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FATTY ACIDS

Using the method of triads in the validation of a food frequency questionnaire to assess the consumption of fatty acids in adults

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Abstract

Background: It is of great value to develop valid instruments to estimate food consumption; for this purpose, the triads method has been applied in validation studies of dietary intake to evaluate the correlation between three measurements [food frequency questionnaire (FFQ), reference method and biomarker]. The main aim of the present study was to validate a FFQ for Brazilian adults by means of the method of triads by estimating the ingestion of total fatty acids based on the level of saturation.
Methods: The present study enrolled 152 Brazilian adults of both sexes, who were residents in the city of Viçosa, Brazil. The ingestion of total saturated, monounsaturated, polyunsaturated, trans, linoleic and linolenic fatty acids was assessed by means of a FFQ, two food records, and biomarkers, which were detected by gas chromatography. The validation coefficients were calculated using the method of triads and concordance was determined by Kappa statistics.
Results: The FFQ was considered an adequate dietary method, because, based on the validation coefficients, it estimates the intake of total fat (0.84), as well as linolenic (0.90) and linoleic acids (0.31). A high concordance rate was confirmed for all nutrients assessed by the FFQ and food records. Regarding the biomarkers, linolenic acid and linoleic acid presented greater concordance.
Conclusions: According to the validation coefficients, the FFQ precisely estimated total fat, linolenic acid and linoleic acid contents.

Introduction

Dietary fatty acids (FAs) play an important role in the development or preventing chronic diseases [1,2]. Currently, the dietary recommendations influence not only the total lipids consumed, but also its quality [3]. This is why objective and precise estimations of fatty acid food ingestion are important when assessing food habit effects on the individuals' nutritional or clinical state.

In general, validation studies of food frequency questionnaires (FFQs) are based on comparisons with reference methods, such as food records [4,5]. Nevertheless, the use of biomarkers in validation studies may reflect a better way of assessing nutrient or food group ingestion because they present measurement errors regardless of their relationship to the inquiries [6,7].

When the ingestion of nutrients is assessed by means of FFQs, food records and biomarkers, the use of the triangulation technique or method of triads [7,8] is recommended because it uses the correlations among them to estimate the validation coefficient [9].

The triads method is a way of validating dietary intake instruments when the quantitative intake information from the three methods is available. This method is an

application of factor analysis to the specific problem. Although it is not possible to directly measure the true intake (the latent variable), it can be estimated by means of indicators [7,8].

Validation studies using nutritional biomarkers may be more advantageous because the use of such markers results in less errors compared to traditional methods of food consumption assessment and many biomarkers provide a measure closer to the nutritional status for some nutrients than dietary intake data [6]. Previous studies that have used the method of triads to estimate fatty acid ingestion in adults showed satisfactory validation coefficients to the FFQ [9–11].

Validation studies of dietary investigation instruments are necessary for determining the precision of the method employed to measure real ingestion in a defined period of time [12]. Furthermore, the validation provides information on possible classification errors, which are especially relevant when associations between diet and disease are investigated [13].

Although the method of triads was described more than a decade ago, few applications of this technique have been used in validation studies of dietary instruments with respect to Brazilian populations [14,15]. Epidemiological studies that provide for intervention protocols in the clinical practice are essential because they help provide estimates that are closer to the real intake of individuals, thus collaborating in disease treatment and prevention strategies. In addition, food intake assessment instruments, such as a FFQ, are frequently used in epidemiological studies [16] when assessing the food intake of population groups and, in this context, it is also paramount that the estimations are as correct as possible so as to subsidise collective-level interventions. Accordingly, the objective of the present study was the validation of a FFQ, which was applied in adult individuals to estimate their fatty acid intake, using the method of triads.

Materials and methods

This research paper is part of the project 'Metabolic syndrome and related factors: a population base study in adults of Viçosa, Minas Gerais, Brazil', a cross-sectional survey performed in the city's urban region between 2012 and 2014, considering a representative sampling plan of the adult population encompassing both sexes and ages between 20 and 59 years old. The project was submitted and approved by the Ethics Committee of the Federal University of Viçosa, under protocol number 008/12. Participants in the study were requested to provide their written informed consent before data collection.

For the validation study of the FFQ using the method of triads, a three-stage follow-up was performed using a stratified random sampling design comprising a subsample of 152 adult individuals of both sexes. From each person, a FFQ, two 24-h food records and biological material (blood) were collected by nutritionists and students of the nutrition course, who had been trained previously. The exclusion criteria were: pregnancy, women who had recently given birth, and individuals with cognitive/intellectual impairment or who presented difficulties in answering the dietary survey.

When the data consistency analysis was performed, four data sets (2.6%) were missing from the second food record (R24h). To avoid reducing the sample size, the missing data referring to the food intake of these individuals were produced using single imputations (i.e. replacements using the mean value according to sex and age group) [17].

The interviews were performed in the homes of participants or in the university premises by nutritionists or trained nutrition students, with a maximum 50-day interval between applications of each dietary survey (Fig. 1). By applying a structured questionnaire, it was possible to ask questions related to the sociodemographic and behavioural characteristics of participants, as well as to make anthropometric measurements.

The sociodemographic characteristics investigated were: sex (female/male), age range (20–39 years old and 40–59 years old) and skin color (white/nonwhite). Education was categorised according to complete years of study into 0–3, 4–7 and ≥8 years, and the socio-economic level was identified according to the Brazilian Association of Survey Companies [18] and classified into 'A and B' (high socio-economic level), 'C' (intermediate socio-economic level), and 'D and E' (low socio-economic level). Behavioural characteristics were also assessed; smoking habits were categorised into smoking, former smoker and nonsmoking [19]. Abusive ingestion of alcoholic beverages was considered present when there was the ingestion of a minimum of four measures on a single occasion in the past 30 days for women, and a minimum of five measures for men, with a measure comprising the ingestion of half a bottle or one can of beer, a cup of wine or a dose of distilled drinks [20]. The physical activity level in free time was assessed by the specific section of the long version of the International Physical Activity Questionnaire, using a weekly time ≥150 min as a cut-off point to classify individuals as physically active [21].

Regarding anthropometric characteristics, weight was obtained in the morning in triplicate using a scale (Ironman BC-554 digital scale; TanitaCorp., Arlington Heights, IL, USA) with a capacity of 200 kg and a precision of 100 g, when the volunteers wore minimal clothing and no shoes. Height was also measured in triplicate, by means of a 2.5-m stadiometer (Welmy, Santa Bárbara

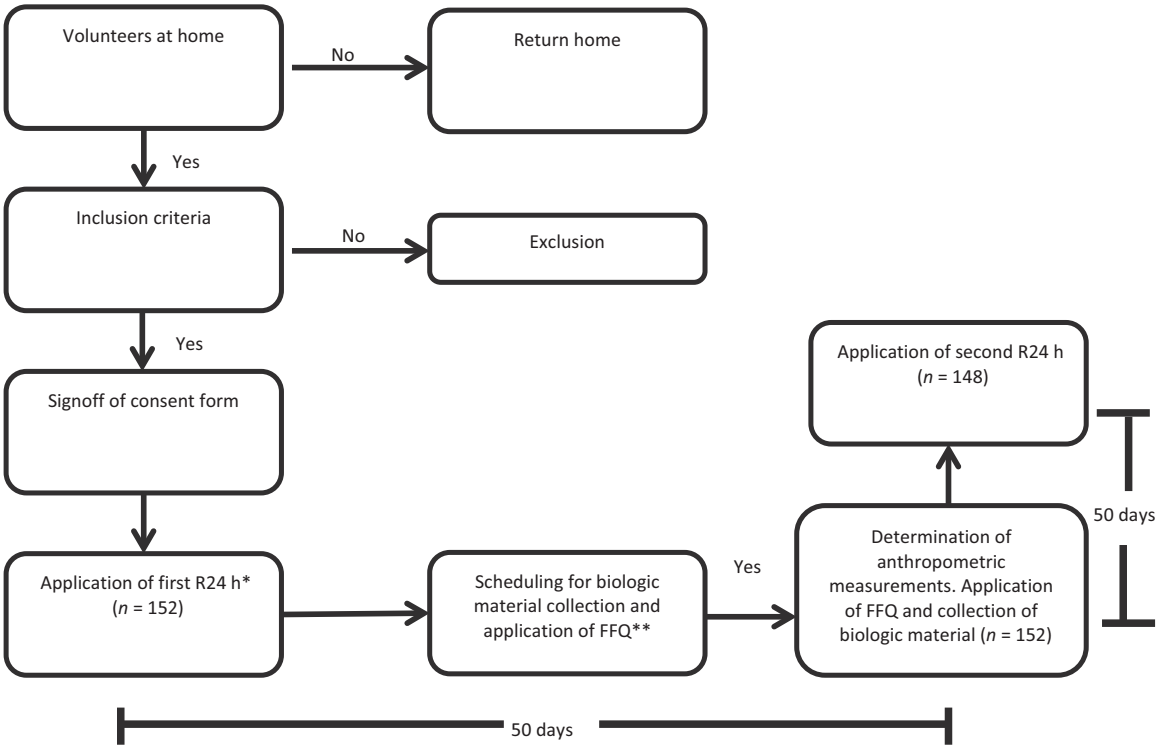

Figure 1 Activities of the food frequency questionnaire validation study using the method of triads, Viçosa, 2014. *R24h, 24-h food record; **FFQ, food frequency questionnaire.

d'Oeste, Brazil), with resolution of 0.1 cm, and when the individuals were standing still on bare feet, joined heels, on a flat, smooth and rigid surface, with the arms pending along the body as described by Jellife [22]. From the mean weight and height, the body mass index (BMI) was determined; individuals with BMI smaller than 25.0 kg m^{-2} were considered as being in eutrophy, and individuals with BMI ≥25.0 kg m^{-2} were classified as being overweight [23].

24-h food record

Food intake was assessed using two R24h, which were based on the food and drink consumption of the previous day, and included the form of food preparation, weight or volume, and portion size. A picture album showing design of foods, utensils and standard measurements was used to minimise memory bias and to help identify the referred portions [24,25].

The application of R24h was carried out by means of the multiple-pass method proposed by the United States Department of Agriculture. This methodology consists of a five-step guide (quick list, quick list review, naming meals, detail cycle and final probe), applied in a standardised process [26,27]. The amounts ingested were estimated in household measurements and converted to measuring units of weight or volume [28].

As a result of the occurrence of intra-individual variability in food intake, the values referring to energy and nutrients in the R24h were adjusted (ratio between intra-individual and interindividual variabilities) [29].

Food frequency questionnaire

The usual intake was assessed using the FFQ developed from the application of 83 R24h during the pilot project of the present study, as detailed in Segheto *et al.* [30]. The application of quantitative FFQ lasted a mean of 30 min and included questions about the regular intake of 95 nutritional items for a 1-year period and established intake frequencies that varied between 0 and 12 times. The time units were day, week, month and year. Subsequently, the time frequencies were transformed on a daily basis. This result was multiplied by the number of times that the interviewee mentioned having ingested an item on the day, as well as by weight of the portion.

The food portions were defined as small, medium, large and extra large, respectively corresponding to the percentiles 25, 50, 75 and 95 of intake (g) of each nutritional item. The mean portion (percentile 50) was defined as

reference, and was presented in household measurements and weight (g). The reference period was the intake for the 1-year period prior to the interview.

The FFQ food items were divided into 15 groups considering their nutritional similarity: soup, pasta, varied dishes and snacks, meat and fish, eggs and legumes, oils and fat, rice and root vegetables, milk and cold meat, vegetables, fruit and juice, sauces and spices, bread and cookies, alcoholic beverages, non-alcoholic beverages, and sweets and desserts. This questionnaire is provided in the APPENDIX S1. A set of utensils was used when applying the FFQ to facilitate the identification of household measurements.

For the quantification of nutrients obtained from the FFQ, a calculation-based spreadsheet in EXCEL, version 2010 (Microsoft Corp., Redmond, WA, USA) was prepared, considering the number of portions consumed per meal, portion weight/measurement, intake frequency and nutritional composition of food portion.

Dietary analysis

Intake estimations of energy, total fat, saturated, monounsaturated, polyunsaturated, trans, linoleic and linolenic FAs were performed with the aid of the *Table of Nutritional Composition of Foods Consumed in Brazil* [31], after tabulation in software containing a list of foods, as obtained from the food acquisition database established by the 2002–2003 Survey on Family Budgets [32]. Foods not belonging to this list were included manually later.

Analysis of lipid biomarkers

Blood samples of participants were obtained by endovenous punction using a vacuum system and disposable material after 12 h of fasting. A blood sample was extracted from each volunteer in two 6-mL amber tubes to serum, aiming to achieve protection from light. After centrifugation at 2000 ***g*** for 15 min, the material was split in aliquots and stored at a temperature of −80 °C.

To perform the serum profile of FAs, referred to here as biomarkers, the material was defrosted and the serum lipids were extracted using the technique determined by Folch [33], and then saponified and esterified as described by Hartmann and Lago [34]. The analyses were performed in a Shimadzu gas chromatograph model CG Solution, equipped with flame ionisation detector (Shimadzu Corp., Kyoto, Japan). The device was coupled to a microcomputer using the GC Solution software for further analysis. The compounds were separated and identified in a Carbowax capillary column (30 m × 0.25 mm) (Dow, Midland, MI, USA). For the chromatographic separation, 1 mL of sample was injected with the aid of a 10-mL Hamilton® syringe (Hamilton, Reno, NV, USA) in a system Split = 5. Nitrogen gas was used as carrier with a linear velocity programmed to 43.2 cm s^{-1}, and hydrogen and synthetic air formed the flame in the detector. Injector and detector temperatures were isothermally controlled at 200 and 220 °C, respectively. The initial column temperature was 100 °C (maintained for 5 min) and was increased at 4 °C min^{-1} until 220 °C was reached (and then maintained for 20 min). The gas flow in the column was 1.0 mL min^{-1}. The peaks were identified by comparing the retention times with the methyl ester standards known as FAME mix (Supelco, Bellefonte, PA, USA) and quantified per automatic integration area.

Statistical analysis

For the statistical analyses, first, the normality of dietary variables and biomarkers was assessed using a Kolmogorov–Smirnov test; the variables that did not demonstrate a normal distribution were log-transformed. The descriptive analysis of the data was obtained by determining means, SDs, medians and interquartile intervals (25th and 75th percentiles). To compare the mean ingestion of nutrients among the FFQ and both R24h, a paired Student's *t*-test was applied.

Given that the composition of fatty acids in the blood was expressed as a percentage of total lipids, the ingestion of nutrients was also analysed as percentage fat, instead of performing classical adjustments to the total energy intake [15].

To correlate the dietary variables (FFQ and R24h) and the biomarkers, Pearson correlation coefficients were calculated and classified as low (>0.1), moderate (>0.3) and high (>0.5) [35,36].

The validation coefficients were calculated among the three studied variables (FFQ, R24h, biomarkers) from the fatty acid ingestion correlations between the estimated dietary and serum level methods, as proposed by the method of triads [8]. The validation coefficients were considered as low (<0.2), moderate (between 0.2 and 0.6) or high (>0.6) [37]. 'Heywood Cases' (validation coefficient ≥1) were also estimated [38]. The equations used were:

$$\rho QT = \sqrt{[(rQR \times rQB)/rBR]}$$
$$\rho BT = \sqrt{[(rQR \times rBR/rQB]}$$
$$\rho RT = \sqrt{[(rQB \times rBR)/rQR]}$$

where *B* is biomarkers, *Q* is FFQ and *R* is 24-h food record.

The validation coefficient varies from 0 to 1. When validation coefficients are high, the correlation between methods is also high. If the correlation between two

methods is low, this suggests that at least one method is not reliable indicator of true ingestion, resulting in low validation [7].

The intake concordance between methods was also determined by means of joint classification analysis. Accordingly, the classification in tertiles was used to compare the FFQ and the R24h, the FFQ and the biomarker, and the R24h and the biomarker, using the Kappa test. The data were analysed with STATA, version 13.1 (StataCorp, College Station, TX, USA) and $\alpha = 5\%$ was considered as statistically significant for all tests.

Results

The sample includes 57% of women; 61.8% of individuals were 20–39 years old and 49.3% reported having white skin. Regarding education, 51.3% reported having studied for a period of ≥11 years, 68.4% of the sample belonged to socio-economic class C, followed by 29.6% of participants presenting a high socio-economic status. Regarding behavioural variables, 68.4% declared being nonsmokers, 61.2% did not abuse alcoholic beverages, 64.5% were rated as physically inactive and 51% were overweight (data not shown).

Table 1 shows the means, standard SDs, medians and interquartile ranges for the data obtained from the FFQ and the R24h. Differences can be observed among the ingestion means of the assessed nutrients, except for the consumption estimation of polyunsaturated, linoleic and linolenic acids as a percentage, which indicates that the FFQ generated energy and nutrient estimations greater than the R24h.

Table 2 shows the values of means, SDs, medians and interquartile ranges for the serum biomarkers.

The correlation coefficients between FFQ and R24h, FFQ and biomarkers, and R24h and biomarkers are shown in Table 3. The correlation between both dietary methods was considered moderate for total fat (g), monounsaturated fatty acids (g), polyunsaturated fatty acids (g, %), saturated fatty acids (g), linoleic acid (g, %), linolenic acid (g) and trans fatty acids (g). Weak correlations were found in all tested biomarkers; however, the correlation between serum monounsaturated fatty acids in grams versus FFQ was considered statistically significant ($P = 0.04$).

In Table 4, the FFQ was considered as the adequate dietary method for estimating the ingestion of total fat, linolenic and linoleic acids based on the validation coefficients. High (≥0.6) validation coefficients were found for total fat (0.84) estimated by the FFQ and for linolenic acid (0.90) estimated by the biomarker.

The validation was considered moderate for linolenic acid (g, %) and linoleic acid (%), both estimated by the FFQ. Total fat and saturated fatty acids (g), as estimated by the biomarkers, presented coefficients higher than 0.3. Validation coefficients considered as weak were observed for all nutrients estimated by the R24h. Heywood Case events were confirmed in the data for monounsaturated and saturated fatty acids (g) estimated by the FFQ, linoleic acid (%) estimated by R24h, and linoleic acid given (g) estimated by the biomarker (Table 4).

It was not possible to determine the validation coefficient with respect to the percentage of total fat and monounsaturated fatty acids, as well as polyunsaturated and trans fatty acids (g, %), because these nutrients presented negative correlations between FFQ and biomarkers, and R24h and biomarkers. Validation coefficients to the FFQ tended to be the highest, followed by the validation coefficients of biomarkers.

Table 5 presents the joint classification of fatty acid consumption estimation (percentage of individuals classified in the same tertile) among the methods investigated. High concordance between FFQ and R24h is confirmed for all nutrients assessed. The concordance analysis between biomarkers and FFQ or R24h confirmed that the linolenic and linoleic acids presented greater concordance because they showed a greater number of individuals in the same tertile.

Discussion

The present study had the objective of validating a FFQ using the method of triads, aiming to estimate the ingestion of fatty acid profile in adults.

Recent literature has shown the importance of FFQ validation studies by correlating three variables (FFQ, reference method of food intake and biomarker) [7,39,40] because the use of biomarkers presents the absence of errors similar to those of the FFQ and good correlation with the true food intake as advantages [41].

The use of biomarkers is an excellent contribution to the assessment studies of ingestion of lipids because some of them may correlate with the development or prevention of noncommunicable chronic diseases [1,2,42].

Some validation studies have used the adipose tissue as fatty acid ingestion biomarker as aq result of the tissue's capability of reflecting 2 years of history of fatty acid ingestion. However, the use of serum in validation studies must be considered in that it it is less invasive, cheaper and allows one the ingestion of lipids to be inferred in a history of weeks up to months [7,10]. Arab and Akbar [43] state that, because of difficulties in estimating the consumption of lipids in national enquiries, the validation studies of FA consumption have chosen to use of serum or adipose tissue biomarkers. It is important to note that lipid biomarkers from blood or adipose tissue have shown similarity when compared [10,11].

Table 1 Means (SDs), medians and interquartile ranges of consumption of total fatty acids of 152 adult individuals estimated by a food frequency questionnaire (FFQ) and 24-h food record (R24h), Viçosa, 2014

	FFQ*				R24h†				
			Interquartile range				Interquartile range		
Nutrients	Mean (SD)	Median	P25	P75	Mean (SD)	Median	P25	P75	*P*-value‡
Energy MJ [(kcal)]	121.53 (43.67) [2904.6 (1043.7)]	111.44 (2663.6)	93.58 (2236.7)	132.84 (3174.9)	87.78 (33.86) [2098.0 (809.3)]	80.79 (1931.0)	66.11 (1580.0)	102.01 (2438.0)	**<0.001**
Total lipids (g)	97.4 (35.5)	89.4	76.1	115.6	72.0 (36.0)	78.5	58.0	93.6	**<0.001**
% Total fat (energy)	30.4 (5.2)	31.1	27.0	34.1	34.1 (6.9)	34.0	29.3	38.2	**<0.001**
Saturated fatty acids (g)	34.4 (16.1)	32.0	23.8	42.0	23.5 (10.9)	22.2	15.4	28.7	**<0.001**
% Total fat	34.7 (6.0)	34.7	31.3	38.0	29.9 (8.0)	30.2	25.1	34.9	**<0.001**
Monounsaturated fatty acids (g)	33.4 (13.9)	31.2	24.1	39.8	23.8 (10.9)	22.3	16.4	29.5	**<0.001**
% Total fat	33.9 (3.9)	34.7	32.5	36.3	30.3 (7.0)	30.3	25.9	34.6	**<0.001**
Polyunsaturated fatty acids (g)	15.6 (6.2)	14.5	11.4	18.3	12.3 (5.6)	11.2	8.2	14.8	**<0.001**
% Total fat	16.2 (3.5)	15.9	13.8	18.3	16.1 (5.1)	15.8	12.4	19.0	0.809
Trans fatty acids (g)	4.7 (3.2	3.8	2.6	6.0	2.4 (1.7)	2.1	1.3	3.0	**<0.001**
% Total fat	4.7 (2.0)	4.3	3.2	5.3	3.4 (2.3)	2.7	1.9	4.0	**<0.001**
Linoleic acid (g)	13.6 (5.5)	12.8	9.9	16.1	10.6 (4.9)	9.9	7.0	12.7	**<0.001**
% Total fat	14.1 (3.1)	14.0	11.9	16.1	13.9 (4.5)	13.7	10.7	16.7	0.575
Linolenic acid (g)	2.5 (6.4)	1.7	1.5	2.2	1.3 (0.6)	1.2	0.9	1.6	**0.028**
% Total fat	3.0 (11.3)	1.9	1.6	2.3	1.8 (0.8)	1.6	1.3	1.9	0.177

*Variables with logarithmic transform.

†Data adjusted according to variability.

‡Paired Student's *t*-test.

P-values in bold are statistically significant ($p < 0.05$).

Table 2 Means (SDs), medians and interquartile ranges of serum concentrations of biomarkers in 152 adult individuals, Viçosa, 2014

Serum biomarkers	Mean (SD)	Median	Interquartile range	
			P25	P75
Total fatty acids (% total fat)	32.32 (8.04)	36.30	27.55	37.52
Saturated fatty acids (% total fat)	13.48 (4.29)	13.69	11.51	15.67
Monounsaturated fatty acids (% total fat)	7.48 (3.37)	7.45	5.11	10.50
Polyunsaturated fatty acids (% total fat)	11.35 (4.23)	11.59	8.92	14.54
Trans fatty acids (% total fat)	2.78 (3.35)	1.00	0	5.35
Linoleic acid (% total fat)	2.16 (2.92)	0	0	4.50
Linolenic acid (% total fat)	0.36 (0.60)	0	0	0.58

Table 3 Correlation coefficients between ingestion and percent distribution of fatty acids, estimated by food frequency questionnaire (FFQ), 24-h food record (R24h) and biomarkers, in 152 adult individuals, Viçosa, 2014

FFQ Nutrients	FFQ and R24h		FFQ and biomarkers		R24h and biomarkers	
	r	*P*†	*r*	*P*†	*r*	*P*†
Total lipids (g)	0.36	**<0.01**	0.15	0.05	0.07	0.33
% Total fat	−0.01	0.88	0.02	0.77	0.00	0.93
Saturated fatty acids (g)	0.43	**<0.01**	0.10	0.21	0.02	0.75
% Total fat	0.29	**<0.01**	0.01	0.82	0.06	0.42
Monounsaturated fatty acids (g)	0.35	**<0.01**	0.16	**0.04**	0.03	0.71
% Total fat	0.06	0.44	0.004	0.95	−0.01	0.88
Polyunsaturated fatty acids (g)	0.40	**<0.01**	0.00	0.96	−0.09	0.22
% Total fat	0.35	**<0.01**	−0.08	0.29	−0.08	0.30
Trans fatty acids (g)	0.31	**<0.01**	−0.02	0.78	−0.06	0.43
% Total fat	0.24	**0.01**	0.02	0.78	−0.06	0.43
Linoleic acid (g)	0.41	**<0.01**	0.06	0.55	0.03	0.75
% Total fat	0.34	**<0.01**	0.03	0.74	0.13	0.24
Linolenic acid (g)	0.35	**<0.01**	0.04	0.75	0.09	0.45
% Total fat	0.18	**0.02**	0.05	0.68	0.04	0.71

†Pearson's correlation coefficient.
P-values in bold are statistically significant ($p < 0.05$).

Table 4 Validation coefficients to the profile of total fatty acids, estimated by the food-frequency questionnaire (FFQ), 24-h food record (R24h) and biomarkers, in 152 adult individuals, Viçosa, 2014

Nutrients	Validation coefficients		
	FFQ ρQT	R24h ρBT	Biomarker ρRT
Total lipids (g)	0.84	0.18	0.42
Saturated fatty acids (g)	1.32	0.07	0.32
Saturated fatty acids (% total fat)	0.28	1.04	0.06
Monounsaturated fatty acids (g)	1.39	0.11	0.25
Linoleic acid (g)	0.27	0.07	1.49
Linoleic acid (% total fat)	0.31	1.12	0.12
Linolenic acid (g)	0.38	0.10	0.90
Linolenic acid (% total fat)	0.45	0.11	0.41

ρQT, validation coefficient of food-frequency questionnaire; ρBT, validation coefficient of the reference method (R24h); ρRT, validation coefficient of biomarker.

The correlations between both dietary methods for the assessed nutrients were greater among the dietary surveys than those performed with biomarkers, similar to the results of a study by Sartorelli *et al.* [15], who sought a validation of an FFQ in expectant mothers for estimating the ingestion of linoleic acid, α-linolenic acid and trans fatty acids using the method of triads. To determine the precision in polyunsaturated fatty acid ingestion of healthy Australian volunteers using a validated, 38-question FFQ, Meyer *et al.* [44] also found overestimation of fatty acids from the FFQ compared to the other methods investigated. In general, the low correlation between dietary instruments and biomarkers can be explained by the influence of other factors, such as individual differences in absorption, metabolism and biochemical adaptations [41,45].

The adequate ingestion of α-linolenic acid has been associated with protection against the main chronic diseases, particularly cardiovascular diseases [3]. From an epidemiological point of view, this is why accurate techniques for determining this nutrient are important [46]. The present study reported validation coefficient values of 0.35 and 0.31, respectively, for α-linolenic fatty acid and linoleic acid. FFQ validation studies by means of α-linolenic fatty acid estimation have been performed in different physiological groups. Kabagambe *et al.* [10] used the method of triads for FFQ validation in a group of healthy adults of both sexes in Costa Rica. Researchers carried out the estimation of fatty acid biomarkers collected from the adipose tissue and multiple R24h, showing validation coefficients of 0.59 and 0.89 to linolenic and linoleic acids, respectively.

When assessing the capability of an FFQ for estimating the ingestion of some polyunsaturated fatty acids using the triangulation technique, McNaughton *et al.* [3] found the validation coefficient of 0.5 for the α-linolenic fatty acid,

Table 5 Classification of 152 participants (*n*) in tertiles of consumption of fatty acids and biomarkers between the means of dietary survey methods, Viçosa, 2014

	FFQ and R24h		FFQ and biomarkers		R24h and biomarkers	
Nutrients	Same tertile	Opposite tertiles	Same tertile	Opposite tertiles	Same tertile	Opposite tertiles
Total lipids (g)	72	22	65	14	62	12
Saturated fatty acids (g)	75	25	68	18	65	15
Saturated fatty acids (% total fat)	74	24	63	11	67	17
Monounsaturated fatty acids (g)	72	22	70	20	66	16
Linoleic acid (g)	73	23	29	6	32	10
Linoleic acid (% total fat)	74	24	101	0	101	0
Linolenic acid (g)	80	30	29	10	29	13
Linolenic acid (% total fat)	69	19	100	0	101	0

FFQ, food-frequency questionnaire; R24h, 24-h food record.

demonstrating that the dietary instrument applied estimated fatty acid ingestion adequately. When estimating the α-linolenic fatty acid intake from expectant mothers, Sartorelli *et al.* [15] confirmed greater precision by applying an FFQ compared to the 24-h food record and breast milk.

A systematic review published by Serra-Majem *et al.* [47], which aimed to determine the validation of dietary methods and biomarkers for omega 3 intake in healthy adults, recommended investigations with different potential biomarkers for these fatty acids, especially in different populations.

Although many fatty acids are synthesised or suffer endogenous chemical alterations, the biosynthesis of the α-linolenic fatty acid is limited [48] because its metabolism depends on adequate food intake. On the other hand, because this is an essential fatty acid and it aids in the formation of anti-inflammatory molecules, its availability in the bloodstream may be quick but still reinforces its correlation with the biomarker.

The determination of linoleic acid ingestion has been associated with the consumption of fats, oils and sauces [49,50]. Andersen *et al.* [51], who assessed 125 healthy adult men, observed a weak correlation (0.16) among the essential fatty acids, including linoleic acid, which was estimated by multiple food records and serum. Astorg *et al.* [52], who measured the correlations between the usual ingestion of linoleic acid and its plasma percentages in French adults, confirmed that the correlations were weak but significant because the biomarker was considered acceptable when measuring the usual levels of linoleic acid ingestion.

The study conducted by Vriese *et al.* [49], which was performed with 30 healthy expectant women, confirmed a positive correlation between the estimation of linoleic acid ingestion by means of an FFQ and its availability in plasma during pregnancy. Sartorelli *et al.* [15] used the method of triads to assess the performance of an FFQ when estimating linoleic acid ingestion during pregnancy and found a determined a coefficient of 0.42 for the dietary instrument, which is not so different from the one obtained in the present study.

One of the strengths of the present study is the validation of the food frequency questionnaire using the method of triads, which is a technique that is hardly ever used currently in Brazilian studies. Another strength is that the instrument test was developed and validated according to the methodological guidance required in validation studies, respecting all required stages and analyses [41].

On the other hand, a limiting factor of the present study was the existence of 'Heywood Case' events in some of the assessed nutrients, which demonstrates correlated errors in the dietary methods applied. Some studies that aimed to validate dietary instruments using the method of triads also observed 'Heywood Case' events [14,15,53] for vitamins and fatty acids. The minimisation of this event may occur with the use of greater sample sizes [54]. Another important limitation worthy of note is that nutrient intake is only one determinate of nutrient status because the concentration of a nutrient in blood is typically influenced by variation in the absorption, transport and excretion of the nutrient [55].

For the present study, the FFQ and the analysis of biomarkers were performed at the same time, although the FFQ expresses habitual consumption over the last 12 months and the serum fatty acids indicate consumption over several months of collection; the R24h were applied at intervals of 100 days. Despite the different periods, the coefficients were adequate for validation.

Conclusions

The FFQ proposed in the present study was validated and therefore is considered as a useful tool for estimating the ingestion of total fat, linolenic and linoleic acids in adults because it showed high validation coefficient values. The food frequency questionnaire provides reliable information

on the regular ingestion of these fatty acids, allowing individuals to be classified into ingestion categories and helping with the tracking of individuals who present low or high ingestion levels of these nutrients. A continuous review of this instrument and a reproducibility study are suggested, as are new validation studies with fatty acids performed in other age groups and populations, with the aim of enabling a better accuracy of the instrument.

Transparency declaration

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported, that no important aspects of the study have been omitted and that any discrepancies from the study as planned have been explained. The reporting of this work is compliant with STROBE guidelines. The lead author affirms that no important aspects of the study have been omitted and that any discrepancies from the study as planned (and registered with) have been explained.

Conflict of interests, source of funding and authorship

The authors declare that they have no conflict of interests.
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DCGS contributed to the data collection and analyses of this study, wrote the initial draft of the manuscript and assembled the final version. WS was responsible for the study design, data collection and analysis. MFCL was responsible for the study design, field work and analysis. MCP and MCGP were responsible for the the study design and analysis. DMLM and DBC were responsible for the analysis and data interpretation. GZL was project leader in the Universidade Federal de Viçosa, contribuited to the study design and data interpretation. All authors critically reviewed the manuscript and approved the final version submitted for publication.

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