n*-9), 10·8 % for linoleic acid (18 : 2*n*-6), 11·3 % for arachidonic acid (20 : 4*n*-6).

Statistical analysis

Fatty acid intakes from the FFQ and every 3 d dietary record were calculated using the China Food Composition 2002/2004[18,19]. The average intake of 3 d dietary records conducted on different time of the study (months 0, 3, 6 and 12) was calculated. Fatty acid data estimated from the FFQ and dietary records were expressed as absolute values and the percentage of total fat. The significance of differences between the absolute intakes of the FFQ and 3 d dietary records was examined by using independent sample t test. Spearman's rank correlation coefficients were calculated between each pair of the three dietary assessment methods (FFQ *v.* dietary records, FFQ *v.* biomarker and dietary records *v.* biomarker) for each fatty acid.

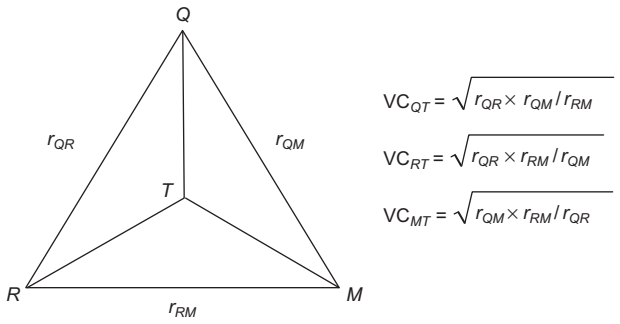

Fig. 1 Diagrammatic representation of the method of triads. *T*, true dietary intake; *Q*, *R* and *M*, measurements of the FFQ, dietary records and biomarker; *r*, sample correlation; VC, validity coefficient (from reference [11])

Validity coefficients (VC) were calculated using the method of triads. This approach assumes that the observed correlations between the measurements are explained by the fact that all assessments are positively linearly correlated to the true intake and that the random errors are mutually independent. Briefly, we referred to the measurements of the FFQ, the 3 d dietary record and the biomarker as *Q*, *R* and *M*, respectively, VC can be calculated as shown in Fig. 1.

The method of bootstrap sampling was used to determine the 95 % CI for VC[10,20]. In all, 1000 bootstrap samples with the equal size (n 125) were obtained by random sampling with the replacement from the study subjects. VC of the FFQ, 3 d dietary records and biomarker obtained from every bootstrap sample were merged into one data set and the 95 % CI was calculated. All statistical analyses were performed using Statistical Package for Social Sciences statistical software package for Windows version 11·5 (SPSS Inc., Chicago, IL, USA). Two-sided P values <0·05 were considered significant.

Results

Details on the general characteristics of 125 subjects for this validation study are presented in Table 1. Table 2 shows the energy, fat and selected fatty acids assessed from the FFQ, 3 d dietary records and erythrocyte membranes. Energy intake assessed from the FFQ and dietary records were similar. For most fatty acids, the average absolute values assessed from the FFQ were significantly lower than those from dietary records, but fat, SFA and *n*-3. Absolute intakes of fat and SFA estimated from the FFQ were higher than dietary record.

The Spearmen correlation coefficients between each pair of the three measurements are presented in Table 3.

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The correlation coefficients between the FFQ and 3 d dietary records ranged from 0·28 to 0·66 with the strongest correlation for MUFA. For most of the fatty acids, the correlations between each pair of the two dietary assessments and erythrocyte membrane contents were poor and insignificant except for some PUFAs. Correlation coefficients between the dietary records and biomarker were 0·15, 0·28 and 0·28 for *n*-6 fatty acids, α-linolenic acid (ALA) and EPA, respectively. Correlations coefficients between the FFQ and biomarker were 0·19, 0·37 and 0·16 for ALA, EPA and DHA, respectively. VC for each method are calculated from the correlations between each pair of three assessments by the method of triads and 95 % CI are calculated by bootstrap steps (Table 4). The range of the VC is also ascertained using the value calculated with the method of triads as the upper limit and the correlation between the FFQ and the biomarker as the lower limit. VC for SFA, total PUFA and *n*-3 fatty acids could not be calculated because of the negative correlations between each pair of the dietary assessments and biomarker, which has been known as one type of Heywood cases[12,21,22]. Estimated 95 % CI of VC calculated by bootstrap sampling steps with values >1 were all set to 1·00. This kind of outcome (VC >1) is another type of situation for the Heywood case. The VC for 3 d dietary records were the highest (ranged from 0·42 to 0·92), followed by the VC for the FFQ (ranged from 0·61 to 0·75), and those VC for the erythrocyte membranes tended to be the lowest (ranged from 0·10 to 0·50). Bootstrap sampling steps were used to obtain the 95 % CI, but it was impossible to estimate the VC in about 22·3 %, 11·8 %, 0·1 %, 0 % and 13·3 % of 1000 bootstrap

Table 1 Characteristics of study participants

	Men (*n* 44)		Women (*n* 81)	
	Mean	SD	Mean	SD
Age at recruitment (years)	57·0	6·2	54·7	4·6
BMI (kg/m^2)	23·1	2·9	23·2	2·8
Waist-to-hip ratio	0·83	0·06	0·88	0·05
	n	%	*n*	%
Education				
≤Elementary school	4	9·1	9	11·3
Middle school	15	34·1	26	32·5
High school	18	40·9	35	42·5
≥College	7	15·9	11	13·8
Monthly income per capita (¥)				
<1000	11	25·0	16	19·8
1000–2000	17	38·6	45	55·6
>2000	16	36·4	20	24·7
Smoking				
Non-smoker	18	40·9	100	100
Ex-smoker	5	11·4	0	0
Current smoker	21	47·7	0	0

Table 3 Spearman's correlation coefficients between each pair of the three measurements

	FFQ *v.* DR	DR *v.* Biomarker	Biomarker *v.* FFQ
SFA	0·30**	0·11	−0·15
MUFA	0·66**	0·09	0·07
PUFA	0·55**	0·02	−0·08
n-6	0·56**	0·15	0·10
n-3	0·56**	−0·01	−0·04
ALA	0·62**	0·28**	0·19*
EPA	0·42**	0·28**	0·37**
DHA	0·28**	0·10	0·16

SFA, saturated fatty acid; MUFA, Mono-unsaturated fatty acid; DR, dietary record.
**Correlation is significant at the 0·01 level (two-tailed).
*Correlation is significant at the 0·05 level (two-tailed).

Table 2 Dietary fatty acid intakes estimated by FFQ and the dietary records and fatty acid composition of erythrocyte membranes

	FFQ†		Dietary records†		Biomarker‡	
Fatty acids	Mean	SD	Mean	SD	Mean	SD
Energy (kcal)	2073·4	431·1	2019·9	390·7		
Fat (g)	77·435	22·827	73·818*	12·845		
SFA (g)	19·056	1·489	19·007*	4·367		
%	24·360	4·290	25·577	2·538	39·997	1·947
MUFA (g)	27·300	11·122	27·968*	7·112		
%	34·974	4·197	37·364	2·941	16·352	1·367
PUFA (g)	18·936	7·183	20·811*	4·883		
%	25·140	6·862	28·176	4·069	40·217	2·515
n-6 (g)	17·715	6·992	19·517*	4·654		
%	23·430	6·854	26·413	4·297	28·666	2·227
n-3 (g)	1·393	1·286	1·455	1·088		
%	1·740	1·275	1·907	1·228	11·551	2·82
ALA (g)	1·352	1·284	1·358*	0·897		
%	1·682	1·278	1·786	0·985	0·114	0·036
EPA (g)	0·013	0·011	0·029*	0·017		
%	0·019	0·017	0·040	0·022	0·312	0·141
DHA (g)	0·024	0·024	0·040*	0·028		
%	0·033	0·033	0·040	0·028	9·714	2·884

ALA, α-linolenic acid.
*Significantly different intakes on dietary records and FFQ ($P<0·01$).
†Present as absolute values and percentage of total fat.
‡Present as the percentage of total fatty acids of erythrocyte membrane.

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Table 4 Validity coefficients of the FFQ, the dietary records and the biomarker estimated by the method of triads*

	FFQ			Dietary records			Biomarker		
	VC_{QT}	95 % CI†	Range‡	VC_{RT}	95 % CI	Range	VC_{BT}	95 % CI	Range
MUFA	0·72	0·25, 1·00	0·07–0·72	0·91	0·25, 1·00	0·09–0·91	0·10	0·01, 0·27	0·07–0·10
n-6	0·61	0·22, 1·00	0·10–0·61	0·92	0·50, 1·00	0·15–0·91	0·17	0·04, 0·37	0·10–0·17
ALA	0·65	0·67, 1·00	0·19–0·65	0·96	0·68, 1·00	0·28–0·96	0·29	0·13, 0·49	0·19–0·29
EPA	0·75	0·25, 0·82	0·37–0·75	0·56	0·38, 1·00	0·28–0·56	0·50	0·25, 0·73	0·37–0·50
DHA	0·67	0·19, 1·00	0·16–0·67	0·42	0·07, 1·00	0·10–0·42	0·24	0·07, 0·92	0·16–0·24

ALA, α-linolenic acid.
*The validity coefficients for SFA, PUFA and total *n*-3 fatty acids could not be calculated for the reason of the negative correlation between the three measurements.
†Values >1 were all set to 1·00.
‡The correlation coefficient between the FFQ and the biomarker was the lower limit and the validity coefficient calculated by the method of triads was the upper limit.

samples for MUFA, *n*-6 fatty acids, ALA, EPA and DHA, respectively, because of the negative sample correlation coefficients between the three measurements.

Discussion

Although several studies have examined the validity of using an FFQ to estimate dietary PUFA intake by comparing the FFQ with biomarkers such as plasma lipids and erythrocyte membranes and (or) dietary records, most of them have been conducted in Western countries[23–27]. It is useful to examine the utility of an FFQ in Chinese, because China is undergoing a remarkably fast, but undesirable, dietary changes towards a stage dominated by a high intake of fat and animal food, as well as a high prevalence of diet-related diseases such as obesity, diabetes mellitus, CVD and cancer[28,29]. In the present study, we evaluated the validity of an FFQ for estimating the dietary intakes of fatty acids. The traditional method for FFQ validity assessment is based on the comparison between the FFQ and the dietary records. In this validation study, we used the method of triads. Erythrocyte membrane fatty acids biomarkers were used as an additional measurement for the triangular comparisons. In the present validation study, the absolute intakes estimated from the FFQ were lower than the dietary records for most fatty acids, such as MUFA, PUFA, *n*-6, EPA and DHA, whereas SFA intake estimated from the FFQ was a bit higher than dietary records. When the comparison was made between FFQ and the 3 d dietary records, the correlations were moderate-to-good for all fatty acids, which indicated that the FFQ could reflect the dietary intakes of fatty acids adequately. The results in the present study were in accordance with the earlier validation studies[20,23].

When compared with the erythrocyte membrane fatty acids, the diet–erythrocyte correlations were not consistent for all fatty acids. Consistent with earlier results, the correlations for SFA and MUFA were both poor for the FFQ and dietary records because of the endogenous synthesis[20,23]. With regard to PUFA, the correlation was weak for total *n*-6 fatty acids ($r = 0{\cdot}15$ and 0·10 for the FFQ and dietary records, respectively) and poor for total *n*-3 fatty acids ($r < 0$). The correlation coefficients were moderate for specific long-chain *n*-3 fatty acids, ALA, EPA and DHA for both the FFQ and dietary records. However, the correlation coefficients observed in our study for ALA, EPA and DHA were not as strong as the results from an earlier study[23]. One explanation for this might be the tissue differences and the time of tests for biomarkers. The fatty acid composition of adipose tissue has been considered a gold standard for the representation of dietary fatty acids due to the slow turnover time in weight stable individuals. The plasma fatty acids reflect the short-term dietary intakes[30]. In the present study, we used fatty acid composition of erythrocyte membranes as the biomarker. As erythrocytes have a half-life in circulation of approximately 120 d, fatty acid composition of the erythrocyte membranes usually reflects the dietary intakes of fatty acids over a period of several months[31]. The measurement for the fatty acid composition of erythrocyte membranes was conducted at the end of the study. Therefore, the biomarker results were likely to represent the status of dietary fatty acid intake during the period of several months, not the whole year before erythrocyte membrane analysis.

The method of triads was applied in this validation study to obtain the VC for FFQ. Instead of traditional validation methods, biomarkers were used as an additional measurement for this triangular approach. An advantage of this method is that the random error occurring with the biomarker measurement is likely to be truly independent with the measurements of FFQ and dietary records, hence it can attenuate the underestimated or overestimated effects, which might be caused by the correlated random errors with the same source between the measurements of FFQ and dietary records. Besides, the triads approach for VC is easy to calculate and requires no specific software for variable analysis. The VC calculated by the method of triads are usually set as the upper limit of VC. The correlation between the FFQ and erythrocyte membrane is considered as the lower limit. The triads approach is now broadly applied in some validation studies[23,27]. However, it still has some limitations. The outcomes of the method of triads could be accompanied by the occurrence of Heywood cases[12,21].

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Heywood cases correspond to two types of situations. One situation is that it is impossible to obtain the VC by the method of triads when one or two sample correlations are negative. Therefore, in the present study, we failed to estimate the VC for SFA, total PUFA and total *n*-3 fatty acids by the triads approach. The other type of Heywood cases occurs when the product of two of the three sample correlations is larger than the third, and it can lead to a coefficient value higher than 1. In such a situation, we set all coefficients with values higher than 1 as 1·00. The major explanation for Heywood cases is the random sampling fluctuations[11]. Increasing the sample size for the validity study may to some extent attenuate the amplitude of random sampling fluctuations.

Compared with the measurements of the dietary records and erythrocyte membrane biomarkers, the VC of the FFQ estimated by the method of triads were good (VC > 0·60) for MUFA, total *n*-6 fatty acids, ALA, EPA and DHA. For the VC of the SFA, total PUFA and total *n*-3 fatty acids that could not be obtained by the triads approach, the correlation coefficients between the FFQ and the dietary records were moderate ($r > 0{\cdot}30$), which indicates that the FFQ was also valid for the estimation of these fatty acids intake.

Conclusions

The present study applied the method of triads to evaluate the validity of the FFQ used in Southern Chinese adults to estimate nutrients, particularly dietary intakes of some specific fatty acids. Our results in this validation study have shown that the FFQ used in earlier cross-sectional study is a valid instrument for assessing dietary fatty acids intake among Chinese people in Guangdong province.

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