Development and validation of a food-frequency questionnaire for the determination of detailed fatty acid intakes

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Abstract

Objective: To validate a fat intake questionnaire (FIQ) developed to assess habitual dietary intake while focusing on the assessment of detailed fatty acid intake including total *trans* unsaturated fatty acids (TUFA).

Design: An 88 food item/food group FIQ was developed using a meal pattern technique. Validation was achieved by comparison with dietary intake assessed by a modified diet history (DH) in a cross-over design. Eighty-four individuals supplied adipose tissue biopsies for linoleic acid and total TUFA analysis as an independent validation of the FIQ and DH.

Setting: Medical Centre, Dublin Airport, Republic of Ireland.

Subjects: One hundred and five healthy volunteers (43 females and 62 males aged 23–63 years).

Results: Significant correlations ($P < 0.0005$) were achieved for intakes of energy (0.78), total fat (0.77), saturated fat (0.77), monounsaturated fat (0.63), polyunsaturated fat (0.73), TUFA (0.67) and linoleic acid (0.71) assessed by the FIQ compared with the DH. Linoleic acid intake assessed by the FIQ and the DH was significantly correlated with adipose tissue concentrations ($r = 0.58$ and 0.49, respectively; $P < 0.005$); however, total TUFA intake was poorly correlated with adipose tissue concentrations ($r = 0.17$ and 0.10 for FIQ and DH, respectively).

Conclusions: The FIQ compared favourably with the DH in assessing habitual diet, in particular fatty acid intake. In addition, the FIQ was successfully validated against the linoleic acid composition of adipose tissue, an independent biomarker of relative fatty acid status. The FIQ could therefore be used as an alternative to the DH as it is a shorter, less labour-intensive method.

Epidemiological and laboratory research suggests that diets high in fat are associated with an increased risk of developing cardiovascular disease, some cancers and possibly obesity[1]. Studies suggest that individual fatty acids within the same class differ in their effects on blood lipids and affect cardiovascular disease risk differently[2]. Hu *et al.*[3] examined dietary intake of saturated fatty acids (SFA) and coronary heart disease (CHD), and showed that while short- to medium-chain (4:0–10:0) SFA were not significantly associated with an increased risk of CHD, longer-chain SFA (12:0–18:0) were each separately associated with a small increase in risk. *Cis* monounsaturated fatty acids (MUFA), e.g. oleic acid, have a lowering effect on low-density lipoprotein (LDL)-cholesterol and a neutral effect on high-density lipoprotein (HDL)-cholesterol levels, whereas *trans* MUFA, e.g. elaidic acid, raise LDL-cholesterol while lowering HDL-cholesterol levels[4]. The assessment of individual fatty acid intake is however highly complex and limited to the nutrient database used to analyse dietary data.

Dietary assessment of fatty acid intake is particularly challenging as the fatty acid composition of certain foods (e.g. margarine, cooking fat and biscuits) differs dramatically between brands. In addition, manufacturers of margarine and cooking fat may change the component oils used depending on production costs. For example,

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the species and content of fish oils used may change depending on availability and cost, thereby changing the fatty acid composition of the margarine or fat produced and finally the products manufactured using this fat. Therefore, dietary data should ideally be analysed using food composition data that have been collected during the same reference period.

The 7-day diet history (DH) method, standardised for use in the Irish National Nutrition Survey[5], has previously been validated for energy intake against the doubly labelled water method in adolescents[6]. The DH assesses habitual dietary intake and can be modified to assess brands of processed foods eaten and therefore fatty acid intake; however, this increases the administration time required. Food-frequency questionnaires (FFQs) are therefore frequently used, as they are less labour-intensive. The relative validity of a newly developed FFQ must in general be assessed by comparison with a reference method (a previously validated dietary assessment method, e.g. DH). However, Hirsch *et al.*[7] suggested the use of a biological index of intake as an independent validity check. It has been demonstrated for example that the fatty acid composition of subcutaneous adipose tissue reflects dietary fatty acid intake over the previous 2 years and can therefore be used as a biological index of fatty acid intake. A biomarker serves as an independent measure of intake unbiased by self-reporting or changes in the fatty acid composition of the food supply from season to season.

The specific objectives of the present paper are:

1. to describe the development of a food/nutrient database which includes the fatty acid and total *trans* unsaturated fatty acids (TUFA) content of 225 foods analysed specifically for this study;
2. to describe the development and validation of a fat intake questionnaire (FIQ) developed to assess habitual dietary intake, with particular emphasis on fatty acid intake, by comparison with the DH method; and
3. to describe the validation of the FIQ by comparison with the fatty acid composition of adipose tissue as an independent marker of relative fatty acid status.

Methods

FIQ development

An FIQ was developed using food intake data assessed in a previous study of 153 healthy Irish adults[8]. This included the development of a comprehensive list of foods that accounted for the total fat intake and 95% of total energy intake of individuals in this study. Food items with a similar nutrient content and of similar portion size were grouped together; for example all types of root vegetable were assessed as one food group. The FIQ was designed to record additional information on method of cooking,

sauces added, the type and amount of fat used both in cooking and at the table, and the consumption of fat on meats.

The FIQ, which was pilot-tested in a group of 20 healthy adults (10 males, 10 females), was modified slightly for clarity. The final questionnaire included 88 food items or food groups and was structured to follow a typical daily meal pattern; i.e. all foods usually consumed at breakfast were assessed first, followed by foods typically eaten at other meals. For example, bread intake was assessed for four eating occasions: breakfast, lunch, dinner and snacks. For certain foods e.g. biscuits, the frequency of consumption of all types of biscuits was recorded and additional questions collected information regarding the amount, type and brand chosen. Intakes of mixed dishes, cakes, confectionery and take-away foods were also assessed in this way. The reference period included the four weeks preceding the interview. The frequency of consumption of each food item was recorded as frequency per day, per week, per fortnight or per month.

Standard portion sizes were derived from commonly used household units for each food listed (e.g. pre-sliced bread, pat of butter, teaspoon, tablespoon, cup, etc.). The FIQ recorded the subject's regular portion size of each food eaten relative to this standard portion size (e.g. 4 slices of bread, 1/2 teaspoon of sugar, etc.). For a limited number of foods (namely breakfast cereals, meat, fish and mixed dishes) a semi-quantitative approach was necessary and portion sizes were described as small, medium and large[9]. To identify brands of food consumed, an atlas of 142 photographs of different brands of biscuits, cakes, pastries, breads, cooking fats, dairy and non-dairy spreads was developed specifically for this study. Finally, the FIQ was developed for administration by a trained nutritionist as a quick method of measuring fat intake.

Compilation of a food/nutrient database

Two hundred and twenty-five foods (cakes, biscuits, pastries, breads, dairy and non-dairy spreads, cooking fats, hard margarines, fruit-filled pies and snack foods) were analysed for their individual fatty acid and total TUFA content[10,11]. Fatty acid methyl esters (FAMEs) were separated by gas chromatography and identified by comparison with standard samples of known methyl esters. Fatty acids with 20–22 carbon atoms were calculated as one group, making no distinction between SFA, MUFA or PUFA, or whether the configuration of the double bonds was *cis* or *trans*. The presence of C_{20} and C_{22} fatty acids indicated the use of marine oils in the production of a food. The total TUFA content was analysed using infra-red spectroscopy[10,11]. The analysis of foods was carried out according to the methods outlined by O'Neill[10] and Cronin and O'Neill[11]. As a result of this analysis, the following fatty acid profile was established for each food: 12:0, 14:0, 16:0, 18:0, 16:1, 18:1, 18:2, 18:3, $C_{20} + C_{22}$ and total TUFA content.

UK food composition tables (FOODBASE) were modified as follows to incorporate the fatty acid data of the 225 Irish foods analysed. The nutrient record of a similar food in the UK database was copied and the fatty acid profile was substituted, producing a new food record that included the carbohydrate, protein and micronutrient content of the UK food and the fatty acid composition of the Irish food. New food records were therefore developed for each of the 225 foods analysed. Checks for internal consistency of the database included comparison of calculated measurements with expected values for each database entry as described by Buzzard et al.[12].

Validation study

Of 2000 employees invited through a mailing system and advertisement in a company magazine, 317 volunteered for the study. One hundred and fifty-two individuals were excluded as a result of the following criteria: taking prescription medication, lipid abnormalities or aged < 20 or > 65 years. Of the 165 suitable healthy volunteers who were recruited, 54 were missing dietary intake data and six reported an implausible dietary intake. Subject information for the remaining 105 volunteers is shown in Table 1. Ethical approval was received from the ethics committee of the Federated Dublin Voluntary Hospitals and all participants provided informed written consent.

The dietary intake of all participants was assessed twice, once with the FIQ and once with the modified DH method (reference method). The DH method described by Livingstone et al.[6] was modified to assess individual fatty acid intake by assessing the brands of foods chosen with the photographic atlas described previously. The study was conducted using a cross-over design whereby equal numbers of participants were assessed using the FIQ and DH history first. A four-week interval between dietary assessments prevented information carryover from the first to the second assessment. Anthropometric measurements were recorded at both interviews. Body weight, without shoes and jacket, was assessed on a SECA scale to the nearest 0.1 kg. Height was recorded in cm.

Laboratory analysis

Subcutaneous adipose tissue samples were aspirated from the abdomen using a modification of the method described by Hirsch et al.[7], and biopsies that were adequate for analysis were obtained from 84 of the volunteers. Accurately weighed samples of approximately 10 mg adipose fat were dissolved in 0.1 ml isopropyl ether and transesterified at ambient temperature with 0.1 ml of 1 M sodium methoxide for 20 min. Methylation of any free fatty acids present in the original adipose sample was completed using 0.2 ml of 5% methanolic HCl, with methylation carried out at 80°C for 1 h. Samples were then evaporated to dryness under nitrogen and dried for 5−10 min in an oven at 80°C. FAMEs were dissolved in hexane to give a concentration of exactly 2% w/v, based on the weight of sample taken. Before sealing, approximately 20 mg of anhydrous sodium sulphate was added to each vial to remove any residual traces of moisture from the extracts. The FAME solutions were stored at −20°C until analysis by gas chromatography under the following conditions: SP2560 (100 m × 0.25 mm id, 20 pm film thickness) flexible fused silica capillary column (Supelco, Bellefonte, PA, USA) in a Pye Unicam 610 FID gas chromatograph; split ratio 50:1; hydrogen (0.8 ml min^{-1}) carrier gas; 1 μl (2% FAME solution in hexane) injected sample; injector temperature of 240°C; detector temperature of 260°C. Resolution of cis- and trans-isomers was optimised using the following temperature programming: 150−180°C at 0.5°C min^{-1}, 180−210°C at 2°C min^{-1}. Peak areas were integrated using a Spectra Physics SP4290 integrator. FAMEs were confirmed by comparison of retention times of authentic standards run under the same conditions. These standards were sometimes 'spiked' into the FAME solution whenever there was doubt about the component identity in groups of closely eluting peaks. The content of linoleic acid and total TUFA was expressed as a percentage by weight (g/100 g) of total fat content.

Data management

To eliminate inter-interviewer bias, the main investigator carried out all interviews, coding and analysis. Nutrient intakes were calculated using the food/nutrient database (FOODBASE), which included analysis of 225 foods described previously. For composite recipe dishes, the nutrient content was calculated as the weighted consumption of each separate food product.

Statistical analysis

The mean difference in intake of each nutrient assessed by both methods was calculated as an estimate of the average bias of one method relative to the other. The limits of agreement (mean difference plus or minus two standard

Table 1 Demographic characteristics of participants who completed the fat intake questionnaire and diet history

	Total (n = 105)	Males (n = 62)	Females (n = 43)
	Mean (range)	Mean (range)	Mean (range)
Age (years)	38.5 (23−63)	40.8 (28−63)	35.1 (23−56)
Weight (kg)	78.3 (48−117)	69.1 (59−117)	62.0 (48−115)
Height (m)	1.72 (1.54−1.94)	1.77 (1.63−1.94)	1.65 (1.54−1.77)
BMI (kg m^{-2})	26.1 (18.9−41.5)	26.7 (20.5−36.3)	25.1 (18.9−41.5)

BMI − body mass index.

deviations of the difference ($\pm 2SD_{difference}$)) were also calculated for each nutrient to consider how well the methods agreed for an individual.

To exclude the possibility of a non-constant bias, i.e. a bias that depends on the level of intake, the difference between the two measures and the mean of the two measures were calculated for each individual[13]. The relationship between these parameters was studied by means of regression analysis, as advocated by Altman and Bland[13]. The ability of the FIQ to classify individuals into the same quartile of intake as the DH was tested for each nutrient to evaluate agreement between both dietary assessment methods.

Pearson product–moment correlation coefficients were calculated to assess the association between the fatty acid composition of the diet, estimated by the FIQ and the DH, with the fatty acid composition of adipose tissue. Finally, the study group was divided into quartiles of long-chain fatty acid ($C_{20} + C_{22}$) intake and a comparison of the total TUFA intake (g/100 g fatty acids) and the total TUFA content of adipose tissue among these quartiles was performed. Statistical analyses were performed using SPSS software (Statistical Package for Social Sciences; SPSS Inc., Chicago, IL, USA).

Results

Validation by comparison with the DH method
The FIQ required on average 29 min to administer compared with 62 min for the DH. The mean differences in macronutrient intake assessed by the FIQ and the modified DH were not significantly different from zero (Table 2), indicating that the methods agreed excellently on average. The limits of agreement indicate that for a new subject we expect the two methods to differ

in the assessment of total fat intake by approximately 8.4, SFA intake by 5, MUFA by 4, PUFA by 3, TUFA by 1.5 and linoleic acid by 3.2 (% energy). Differences between individual pairs of intake estimates were not significantly related to the means for the majority of macronutrients, as demonstrated by regression analysis (Fig. 1). However, low intakes were underestimated and high intakes overestimated for polyunsaturated fat (% energy) and vice versa for carbohydrate (% energy) intake, resulting in a regression equation in which β was 0.21 and -0.23, respectively.

Mean differences in micronutrient intakes assessed by both methods were also comparable (Table 3), with the exception of vitamin D. A non-constant bias was also detected for vitamin D, as low intakes were underestimated and high intakes overestimated ($\beta = 0.45$).

The ability of the FIQ to classify individuals into the same or adjacent quartile of intake as the DH ranged from 76% for linoleic acid (% energy) to 91% for energy (MJ), total fat (g day^{-1}) and saturated fat (g day^{-1}), shown in Table 4. The highest proportion of misclassification (i.e. classified from one extreme category to the other extreme category of intake) was 5% for total TUFA (% energy).

Validation by comparison with adipose tissue biopsies
Linoleic acid intake (g/100 g total fatty acids) assessed by the FIQ and the DH was significantly correlated with adipose tissue concentrations (g/100 g FAMEs) for the total group, with $r = 0.58$ and 0.49 ($P < 0.005$), respectively (Table 5). In contrast, total TUFA intake assessed by the FIQ and the DH were poorly and not significantly

Table 2 Intakes of energy, macronutrients and dietary fibre, and ratio of energy intake to basal metabolic rate (EI/BMR), estimated using the fat intake questionnaire (FIQ) and diet history (DH) in 105 healthy adults

Intake	FIQ Mean (SD)	DH Mean (SD)	Mean difference (limits of agreement)†	Correlation‡
Energy (MJ)	10.6 (3.0)	10.6 (2.7)	−0.02 (−3.3, 3.3)	0.78***
Fatty acids (%E)				
Total	33.9 (6.2)	34.2 (6.1)	0.28 (−8.1, 8.6)	0.77***
SFA	12.7 (3.8)	12.6 (3.7)	−0.10 (−5.1, 4.9)	0.77***
MUFA	10.4 (2.2)	10.8 (2.5)	0.39 (−3.7, 4.5)	0.63***
PUFA	5.5 (1.9)	5.7 (2.2)	0.23 (−2.8, 3.3)	0.73***
TUFA	1.9 (0.9)	1.8 (0.9)	−0.10 (−1.6, 1.4)	0.67***
Linoleic	4.8 (2.1)	4.9 (2.1)	0.05 (−3.1, 3.2)	0.71***
Alcohol (%E)	6.3 (5.1)	5.7 (4.3)	−0.04 (−7.5, 6.4)	0.71***
CHO (%E)	45.9 (6.8)	45.5 (5.9)	−0.43 (−8.3, 7.4)	0.82***
Protein (%E)	14.8 (2.6)	15.2 (2.5)	0.38 (−3.9, 4.7)	0.64***
Fibre (g)§	26.9 (9.2)	26.9 (9.7)	0.07 (−14.0, 14.2)	0.73***
EI/BMR	1.50 (0.4)	1.51 (0.4)		

SD – standard deviation; %E – percentage of energy; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; TUFA – *trans* unsaturated fatty acids; CHO – carbohydrate.
† Limits of agreement = mean difference (DH − FIQ) $\pm 2SD_{difference}$.
‡ Pearson correlation.
§ Southgate method.
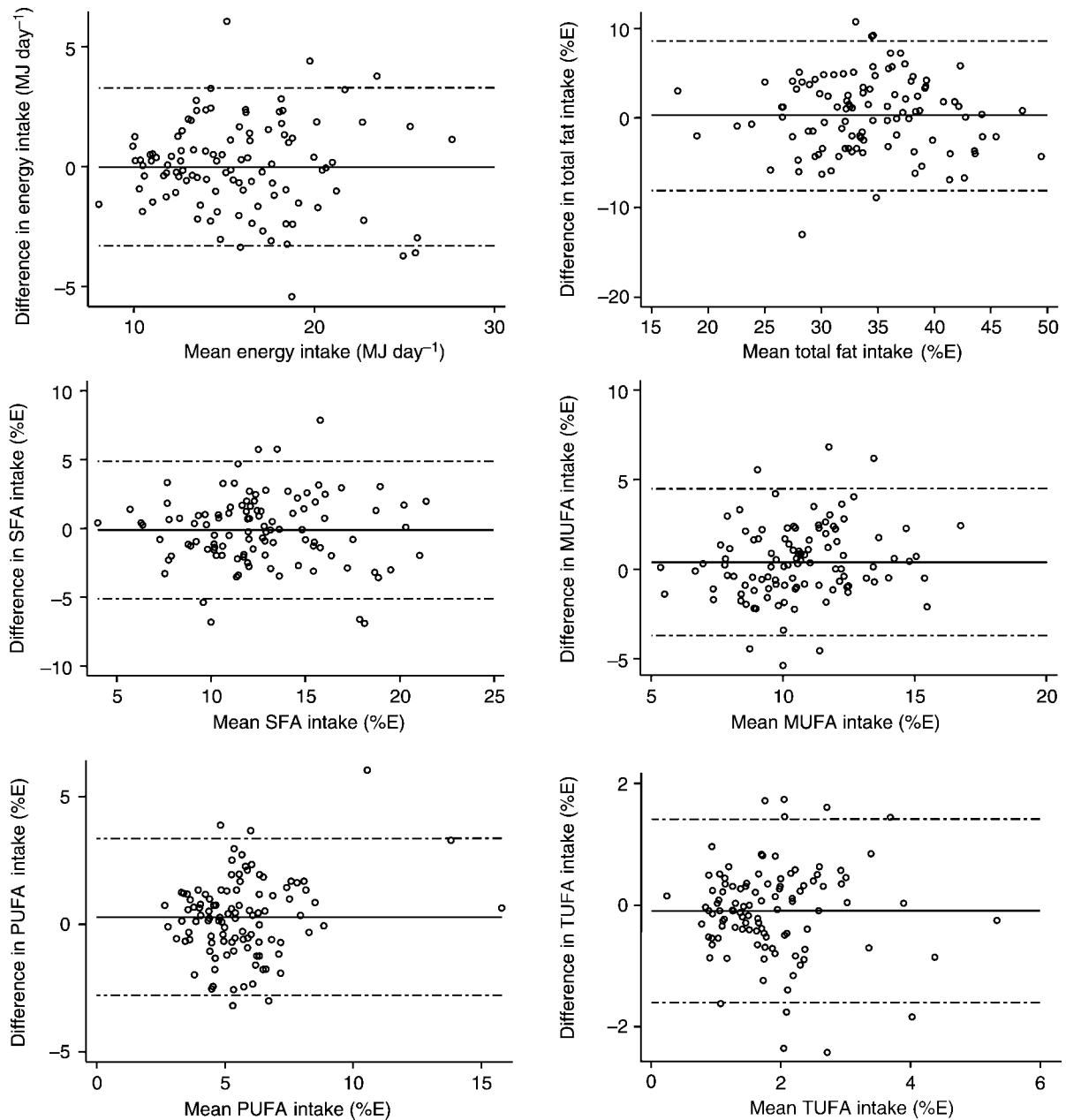*** Significant correlation between FIQ and DH: $P < 0.0005$.

Fig. 1 Bland−Altman plots showing the relationship between the mean nutrient intake and the difference in nutrient intake assessed by the fat intake questionnaire and the diet history. Separate plots are shown for energy intake and for intake of each of the macro- and micronutrients assessed. The solid line indicates the mean difference between the two measures and the dashed lines indicate plus or minus two standard deviations of the difference ($\pm 2SD_{\text{difference}}$). %E − percentage of energy; SFA − saturated fatty acids; MUFA − monounsaturated fatty acids; PUFA − polyunsaturated fatty acids; TUFA − *trans* unsaturated fatty acids; ret equiv − retinol equivalents

correlated with adipose tissue concentrations ($r = 0.17$ and 0.10, respectively).

The study group ($n = 84$) was divided into quartiles based on their $C_{20} + C_{22}$ fatty acid intake (Table 6). Analysis indicated that total TUFA intake significantly differed between quartiles, with those in the top quartile of $C_{20} + C_{22}$ fatty acid intake having the greatest total TUFA intake. In addition, those in the top quartile of intake had the greatest total TUFA adipose tissue content. There was, however, no significant difference in adipose tissue TUFA amongst the quartiles.

Discussion

The results of this study indicate that the FIQ and the DH agreed excellently on average, with negligible mean differences in intake. However, the limits of agreement, which were calculated to assess the performance of the FIQ at the individual level, indicate that the FIQ does not assess intake of some nutrients as well as at the group level. Nevertheless, the FIQ's ability to classify individuals into the same or adjacent quartile of intake as the DH ranged between 76 and 91% for energy, total fat, SFA,
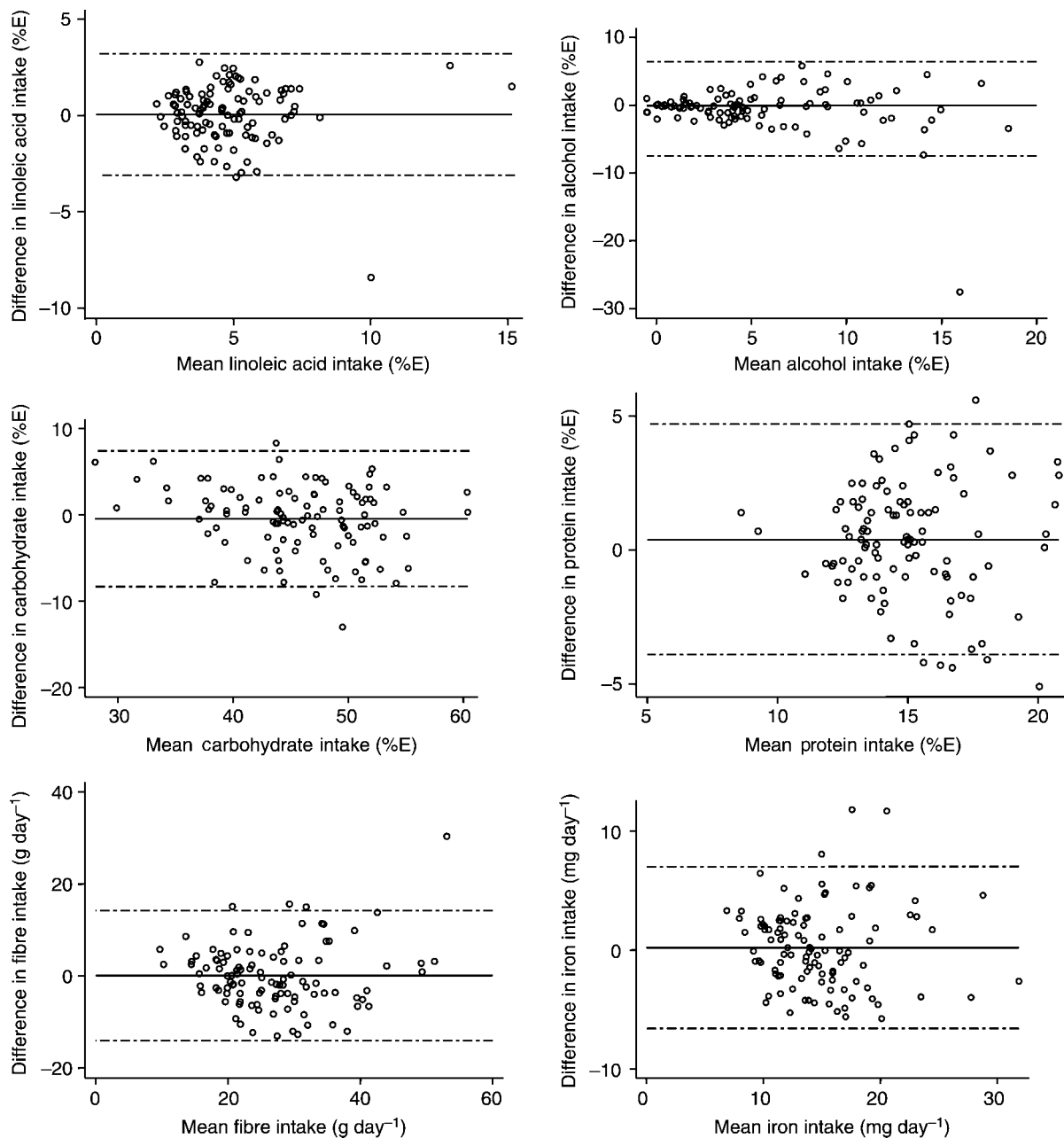
Fig. 1 *Continued*

MUFA, PUFA, linoleic acid and TUFA. Furthermore, the absolute validity of the FIQ was assessed as linoleic acid intake, which was significantly correlated with adipose tissue concentrations, a reliable indicator of long-term intake. In addition, the FIQ required far less time to complete compared with the DH, and would therefore be preferable for use in large studies.

The relative validity of a new dietary assessment method is generally achieved by comparison with a reference method (a dietary assessment method considered to be more accurate), or absolute validity can be achieved by comparison with an independent marker of intake such as biomarker concentrations. The DH was chosen as the reference method in this study as it assesses habitual

intake and had previously been validated against the doubly labelled water method for energy intake in Irish adolescents[6].

The limits of agreement calculated in this study indicate that for a new subject we expect the two methods to differ in the assessment of total fat intake by approximately 8.4, SFA intake by 5, MUFA by 4, PUFA by 3, TUFA by 1.5 and linoleic acid by 3.2 (% energy). Validation studies are difficult to compare due to differences in the study population, the distribution of nutrient intakes and the methodology and statistical analyses used. However, most validation studies assess the validity at the individual level by assessing the questionnaire's ability to place individuals along a distribution of intake from low to high and by
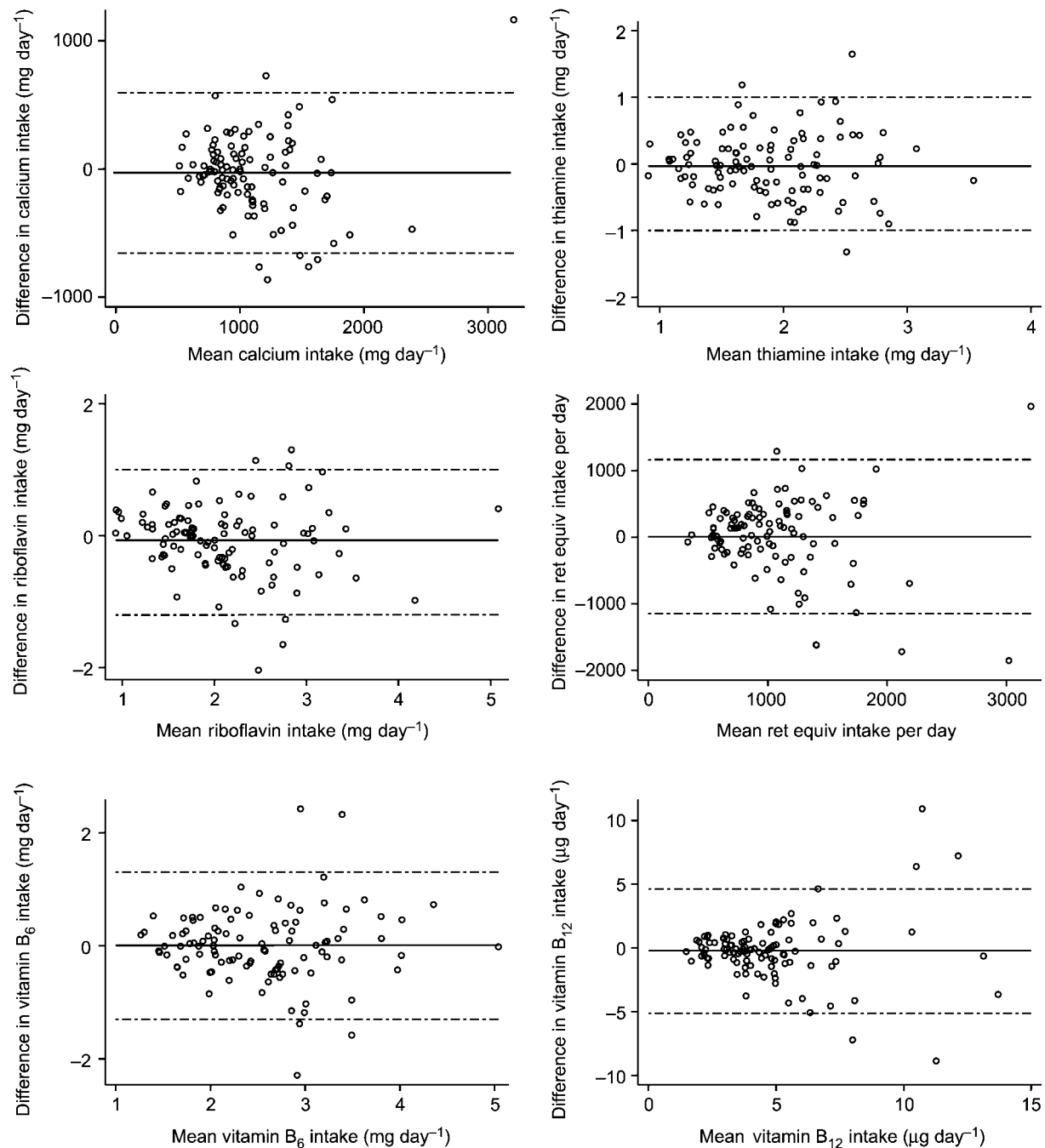
Fig. 1 *Continued*

using cross-categorisation by the two methods into tertiles, quartiles or quintiles[14]. The results of this study indicate that the FIQ's ability to assess the relative magnitude of an individual's intake of energy and their fatty acid profile is comparable to that of other studies which have used the diet history[15] and dietary records[16] as the reference method (Table 4).

This study showed that, for the majority of macro- and micronutrients, intake assessed by the FIQ was not affected by a non-constant bias. Exceptions were PUFA (% energy) and vitamin D intakes, where low intakes were underestimated and high intakes were overestimated, and carbohydrate (% energy) intake, where low intakes were overestimated and high intakes were underestimated.

Validity of the FIQ was also assessed by comparison with adipose tissue fatty acid concentrations. The half-life of adipose tissue in humans in energy balance is approximately 600 days and its composition should reflect the dietary fatty acid intake over the preceding 2.5 years[7]. Linoleic acid is generally accepted as a good indicator of intake since its appearance in adipose tissue is due primarily to dietary intake. The correlation coefficient
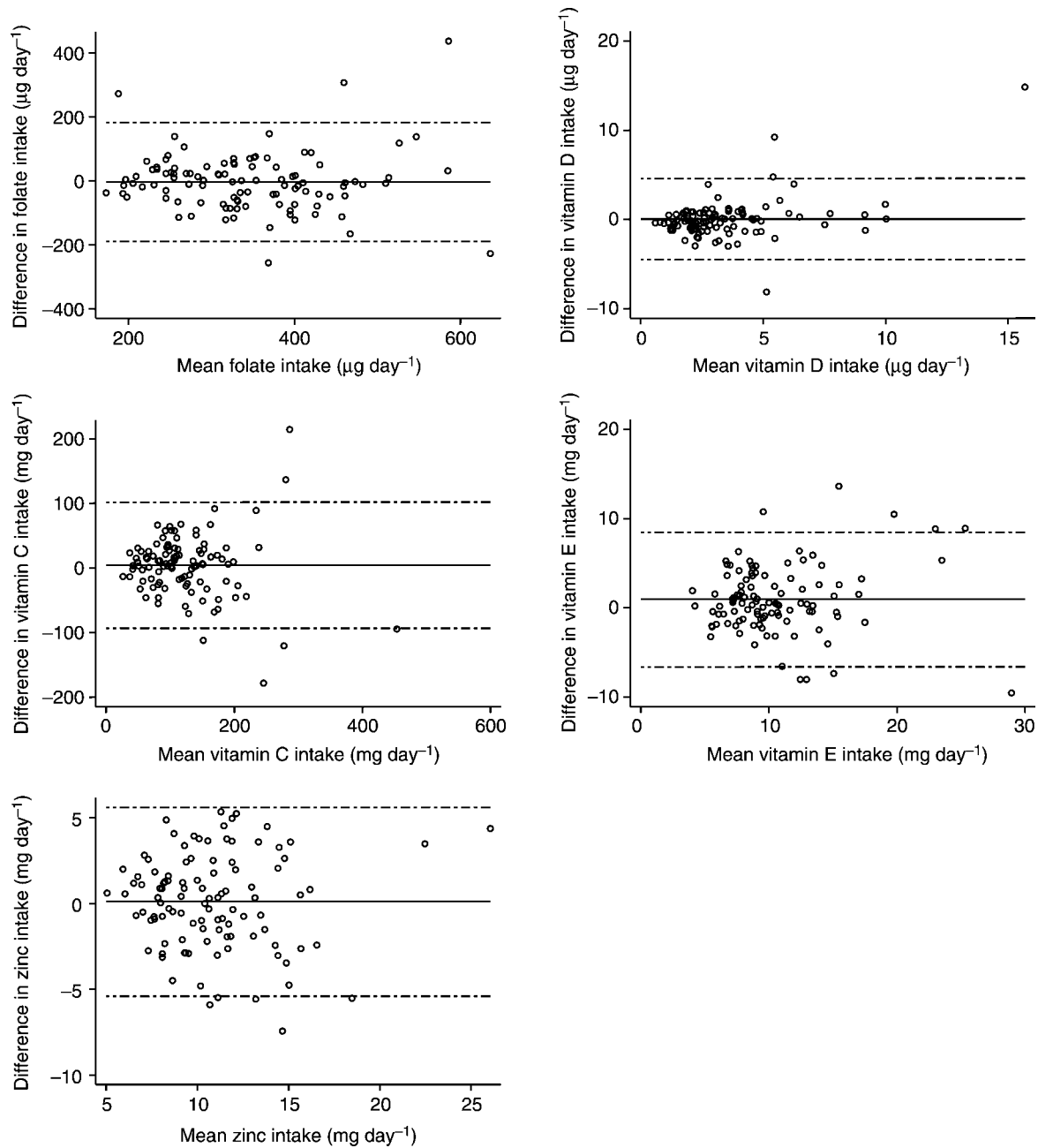
Fig. 1 *Continued*

($r = 0.58$) of linoleic acid intake assessed by the FIQ and the adipose tissue content is comparable to previous studies, where correlations ranged from 0.28 to 0.70[17–20]. This provides additional support for the validity of the FIQ described in this study.

Five studies[17,18,21–23] carried out in the USA and the UK have examined total TUFA intake in relation to adipose tissue concentrations and shown correlations of the order of 0.17–0.67. The main source of TUFA in these studies was from hydrogenated vegetable oils, which produces *trans*-isomers of 18:1 and 18:2. However, in the Republic of Ireland, hydrogenated vegetable and marine oils are used

in the food industry, providing – in addition to the *trans* 18:1 and *trans* 18:2 isomers – a variety of both positional and geometric isomers of longer-chain fatty acids (C_{20} and C_{22}). In the present study, gas chromatographic analysis of adipose tissue biopsies revealed an almost complete absence of TUFA other than geometric and positional isomers of 16:1, 18:1 and 18:2. Results showed that total TUFA in the diet was poorly correlated ($r = 0.17$) with total TUFA in adipose tissue. However, the study group ($n = 84$) was divided into quartiles based on their $C_{20} + C_{22}$ fatty acid intake. As expected, the total TUFA intake differed significantly between quartiles, with those subjects in

Table 3 Intakes of micronutrients estimated using the fat intake questionnaire (FIQ) and diet history (DH) in 105 healthy adults

Intake	FIQ Mean (SD)	DH Mean (SD)	Mean difference (limits of agreement)†	Correlation‡
Iron (mg)	14.6 (4.9)	14.8 (4.7)	0.2 (−6.6, 7.0)	0.74***
Calcium (mg)	1115.6 (427)	1080.2 (437)	−31.3 (−657, 595)	0.71***
Thiamine (mg)	1.9 (0.6)	1.9 (0.6)	−0.03 (−1.0, 1.0)	0.64***
Riboflavin (mg)	2.2 (0.8)	2.1 (0.7)	−0.07 (−1.2, 1.0)	0.75***
Retinol equivalents	1058 (521)	1048 (589)	10.5 (−1145, 1166)	0.47***
Vitamin B_6 (mg)	2.5 (0.8)	2.5 (0.8)	0.008 (−1.3, 1.3)	0.67***
Vitamin B_{12} (μg)	4.9 (2.8)	4.6 (2.8)	−0.23 (−5.0, 4.6)	0.60***
Folate (μg)	343.6 (106.9)	340.2 (107.4)	−3.01 (−189, 183)	0.62***
Vitamin D (μg)	3.3 (2.0)	3.2 (2.0)	0.05 (−4.5, 4.6)**	0.64***
Vitamin C (mg)	120.8 (71.2)	125.7 (66.9)	4.2 (−93, 102)	0.75***
Vitamin E (mg)	9.8 (4.0)	10.9 (4.9)	0.94 (−6.6, 8.5)	0.68***
Zinc (mg)	10.8 (3.6)	10.9 (3.6)	0.11 (−5.4, 5.6)	0.70***

SD − standard deviation.
† Limits of agreement = mean difference (DH − FIQ) ± 2SD$_{difference}$.
‡ Pearson correlation.
** FIQ and DH estimates significantly different based on a paired t-test: $P < 0.005$.
*** Significant correlation between FIQ and DH: $P < 0.0005$.

Table 4 Ability of the fat intake questionnaire to classify individuals into the same or adjacent quartile of nutrient intake as the diet history method ($n = 105$), expressed as % (n)

Nutrient	Same quartile	Same or adjacent quartile	Grossly misclassified†
Energy (MJ)	51 (53)	91 (96)	2 (2)
Total fat (g day^{-1})	53 (56)	91 (96)	2 (2)
Total fat (%E)	44 (46)	86 (90)	2 (2)
SFA (g day^{-1})	53 (56)	91 (96)	0 (0)
SFA (%E)	47 (49)	90 (95)	1 (1)
MUFA (g day^{-1})	60 (63)	85 (89)	0 (0)
MUFA (%E)	41 (43)	88 (92)	3 (3)
PUFA (g day^{-1})	44 (46)	86 (90)	4 (4)
PUFA (%E)	45 (47)	83 (87)	4 (4)
Total TUFA (g day^{-1})	48 (50)	89 (93)	2 (2)
Total TUFA (%E)	45 (47)	91 (95)	5 (5)
Linoleic acid (g day^{-1})	37 (39)	87 (91)	2 (2)
Linoleic acid (%E)	48 (50)	76 (80)	3 (3)

%E − percentage of energy; SFA − saturated fatty acids; MUFA − monounsaturated fatty acids; PUFA − polyunsaturated fatty acids; TUFA − *trans* unsaturated fatty acids.
† Classified from one extreme quartile to the other extreme quartile.

the top quartile of $C_{20} + C_{22}$ fatty acid intake having the greatest total TUFA intake and total TUFA adipose tissue content. However, there was no significant difference in total TUFA adipose tissue concentrations amongst quartiles. Clearly, longer-chain TUFA are not incorporated into adipose tissue to the same extent as *trans*-isomers of C_{18}. This concept, which has been discussed previously[24], can be explained by a decreased absorption of long-chain fatty acids with increasing chain length in humans[25] and a reduced retention of 22:0, when fed as a component of hydrogenated fish oil, in the carcass fat of rats[26].

It is possible that nutrient intakes assessed in the present study may be slightly biased because the study participants, who were volunteers, could be nutritionally more aware than persons who did not respond. However, it does not appear that total fat intake or the fatty acid profiles assessed in the present study differ substantially from population estimates in 2001 assessed by the North/South Ireland Food Consumption Survey[27]. For example, in the present study, total fat intake (% energy) was 33.9 versus 34.8 and 35.6 for men and women, respectively, in the nationally representative sample. The FIQ, which was developed for use in Ireland, could be used by other investigators without additional changes, but would need to be re-validated for use in other ethnic or culturally different groups. As previously mentioned, if food manufacturers change the oils they use in margarine

Table 5 Comparison of fatty acid intake (g/100 g fat) assessed by the fat intake questionnaire (FIQ) and diet history (DH) with adipose tissue biopsies (g/100 g fatty acids) ($n = 84$)

Fatty acid	Adipose tissue Mean (SD)	FIQ Mean (SD)	DH Mean (SD)	Correlation† FIQ and adipose tissue	Correlation† DH and adipose tissue
Linoleic acid	13.8 (2.5)	16.1 (5.9)*	16.6 (6.4)*	0.58**	0.49**
Total TUFA‡	4.2 (0.85)	5.8 (2.4)*	5.4 (2.5)*	0.17	0.10

SD − standard deviation; TUFA − *trans* unsaturated fatty acids.
† Pearson correlation.
‡ Gas chromatography method.
* Significantly different from adipose tissue concentration by paired t-test: $P < 0.001$.
** Significant correlation: $P < 0.005$.

Table 6 Comparison of total *trans* unsaturated fatty acids (TUFA) intake (g/100 g fat) and total TUFA content of adipose tissue (g/100 g fatty acids) based on quartiles of *trans* C_{20} and C_{22} fatty acid intake

Quartile of C_{20} + C_{22} intake	Total TUFA intake Mean (SD)	Adipose tissue TUFA content Mean (SD)
1	3.76 (1.04)	4.01 (1.06)
2	4.99 (1.46)[*,a]	4.10 (0.93)
3	6.23 (1.72)[*,a,b]	4.35 (0.73)
4	8.18 (2.38)[*,a,b,c]	4.42 (0.58)

[*] One-way analysis of variance with Bonferroni adjustment.
[a] Significantly different from quartile 1: $P < 0.05$.
[b] Significantly different from quartile 2: $P < 0.05$.
[c] Significantly different from quartile 3: $P < 0.05$.

production then the fatty acid composition of all products manufactured from this margarine will also vary. Investigators must acknowledge this and if detailed fatty acid intake data are required, then some direct analysis of foods may be necessary.

Finally, the excellent agreement between mean nutrient intakes assessed by the FIQ and the DH, the absence of a non-constant bias for most nutrients and the ability of the FIQ to classify individuals adequately demonstrate an acceptable relative validity. In addition, the independent validation of the FIQ for linoleic acid intake by comparison with adipose tissue concentrations provides additional evidence that the FIQ could be used in studies that require a shorter dietary assessment method.

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