Original Article

Development and evaluation of a semiquantitative food frequency questionnaire for estimating omega-3 and omega-6 fatty acid intakes in Indonesian children

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Background and Objective: A balance ratio of dietary omega-3 (n-3) and omega-6 (n-6) fatty acids reduces childhood obesity. However, few studies have focused on validation of semiquantitative food frequency questionnaire (SFFQ) for determining the n-3 and n-6 intakes in children. Therefore, a valid SFFQ for assessing n-3 and n-6 intakes among Indonesian children is required. **Methods and Study Design**: A cross-sectional study was conducted by selecting 89 healthy children through multistage random sampling. Dietary intakes were assessed using the SFFQ and a 3-day non-consecutive 24-h recall. Randomly selected children (n=35) were assessed for plasma phospholipid fatty acid (PFA). In total, 78 food items in the SFFQ, as in the Thai, Vietnamese, and American food composition databases, were validated using dietary recall and PFA. The SFFQ was readministered after 4 weeks to assess its reproducibility. The validity and reproducibility of the SFFQ were determined by Bland–Altman analysis. **Results**: Favourable agreement was found between the SFFQ and recall for docosahexanoic acid, eicosapentanoic acid, docosapentanoic acid, and arachidonic acid, but not for total n-3, n-6, α-linolenic acid, or linoleic acid. Significant correlations were found between the SFFQ estimations and plasma n-6 and LA (r=0.40, p=0.025; and r=0.42, p=0.018, respectively). A 95% limit of Bland–Altman agreement was observed between the first and repeat SFFQ for all fatty acids. **Conclusion**: The proposed SFFQ is sufficiently valid and reliable for assessment of essential fatty acids intakes in Indonesian children.

Key Words: omega-3, omega-6, reproducibility, semi-quantitative food frequency questionnaire (SFFQ), validity

INTRODUCTION

The increasing prevalence of paediatric obesity is a major public health concern worldwide,[1,2] particularly in developing countries. In 2013, the prevalence of obesity in children aged younger than 5 years was 11.9%, and children aged 6–23 months exhibited the highest prevalence compared with those in other age groups.[3] Community behavioural programmes like the EPODE approach which is ecological and is currently the most promising strategy for the prevention and reduction of paediatric and childhood obesity.[4] Several studies indicated of the health benefit of consuming a balanced ratio of omega 3 (n-3) and omega 6 (n-6) fatty acids in preventing paediatric obesity.[5-7] Therefore, a clearer understanding of current n-3 and n-6 intakes in children is required to apply an appropriate intervention and establish conclusive public health recommendations.

Estimating n-3 and n-6 intake levels by using accurate and non-invasive tools in large epidemiological studies is challenging. Semiquantitative food frequency questionnaire (SFFQ) is easier to use and relatively non-invasive compared with other methods;[8] however, its validity must be assessed. The validity and realiability of applying a SFFQ for assessing n-3 and n-6 intake in children aged 6-23 months have yet to be determined. Therefore, this study developed a valid and suitable SFFQ for assessing dietary n-3 and n-6 intake in Indonesian children.

A systematic review revealed that 75% of previous studies have validated the FFQ against another dietary estimation method and 19% against a biomarker.[9] Validating the FFQ against another dietary estimation method alone is not sufficient because a favourable agreement in the dietary intake results between the test and reference methods does not necessarily indicate validity, but merely indicates similar error.[10] Therefore, the present study

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combined the dietary method and biomarker reference to validate the developed SFFQ. This study had the following objectives: 1) to develop an Indonesian polyunsaturated fatty acid (PUFA) SFFQ for assessing the dietary intake of n-3 and n-6 PUFAs in Indonesian children aged 6–23 months and 2) to assess its validity and reproducibility.

MATERIALS AND METHODS

Study design and sampling

This was a preliminary study aimed at confirming the validity of methods to be used for assessing PUFA intake in a larger study will investigate the effect of a balanced ratio n-3 and n-6 diet on the nutritional status of obese children aged 6–23 months. This preliminary study involved a cross-sectional design. Data were collected in two villages of East Jakarta, Indonesia, from 4 February to 18 April, 2015. Sampling was performed using multistage random sampling. The list of all children aged 6–23 months in each village was obtained through anthropometric screening. A total of 1199 children were screened and randomly listed.

Participants

As recommended by Bland and Altman,[11] 100 children who met the inclusion criteria were randomly selected at the initial of the study by using a random number table function in NutriSurvey for emergency nutrition assessment software. This sample size yielded a 95% CI +0.34 standard error. Children who met the following criteria were included in the analysis: apparently healthy children with caregivers willing to be respondents by signing the informed consent and who had completed the first and repeated SFFQ assessments as well as the 3-day nonconsecutive 24-h recall. Of all the children who met the inclusion criteria, 35 were asked to undergo blood sampling. Children who had congenital abnormalities; whose mothers were unable to communicate properly (had a mental disability alone or with deaf/mute); who had specific food taboos on PUFA-rich food and suffered infectious diseases, which could affect the lipid levels (diarrhoea, acute respiratory infection, and measles); and who refused to be interviewed were excluded from the study. Moreover, 4 out of 100 respondents were excluded because of incomplete test and reference assessments; two children moved to other areas and the other two refused to continue the assessment. After the data were screened, seven respondents were excluded from analyses: Six children were excluded because of underreporting of energy intake and one respondent only consumed less than 5% of the listed food items in the SFFQ. Finally, 89 children were included in the final analysis.

The ethical review boards of Faculty of Medicine Universitas Indonesia and Dr. Cipto Mangunkusumo General Hospital, Indonesia, approved the study protocol no. 858/UN2.F1/ETIK/2014.

Anthropometric screening

Anthropometric assessment was conducted for screening and sampling frame development purposes. In addition, the result of the anthropometry assessment was used to calculate the Goldberg ratio to manage the underreporting of dietary intake. Recumbent length and body weight were measured in children aged 6–23 months at community health centres or their homes.

Food list development

To develop a food list, a list of food items that were important nutrient sources was first constructed on the basis of food composition tables (FCTs), and subsequently, items were systematically omitted from or added to the list.[12] Specifically, to construct the food list, potential food sources of PUFAs for children aged 6–23 months were examined from the Thai, Vietnamese, and US FCTs; these FCTs were used because an Indonesian FCT is unavailable.

Initially, the list of food items that were collected and considered as important food sources of PUFAs included 84 items. Subsequently, the food list was systematically validated against the results of the predietary survey and group interview (GI).[12] The predietary survey (single 24-h food recall) was conducted on 30 respondents purposively in another village (the nearest area to the study site). The information from this predietary survey was used to determine which listed food items should be omitted or added on the basis of the frequency of consumption. In addition, the predietary survey result was used to generate a child-specific portion size for each listed food item and to identify the variation in milk brand, food supplement-rich PUFAs, and type of complementary food consumed by children aged 6–23 months in that area. Group interviews were conducted among caregivers in each village to determine the common food type that was never consumed by children and the reference market to buy the food items.

Ultimately, 19 food items were omitted from and 12 items were added to the initial food item list (84 items). Therefore, the final n-3 and n-6 SFFQ in this study comprised 78 food items with a complete set of standard portion sizes and household measurement units. The final food list was grouped into eight food groups: staple food, animal proteins, plant proteins, breast milk and milk products, fruits and vegetables, oil and fat, snacks, and supplements.

Standard portion size generation and database development

Cade et al suggested specifying a standard portion size in the SFFQ so that participants can select a frequency category according to how often they consumed the specified portion size.[9] In this study, the standard portion size was calculated as the mean portion size from the predietary survey.[12] The amount of each food item reported by each participant during the single 24-h recall was recorded to calculate the mean portion size (total reported amount divided by the number of persons reporting the item).[13] However, not all mean portion sizes of food items can be calculated in the SFFQ. A single 24-h recall cannot cover all information regarding the amount or the actual weights of all food items. Therefore, some food items with no standard portion sizes were weighed on the basis of certain usual household serving units (such as spoon, pieces, bowl, and slice) in a market survey. Furthermore, to determine the usual household serving unit per food

item in the SFFQ, we compiled all household serving units per food item from all respondents in the predietary survey. Subsequently, the standard household serving unit was determined on the basis of the most frequently used serving unit by the caregivers.

Food composition data were obtained using NutriSurvey for Windows (SEAMEO TROPMED University of Indonesia, 2004 Version) and the Indonesian Food Database. Some packaged foods were added to the database, and recipes were formulated for mixed dishes that were not found in the available databases.

Structured interview and SFFQ

The SFFQ consists of questions on the following respondent characteristics: socioeconomic and demographic information, food taboo, and disease history. Subsequently, caregivers were interviewed for the first administration of the SFFQ, followed by a 3-day 24-h recall as well as plasma phospholipid fatty acid (PFA) assessment for selected children. To minimise interviewer bias, different interviewers were assigned for conducting the SFFQ (test) and dietary recall (reference). The second SFFQ was conducted 4 weeks after the first SFFQ by the same interviewer to assess the reproducibility of the questionnaire.

The SFFQ comprises five components: a food list, cooking method, standard portion size, the amount of food consumed, and a frequency response section for the respondent to report the frequency of consumption of each food item. The cooking method column was provided to fulfil a specific objective. For food items prepared using the frying method, the cooking oil consumption was predicted by referring to a table listing the percentage of oil absorption for Indonesian foods.[14] The percentage of oil absorption was defined as the amount of oil absorbed per 100 g of cooked food. The predicted oil consumption per food item was calculated by multiplying the percentage of oil absorption by the amount of cooked food consumed by the participant. For example, if the amount of soy cake consumed is 50 g and the percentage of oil absorption by the cake is 14%, the cooking oil consumption can be derived as follows: $14/100 \times 50 = 7$ g. In addition, to calculate the predicted amount of breast milk consumption (mL/day), the standard amount of breast milk consumption in developing countries was adjusted according to a previous review by the WHO.[15] Children aged 6–8 months consumed 776 and 660 mL of breastmilk exclusively and partially, respectively. Children aged 9–11 and 12–23 months consumed 616 and 549 mL of breastmilk partially, respectively.

The SFFQ was completed using the following protocol: 1) The caregivers were asked to recall the frequency of their children's consumption of the food items over the past 2 months[16] either per day, per week, or per month. 2) The caregivers were asked to indicate the usual serving based on the standard portion size provided. Serving or portion size estimates were requested in household measurement or standard quantities (e.g., 1 tablespoon, a glass, a cup, a slice, and a scoop). Visual aid of food photographs (National Institute of Health Research and Development, Ministry of Health Republic Indonesia, 2014) were used to estimate serving size. The total nutrient intake of each participant were computed by multiplying the relative frequency with nutrient content of each food item consumed according to specified standard portion size.[9] Open-ended questions were also included in the questionnaire to accommodate the food items that were frequently consumed by the children but were not listed in the SFFQ, such as special supplement-rich n-3 and milk for children.

Dietary recall

A 3-day nonconsecutive 24-h dietary recall (weekdays or weekend) was conducted to estimate the habitual n-3 and n-6 intakes as a reference dietary method for SFFQ validation. Dietary recall data were collected on the next day after the first SFFQ administration. Caregivers were asked by field workers, who had been trained in interviewing techniques through a multiple pass 24-h dietary recall method,[17] to recall the children's food intakes during the previous 24-h period.

Detailed descriptions of all food and beverages consumed, including the cooking method, brand names (if possible), and supplements used, were recorded by the field workers. For the food that were not available in the database, the ingredients of the cooked food were requested for calculating the fatty acid analysis from each ingredient. Cooking oil absorption and raw–cooked weight conversion tables were used to reduce the error in weight estimation. Consumed food items, particularly fish products, that had no information on their PUFA content in the PUFA content database were categorised as another variety of fish whose characteristics are closest to those of the ordo or family of these fish products.

To reduce the error estimation, the field workers enumerator verified the gram equivalents of food item that could not be estimated using food photograph. This purpose, the field workers were equiped by food weighing instrument (TANITA KD-160 WH, Illinois 60005, USA) to weight the actual amount of food sample obtain from the cares or from community store. Field workers also recorded the actual weight of the manufactured or processed food products. Similarly, brand names, price, and flavour details of manufactured or processed foods or street foods were obtained.

Plasma phospholipid assessment

Blood samples were collected from children (n=35) two weeks after the first administration of the SFFQ. Some caregivers and their children were invited to Posyandu (Integrated Health Post), and the rest were visited at their houses for blood sample collection. Blood samples were obtained through venipuncture by a phlebotomist. The required amount of blood for this test was 3 mL. The samples were collected in a labelled vacutainer tube containing 0.1% EDTA and then directly stored in a cool box. On the same day, the blood samples were directly transported to the DKI Jakarta Provincial Health Laboratory (Labkesda DKI) for further analysis. The concentration of PUFAs and their derivatives in plasma were analysed using the gas chromatography-fatty acid methyl ester method. The analysis results are expressed in percentage (%) and milligram per millilitre units for total n-3, docosahexanoid acid (DHA), eicosapentanoic acid (EPA), α-linolenic acid (ALA), total n-6, arachidonic acid (AA),

and linoleic acid (LA).

Data presentation and analysis

Variables with normally distributed data are presented as the mean ± SD, whereas those without normally distributed data are presented as the median (25th–75th percentile). The Kolmogorov–Smirnov test was used to analyse the normality of the data distribution, whereas data log10 (x + 1) was used for data transformation of intake variables. Anthropometric data were analysed using WHO Anthro 2005, whereas statistical analyses were performed using SPSS for Windows version 20.0 and Prism 5.0.

The relative validity and reproducibility of the SFFQ were assessed using Bland–Altman plots by calculating the limit of agreement, whereas the Spearman rank test was performed to assess the absolute validity of the SFFQ. Bland and Altman recommended the use of a log transformation scale for any linear relationship between the mean difference and average in both the test and reference methods.[18] To facilitate the interpretation of Bland–Altman plots in log form, the mean bias and limit of agreement were transformed back to the original scale by taking the antilog. This antilog yielded an interval for the ratio between the two measurements.[19] The closest agreement was shown by a 100% mean of the ratio; in other words, the closer the ratio was to 1, the closer the agreement was.

To reduce bias in the reference data because of underreporting, the means of the 3-day 24-h recall data were assessed by using the Goldberg ratio. Underreporting was identified by calculating the ratio between the reported energy intake and basal metabolic rate (EI_{rep}:BMR_{est}) for each individual. These ratios were then compared with the Goldberg cutoff for the 3-day dietary assessment.[20,21] Participants who reported an energy intake lower than the minimal basal metabolic rate (BMR) or out of the Goldberg cutoff range were excluded from the data analysis. The BMR was predicted according to the standard age- and sex-specific equations derived by Schofield.[22]

Table 1. Characteristics of healthy children participant in the PUFA SFFQ validation study

Variable	n (%)
Children's sex	
Boy	55 (61.8)
Girl	34 (38.2)
Children's age (month)	
6–8	16 (18.0)
9–11	15 (16.9)
12–23	58 (65.2)
Current breastfeeding status	
Breastfed	63 (70.8)
Non-breastfed	26 (29.2)
Nutritional status (BAZ)	
Wasting (-3 SD –<-2 SD)	12 (12.9)
Normal (-2 SD–2 SD)	76 (84.9)
Overweight (>2 SD)	2 (2.2)
Mothers's age (years)†	31 (29, 37)
Mothers' education (years)	
0–6	4 (4.5)
7–9	16 (18.0)
10–12	53 (59.6)
>12	16 (18.0)
Mothers's occupation	
Working	29 (32.5)
Not working	60 (67.4)
Household's monthly income (USD)†	219 (197, 291)

BAZ: body mass index for age z score.
†Values are median (25th, 75th percentile).

RESULTS

Respondent characteristics

Most of the participants were boys (61.8%), were aged between 12 and 23 months (65.2%), were currently breastfed (70.8%), and had normal weight according to the BMI-for-age z scores (84.9%). Most mothers graduated from junior high school (>75%) and are currently housewives (67.4%). The median monthly income was approximately US$219 (Rp 3,066,000).

The median intakes of almost all essential fatty acids in the first SFFQ and the mean values of the 3-day 24-h recall data were comparable (no significant difference; p>0.05), except for the total n-3 PUFA, ALA, total n-6 PUFA, and LA; Table 2). A large discrepancy in the total n-3 and n-6 PUFA intakes was observed, followed by the ALA and LA intakes, because ALA and LA were the

Table 2. PUFA SFFQ and 3-day 24-hour food recall intakes and plasma fatty acid in children aged 6-23 months

Fatty acid	1st SFFQ g/day	2nd SFFQ† g/day	Mean 3–d 24–h FR‡ g/day	PFA§ mg/mL
n–3 PUFA	2.29 (0.90–4.21)	2.44 (1.36–3.77)	1.37 (0.75–1.85)	0.074 (0.057–0.103)
DHA*	0.20 (0.15–0.27)	0.22 (0.17–0.30)	0.18 (0.15–0.28)	0.053 (0.040–0.083)
EPA*	0.03 (0.02–0.06)	0.03 (0.03–0.10)	0.03 (0.02–0.05)	0.004 (0.003–0.006)
DPA*	0.06 (0.05–0.07)	0.07 (0.05–0.09)	0.06 (0.04–0.09)	–
ALA	1.94 (0.60–3.68)	1.88 (0.86–3.23)	0.99 (0.48–1.36)	0.005 (0.003–0.007)
n–6 PUFA	19.0 (7.24–35.1)	19.4 (10.8–30.4)	11.0 (6.06–16.8)	0.400 (0.341–0.512)
AA*	0.16 (0.10–0.23)	0.20 (0.11–0.28)	0.13 (0.09–0.26)	0.009 (0.007–0.012)
LA	17.9 (6.71–34.6)	19.1 (10.3–30.1)	10.8 (5.66–16.4)	0.390 (0.327–0.491)

PFA: plasma fatty acid; DHA: docosahexanoid acid; EPA: eicosapentanoic acid; DPA: docosapentanoic acid; ALA: α-linolenic acid; AA: arachidonic acid; LA: linoleic acid; PUFA: polyunsaturated fatty acid..
Data are presented as median (25th- 75th percentile), n: 89 children.
*The wilcoxon signed rank test showed no significant differences (p>0.05) between 1st SFFQ and 3d 24h recall for DHA, EPA, DPA, and AA.
†Administered 4 weeks apart from 1st SFFQ.
‡Collected on 3-day non-consecutively, including 1 weekend and 2 weekdays and those conducted in between administration of the SFFQ.
§Total number of respondents were 31 children.

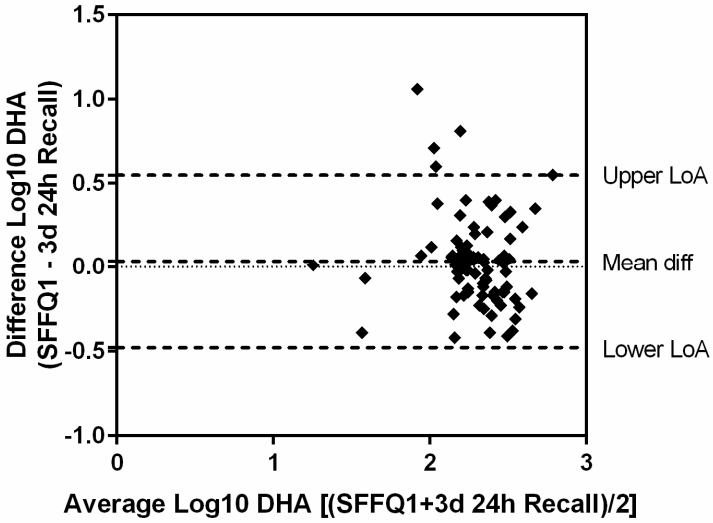

Figure 1. Bland Altman plot assessing the agreement between 1st SFFQ and mean 3-day 24-hour recall for DHA after log transformation [log10 (x+1)].

Table 3. Agreement between 1st SFFQ vs 3-day 24-hour recall in measuring PUFA intakes

Fatty acid	Bland-Altman (diff vs average in g/day)				Bland-Altman log transformation		
	Mean diff (SD)†	Lower LoA‡	Upper LoA	*p* linear trend	Antilog ratio (SD)§	Lower LoA	Upper LoA
Total n-3	1.27 (2.10)	-2.86	5.40	0.001*	1.56 (2.31)	0.30	8.13
DHA	0.01 (0.15)	-0.28	0.32	0.022*	1.08 (1.83)‖	0.33	3.54
EPA	0.00 (0.06)	-0.12	0.13	0.001*	1.16 (3.06)‖	0.13	10.4
DPA	0.00 (0.07)	-0.14	0.16	0.001*	1.21 (2.89)‖	0.15	9.73
ALA	1.22 (2.05)	-2.79	5.25	0.001*	1.78 (2.95)	0.21	14.7
Total n-6	10.7 (16.9)	-22.6	43.9	0.000*	1.61 (2.76)	0.34	7.59
AA	-0.01 (0.22)	-0.45	0.43	0.77	1.08 (2.76)‖	0.15	7.89
LA	10.8 (16.9)	-22.5	44.0	0.000*	1.69 (2.29)	0.33	8.51

LoA: limit of agreement; DHA: docosahexanoid acid; EPA: eicosapentanoic acid; DPA: docosapentanoic acid; ALA: α-linolenic acid; AA: arachidonic acid; LA: linoleic acid; PUFA: polyunsaturated fatty acid.
*$p<0{\cdot}05$, there is any linearity/magnitude dependency should be converted into log transformation as suggested by Bland-Altman.
†The mean difference between 1st SFFQ and 3d 24h recall.
‡Mean difference±1.96*SD
§Antilog Ratio (10^y), y = log10 (x+1). Ratio between mean 3d 24h recall and 1st SFFQ, the closest agreement is "1".
‖There is an agreement after converted back into antilog ratio, shown by the mean antilog ratio close to 1.

main sources of total n-3 and total n-6 PUFAs, respectively. The SFFQ tended to record higher estimations than did the 3-day 24-h recall for the aforementioned nutrients. Furthermore, the median intakes of all essential fatty acids between the first and repeated SFFQ administration were comparable.

Validity

According to the Bland–Altman and prior log-transformed graphs (not shown) as well as the results in Table 3, the mean differences for all essential fatty acids between the first SFFQ administration and 3-day 24-h recall were nearly zero, except for total n-3 and n-6 PUFAs, ALA, and LA. To ensure that the bias or difference was acceptable from a nutrition perspective, the regression line was subjected to a fitting process to assess whether there was any correlation between the differences and averages from both methods. Most essential fatty acids, except for AA, exhibited positive linearity or magnitude dependency ($p < 0.05$). The differences between the SFFQ and 3-day 24-h recall intakes increased positively with the mean intakes. As suggested by Bland–Altman, log transformation was then used to obtain a clearer interpretation. As illustrated in Figure 1, more than 95% of the dots fell within the limit of agreement, and the dots were located equally in the left and right parts, thus signifying excellent agreement. However, the antilog ratios for total n-3 and n-6 PUFAs, ALA, and LA were too far from 1 (ratio between SFFQ and 3-day 24-h recall) to exhibit ideal agreement (Table 3). This implies that the amounts of these essential fatty acids estimated in the SFFQ were approximately >1.5 times higher than those estimated in the 3-day 24-h recall.

Furthermore, this study estimated the absolute validity by comparing the intake from the first SFFQ with plasma phospholipid fatty acid content through a correlation test. The correlation test was divided into two parts: nonadjusted and adjusted (Table 4). The nonadjusted correla-

Table 4. Absolute validity estimation between 1st SFFQ and plasma fatty acid in measuring PUFA intakes

Fatty acid	1st SFFQ vs PFA†			
	Spearman coefficient	*p* value	Adjusted Spearman coefficient	*p* value
Total n-3	0.13	0.45	0.01	0.95
DHA	0.20	0.26	0.10	0.60
EPA	0.12	0.52	0.13	0.49
ALA	-0.07	0.69	0.22	0.24
Total n-6	0.34	0.06	0.40	0.03*
AA	-0.02	0.91	-0.03	0.87
LA	0.37	0.04*	0.42	0.02*

PFA: plasma fatty acid; DHA: docosahexanoid acid; EPA: eicosapentanoic acid; DPA: docosapentanoic acid; ALA: α-linolenic acid; AA: arachidonic acid; LA: linoleic acid; PUFA: polyunsaturated fatty acid.
Total number of respondents included in analysis were 31 subjects.
*$p<0.05$, significantly correlated after adjusted by weight for age z score and age by partial correlation.
†Intake and plasma were log transformed prior to analysis. Fatty acid intake were expressed in g/day; while plasma fatty acid in mg/mL.

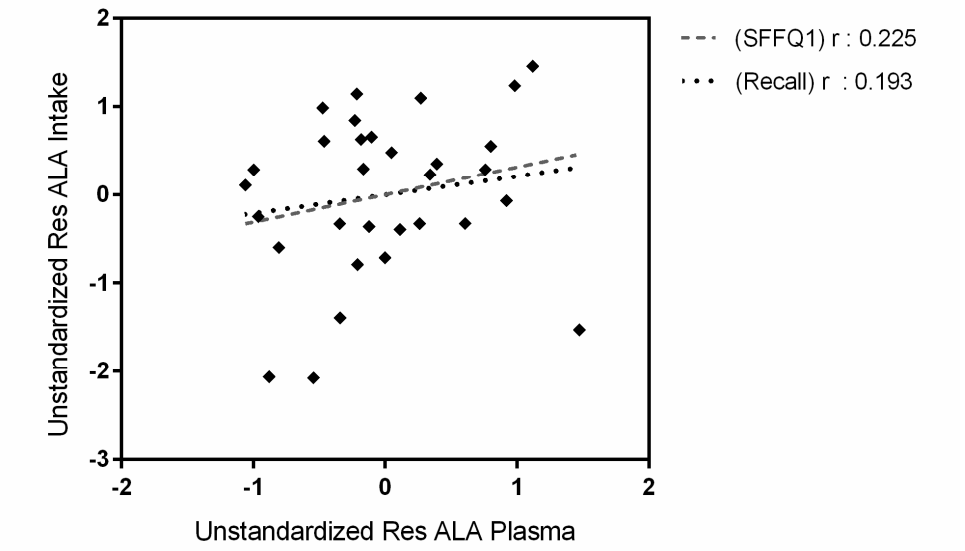

Figure 2. Correlation between plasma and dietary intake of α-linoleic acid (ALA) after adjusted by age of children and weight for age z-score.

tion test revealed a nonsignificant correlation between the first SFFQ and plasma fatty acid content for almost all essential fatty acids, except for LA (r=0.37, p=0.04), thus indicating a moderate correlation. This result was then confirmed through further analysis by performing a partial correlation test to adjust for other variables that can be considered as possible factors affecting the correlation between dietary intake and plasma fatty acid content. The adjusted correlation coefficients exhibited significant correlation for total n-6 PUFA and LA, after controlling for the weight-for-age z score and age. A positive correlation was identified between the SFFQ and plasma concentration of DHA, EPA, and ALA, indicating that the higher the intake of DHA, EPA, and ALA was, the higher the plasma concentration was, except for total n-3 PUFA and AA (Figure 2). However, these correlations were not statistically significant.

Reproducibility

Table 5 presents the reliability of the SFFQ obtained through Bland–Altman analysis. According to the Bland–Altman graph and prior log-transformed graph (not shown) as well as the results in Table 5, the mean differences for all essential fatty acids were minor and even nearly zero for some nutrients. To fit the regression line, the magnitude dependency between the differences and averages of both SFFQs was assessed. Only total n-3 PUFA, DHA, and ALA demonstrated a significant linearity level. Log transformation was then employed to obtain a clearer interpretation. The antilog ratios for all essential fatty acids approached 1 (ratio between the first and second SFFQs), thus implying ideal agreement (Table 5). This indicates that the SFFQ was reproducible and sensitive to change at various time points.[23]

DISCUSSION

The present study developed a relatively valid and reliable SFFQ for assessing PUFA intake in Indonesian children aged 6–23 months. According to the Bland–Altman analysis, the SFFQ exhibited favourable agreement with the 3-day 24-h recall for DHA, EPA, DPA, and AA, but not for total n-3 PUFA, ALA, total n-6 PUFA, and LA. However, this SFFQ did not yield the same result for the correlation between the SFFQ and PFA, except for plasma total n-6 long-chain (LC) PUFA and LA. Furthermore, the developed SFFQ demonstrated a 95% limit of Bland–Altman agreement, which was clearly observed at different time points of SFFQ administration, indicating excel-

Table 5. Agreement between 1st and 2nd SFFQ in measuring PUFA intakes

Fatty acid	Bland-Altman (diff vs average in g/day)				Bland-Altman log transformation		
	Mean Diff (SD)[†]	Lower LoA[‡]	Upper LoA	*p* linear trend	Antilog ratio (SD)[§]	Lower LoA	Upper LoA
Total n-3	0.17 (2.93)	-5.57	5.93	0.02[*]	1.13 (2.57)[‖]	0.18	7.19
DHA	0.04 (0.25)	-0.44	0.54	0.000[*]	1.17 (1.86)[‖]	0.35	3.97
EPA	0.02 (0.11)	-0.19	0.25	0.05	1.33 (2.98)[‖]	0.16	11.2
DPA	0.01 (0.08)	-0.14	0.16	0.06	1.19 (2.39)[‖]	0.22	6.54
ALA	0.11 (2.82)	-5.42	5.66	0.01[*]	1.16 (2.84)[‖]	0.14	9.58
Total n-6	1.44 (23.2)	-44.1	47.0	0.59	1.11 (2.46)[‖]	0.19	6.49
AA	0.03 (0.17)	-0.31	0.38	0.99	1.22 (2.25)[‖]	0.25	6.00
LA	1.39 (23.2)	-44.0	46.8	0.06	1.12 (2.47)[‖]	0.19	6.62

LoA: limit of agreement; DHA: docosahexanoid acid; EPA: eicosapentanoic acid; DPA: docosapentanoic acid; ALA: α-linolenic acid; AA: arachidonic acid; LA: linoleic acid.
[*] $p<0.05$, there is any linearity/magnitude dependency should be converted into log transformation as suggested by Bland-Altman.
[†] The mean difference between 1st SFFQ and 3d 24h recall.
[‡] Mean difference ±1.96*SD
[§] Antilog ratio (10^y), y = log10 (x+1). Ratio between mean 3d 24h recall and 1st SFFQ, the closest agreement is "1".
[‖] There is an agreement after converted back into antilog ratio, shown by the mean antilog ratio close to 1.

lent reproducibility. The study results reveal that the SFFQ is sufficiently reliable and sensitive for use in further intervention studies.

The proposed SFFQ was constructed systematically to maintain the internal validity of this study. The list of PUFA-rich foods was developed by adopting the approach used by Magkos et al in their study on validating the FFQ for assessing calcium intake.[24] The initial food list was derived from food composition tables; subsequently, the list was shortened by conducting a predietary survey and a series of GIs. Some studies have proposed alternative methods of developing food lists in FFQs. For example, Hinnig et al[25] identified the most representative food items that represented up to 95% of the energy and macronutrient consumption by using a formula proposed by Block et al.[26] In addition, Omidvar et al conducted principal component analysis with varimax rotation on food frequency items to identify main factor groupings and generate the final food list for a short questionnaire.[27] However, the aforementioned approaches are more complex and require a higher number of respondents for the predietary survey.[12] Hence, the approach used in the present study is more appropriate because it is easier and does not require a high number of respondents.

For total n-3 PUFA, ALA, total n-6 PUFA, and LA, the proposed SFFQ tended to record higher mean estimations compared with the means derived from the 3-day nonconsecutive 24-h recall in this study. This finding is similar to those of previous studies[28,29] on SFFQ validation; even the previous studies applied more accurate food data collection namely 3-day nonconsecutive weighed food record (WFR)[28] and 2-day nonconsecutive WFR.[29]

These discrepancies were observed because of the high variability of the concentrations of those fatty acids in a few foods such as formula milk, seafish, and tofu. PUFA nutrients usually exhibited large within-subject variation, which could affect the variation of intake estimations between the two methods.[9] Tofu of various sizes are available in the market. Because the ALA and LA contents are high in tofu, a small difference in reporting its portion size affects the total n-3 and n-6 intake estimation in the children. Furthermore, a variety of brands and types of milk are available for children. The SFFQ developed in the present study accommodated many varieties and types of milk; however, nutrition information of all brands could not be obtained for data analysis. Therefore, including complete nutrition content information of milk consumption in the SFFQ is recommended for future research.

The discrepancy between the SFFQ and 3-day 24-h recall in assessing total n-3 PUFA, ALA, total n-6 PUFA, and LA can be further explained as follows. First, according to the dietary recall, the children's diet included a variety of composite dishes, and the SFFQ provided few options of such dishes, while the SFFQ questionnaire mostly consisted of non-composite dishes. This may have led to misclassification and affected the correlation observed between the SFFQ and the 3-days consecutive 24-h recall. Second, the different method to estimate the weight of the food by SFFQ and food recall. In the SFFQ, the way to estimate the weight of food based on standard portion size which categorize into small, medium and large while food recall based on actual food quantity consumed by the respondent. It triggered the observed response bias. The size variation of tofu and seafood, which contain high amounts of ALA and LA, was a major source of error in determining agreement between the SFFQ and 24-h dietary recall.

The present study used the 3-day nonconsecutive 24-h recall as the reference test to record within- and between-person variations of PUFA intake in children. The formula by Black, Cole et al in Pereira et al was used to determine the number of measurement days or replications of the 24-h food recall.[30] According to the finding of the present study, the used of a 3-day food recall measurement were suitable to represent the habitual intake of some PUFA nutrients. Cade et al[9] suggested increasing the number of measurement days in the reference test with sufficient time intervals corresponding to the questionnaire to obtain typical PUFA intakes. Some studies have demonstrated that sufficient intervals in the reference method can improve the apparent validity of the SFFQ.[29,31] The most favourable recommended references are 7-day 24-h recalls and WFR specific to fatty acids;

however, further research is required to validate these recommendations.[32]

The present study determined the relative validity of the SFFQ on the basis of the Bland–Altman analysis. The Bland–Altman analysis was recommended as the most favourable approach for assessing agreement because it is not influenced by sample size and it entails quantifying the variation between different methods for each individual.[18] The relative validity of this SFFQ was not consistent with the correlation between the SFFQ and plasma fatty acid content. The plasma fatty acid content did not reflect the intake during the previous 2 months for all essential fatty acids, except for total n-6 PUFA and LA. The developed SFFQ demonstrated a moderate correlation for total n-6 LC PUFA and LA after adjustment for age and nutritional status. Therefore, this SFFQ should be interpreted with caution by considering the objective markers as a reference.

Plasma phospholipid was used as a reference biomarker in the present study because it has been frequently used and can be easily sampled in large populations,[33] it is considered a reliable biomarker for reflecting n-3 status for a short period (past few days or more),[34] it is largely derived from exogenous sources,[35] and it is reported with a moderate correlation for EPA and DHA.[29,36,37] However, the ideal correlations between plasma fatty acids in tissues and intakes measured using FFQs are sometimes unrealistic.[36] In this study, all possible factors influencing the plasma fatty acid composition could not be controlled. Factors such as absorption, metabolism, genetic factors, sex, hormones, and lifestyle, which may act as determinants for plasma fatty acid concentrations in tissues, must be considered.[13,38] In general, three factors can affect the plasma fatty acid levels, namely nutritional, hormonal or biological, and physiological factors.[39]

After adjustment for confounding factors such as age and nutritional status, a higher correlation coefficient was obtained between the SFFQ and plasma content. Physiological factors such as age can affect the plasma fatty acid levels, particularly in children.[39] PUFA requirements in children are critical for their growth and development, particularly brain development.[39] Moreover, the nutritional status of the participants adversely influenced the fatty acid profiles of dietary fat intakes. Because desaturases are metalloenzymes, adequate amounts of iron, zinc, copper, and magnesium may be required for normal fatty acid metabolism.[40]

The present study did not consider the effects of gene mutations that affect the association between dietary and plasma fatty acids, such as polymorphisms in delta-5 (FADS1) and delta-6 (FADS2) fatty acid desaturase genes and the contribution of the apolipoprotein E (APOE) genotype. These modifications can alter the EPA and DHA levels.[37,41,42] Moreover, fasting conditions must be managed to maintain homeostatic condition in order to accurately reflect intake levels and prevent lipolysis-induced bias.[40] However, the present study could not collect plasma from children under fasting conditions.

The low correlations between most n-3 essential fatty acids such as ALA, EPA, and DHA in the present study can be due to the following reasons: (1) competition between ALA and LA to use the enzyme D6 desaturase for further metabolism,[43] inhibition of the conversion of ALA into EPA and DHA because of the increased LA concentration,[39,44] and (2) high ALA oxidation rate.[45] The concept of loss of dietary ALA is consistent with the finding of this study (Table 1), in which each gram of daily LA intake translates into approximately 0.390 mg/mL of plasma. By contrast, each gram of daily ALA intake translates into only 0.005 mg/mL of plasma. This is partly because most dietary ALA undergoes beta-oxidation in the mitochondria, and only a limited amount is available for conversion to EPA and DHA.[46]

The strength of the present study is the prevention of bias regarding the actual portion size and nutrition composition labels of commercial foods; this was achieved by employing food photographs and food weighing of some purchased food item as well as recording the actual nutrition information or labels. Moreover, potential confounders of plasma-diet analysis were controlled in this study. According to our review of the relevant literature, this is the first study to particularly develop and validate the PUFA SFFQ for Indonesian urban children aged 6–23 months.

In conclusion, the developed SFFQ is relatively valid and reliable for estimating PUFA intakes in Indonesian children aged 6–23 months. However, total n-3 PUFA, ALA, total n-6 PUFA, and LA were poorly estimated by this SFFQ. The application of the proposed SFFQ to other populations should be adopted and interpreted with caution, because this SFFQ was developed by including mothers or cares with a medium-high education level and because the SFFQ estimation was not consistent with the plasma phospholipid content for some essential fatty acids.

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AUTHOR DISCLOSURES

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