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Validation of Food Frequency Questionnaire in Estimating Docosahexanoic Acids (DHA) Intake among Malay Primary School Children

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ABSTRACT

Introduction: Omega-3 Polyunsaturated Fatty Acids (PUFAs) play an important role in developing cognitive function in children, especially Docosahexanoic Acid (DHA). However, there is no suitable dietary assessment tool to assess DHA intake among Malaysian children. Thus, this study assessed the validity of an interviewer-administered semi-quantitative Food Frequency Questionnaire (FFQ) for estimating DHA intake among Malay school children in the Sepang District. **Methods**: Thirty 12-year-old Malay children (13 boys and 17 girls) were recruited through purposive sampling. Their DHA intake for one month duration was assessed using a 30-food item FFQ and validated against a 3-day food record. **Results**: The majority of the subjects (70%) had normal body mass index, 6.7% were overweight, 16.7% obese, whilst the rest were in the thin category. The reliability of FFQ was found to be good with a Cronbach's α coefficient value of 0.815. Wilcoxon Signed Rank Test indicated no significant difference in mean intake between the two assessment methods. Significant and strong correlation between FFQ and the 3-day food record was found for total omega-3 PUFAs (r=0.812)and DHA (r=0.839) using both methods. Bland-Altman analysis exhibited no apparent systematic bias between the two methods for DHA and total omega-3 PUFAs intake, whilst a quartile analysis assigned 73.3% of the subjects into the same quartile. **Conclusion**: The FFQ was found to be valid in estimating DHA intake among Malay school children, and it is recommended that its validity be tested on other ethnic population groups.

Key words: Children, food frequency questionnaire (FFQ), Docosahexaenoic acid (DHA), school, validation

INTRODUCTION

The health benefits of consuming long-chain (LC) omega-3 Polyunsaturated Fatty Acids (PUFAs), consisting mainly of α-linolenic acid (ALA, 18: 3n3), and the longer chain of eicosapentanoic acid (EPA, 20: 5n3) and docosahexaenoic acid (DHA, 20: 6n3) are frequently reported,

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particularly its capability to aid in brain development (Dangour & Uauy, 2008), protect against hemorrhagic stroke (Parka *et al*., 2009), coronary heart diseases (Logan *et al*., 2009), rheumatoid arthritis (Cleland ,James & Proudman, 2003), depression (Su, 2008) and Alzheimer's disease (Freund-Levi *et al*., 2006).

Considering the importance of DHA in the human diet, an accurate assessment of their intakes could be useful in healthcare settings and nutritional research. Possible dietary assessment methods are the 24-h diet recall, diet history, food frequency questionnaire (FFQ) and food records. Overall, the FFQ may be the best dietary assessment method, particularly for nutrients such as DHA, as its intake varies considerably from day to day. It also has a lower participant burden than other methods, is inexpensive and quick to administer (Eck, Klesges & Klesges, 1996). However, from the point of view of nutritional epidemiology, any newly developed FFQ must be assessed for validity and reproducibility. The 24-h dietary recall has been reported as a suitable reference method to assess the validity of the FFQ (Cade *et al*., 2002).

Measuring dietary intakes in children and adolescents is challenging. For example, children may have difficulty remembering quantities, and they are not always able to describe in detail the food that they eat (Livingstone & Robson, 2000). Although Stiegler *et al.* (2009) have conducted a validation study in Germany for evaluating fatty acids intake among children, the available FFQ could not be adopted for all populations due to different habitual dietary patterns and food sources (McNutt, Zimmerman & Hull, 2008). Formulation of a FFQ must be tailored to a specific targeted population due to varying dietary practice habits across populations of different ethnicity, social and cultural practices (Cade *et al*., 2002). In Malaysia, population-focused FFQ for omega-3 PUFAs has been developed for older adults, particularly among the Chinese and Malays (Lee *et al*., 2013).

The focus of this research was to develop an interviewer-administered FFQ for estimating DHA intake among Malay children. The validity of this newly developed FFQ was analysed using statistical analyses and compared against a 3-day-food record (FR). In this context, we hypothesised that there would be no significant difference between the newly developed FFQ with the food record (FR) for evaluation of DHA and omega-3 PUFAs intake among children in Malaysia.

METHODS

Recruitment of subjects

The participants in this study were recruited from a convenience group of children aged 12 years studying in Sekolah Kebangsaan Taman Seroja in Salak Tinggi, Sepang District, Malaysia. The inclusion criteria were Malaysian citizen, Malay, able to communicate, and apparently healthy Year Six children. The exclusion criteria were children who were absent during data collection and had been diagnosed with mental health problems or learning disabilities such as dyslexia, autism and others.

The subjects were interviewed to obtain detailed information using a structured questionnaire. The questionnaire consisted of socio-demographic details. Informed consent for participation in the study was obtained from each subject and their parents. The study was approved by the Ethics Committee, National University of Malaysia.

Development and administration of DHA FFQ

The specified semi-quantitative FFQ (Appendix 1) was designed to assess omega-3 PUFA intake over the past month among the selected 12-year-old Malay children. Specific food items were listed

according to seven categories (ocean fish, seawater fish, seafood, egg and chicken, omega-3 egg, canned products and cod liver oil) and the amount of consumption was recorded. The list of food items was developed based on previous studies related to the potential sources of Omega-3 PUFAs available in Malaysia (Lee *et al.,* 2013; Ng, 2006; Wan Rosli *et al.,* 2012). Then, the food items were shortlisted to foods containing DHA. In order to avoid under-reporting, detailed information was included such as brands, methods of cooking, and parts of food being consumed. Household measuring utensils, food atlas (Suzana *et al.,* 2009), printed visuals and open-ended questions were utilised to assist the participants. A pilot test was carried out among ten Year Six primary school children to assess the participants' understanding of the newly developed FFQ and the Cronbach Alpha was found to be 0.815. Improvements were made based on the feedback given. The improved FFQ was used for interviewing the children. Subsequently, every guardian was requested to verify the information provided by their child, based on their knowledge regarding the usual dietary intake patterns of the family. The children were asked to return the FFQ to the researcher the following day. The guardians were contacted by the researcher through telephone whenever information was not clear.

The 3-day food record

In this study, the 3-day food record (FR) was used as a standard method to assess the validity of the FFQ. Food records were filled by the subjects for three days; two weekdays and one weekend. Subjects were advised to adopt a regular eating habit and record it shortly after taking meals. Serving size for food and drinks had to be specified using household measurements such as tablespoons, teaspoons, and bowls. Pictures of household measurements were provided in the FR booklet to assist subjects to determine the portion sizes consumed The limitations of this method are (a) subjects tend to misreport any of the food they think is unimportant; (b) they may incorrectly identify foods because of a lower level of knowledge on food and preparation methods (Livingstone & Robson, 2000). In view of these limitations, subjects in this study were asked to hand over the FR to their parents to review after it was completed. The researchers checked the form for completeness and clarified with subjects and their guardians when necessary.

Omega-3 PUFAs intake

The average daily intake of DHA and omega-3 PUFAs were analysed according to the United States Department of Agriculture Nutrient Database (USDA, 2007). Meanwhile, standard serving size of each food item was referred to the Malaysian Food Composition Table (Tee *et al.,* 1997). The composition of fatty acids in seawater fish was gathered from a previous study (Wan Rosli *et al.,* 2012). Additional information including labeling on canned and fortified food was adopted in the database system. Mean daily intake of DHA and total omega-3 PUFAs was derived as follows:

Mean daily DHA intake (g/day) = Docosahexanoic Acid content (g) X frequency of consumption (1)

Statistical analysis

All statistical tests were carried out using Statistical Package for the Social Sciences (SPSS) version 22.0 and two-sided p value <0.05 was considered to indicate the statistical significance. Since the data were not normally distributed, Mann-Whitney test was performed to measure the mean difference (±SD) of DHA and total omega-3 PUFAs intake. Mean differences (SD) of DHA and omega-3 PUFAs between FFQ and FR were assessed using the Wilcoxon signed rank, while Spearman's correlation

Table 1. Socio-demograpics and nutritional status characteristics of the participants (n=30)

Characteristics	*n*	*(%)*
Gender		
Male	17	56.7
Female	13	43.3
Father's education level		
Primary school	3	10.0
Secondary school	21	70.0
College/university	6	20.0
Mother's education level		
Primary school	1	3.3
Secondary school	25	83.4
College/university	4	13.3
Family household income		
< RM 720 (Poor)	5	17.6
RM 721 – RM 2299 (Low)	11	36.7
RM 2300 – RM 6999 (moderate)	14	46.7
Father's employment		
Yes	30	100.0
No	0	0.0
Mother's employment		
Yes	12	40.0
No	18	60.0
Supplement intake		
Yes	5	16.7
No	25	83.3
Tuition attendance		
Yes	28	93.3
No	2	6.7

was performed to assess the strength of association between FFQ and FR. In order to assess the agreement between FFQ and FR and to detect systematic bias with FFQ relative to FR, Bland-Altman analyses were used (Bland & Altman, 1986). By plotting the difference between the methods against the average of fatty acid intake from the two methods, the dispersion and the extent to which the two methods agreed can be visualised. Quartile's cross-classification analysis was applied based on DHA and total omega-3 PUFAs intake from both instruments. The degree of mis-classification across categories between the FFQ and the reference method was examined by dividing nutrient intake values into quartiles. The proportion of subjects classified into the same, adjacent and extreme quartiles was calculated.

RESULTS

A total of 30 year-six primary school Malay children (56.7% boys and 43.3% girls) participated in this study. As shown in Table 1, 70% of the participants were in normal BMI category, followed by 16.7% obese and 6.7% overweight. There were no significant differences in anthropometric profile according to gender.

A pilot test was carried out before the validation process began and the value of Cronbach's alpha was found to be 0.815. A Cronbach alpha value that is higher than 0.70 is considered the standard for good

internal consistency of the instrument (Leech, Barett & Morgan, 2007). Total omega-3 PUFAs intake from both FFQ and FR ranged from 90 to 2090 mg/d and 65 to 1495 mg/d respectively. Meanwhile, DHA intake from FFQ and FR ranged from 60 to 1520 mg/day and 63 to 1370 mg/day, with the mean differences being 285 and 167 mg/d for total omega-3 PUFAs and DHA respectively. DPA intake from FFQ and FR ranged from 6.1 to 228.9 mg/day and 6 to 72 mg/day; meanwhile the EPA intake from FFQ and FR ranged from 24 to 885 mg/day and 5 to 356 mg/day. Wilcoxon Signed Rank Test indicated no significant mean difference in the fatty acids intake between the two assessment methods (Table 2). Median for total omega-3 was 559 mg for FFQ and 465mg for FR. Meanwhile, median for DHA was 392 mg for FFQ and 339 mg for FR.

Unadjusted correlation coefficients for total omega-3 and DHA were 0.7630 (p<0.001) and 0.812 (p<0.001). Bland-Altman plot and variation spread around the means exhibited no apparent systematic bias between the two methods for average total omega-3 PUFA intake (Figure 1). The top line is the maximum value, while the bottom line is the minimum value. All the values have to be in between the maximum and minimum value, to indicate a good agreement between two methods. Table 3 shows analysis of agreement (25th quartile, 75th quartile and 100th quartile) for FFQ and FR (total omega-3 PUFAs intake). In general, both FFQ and FR classified 73.3% subjects into the same quartile and 36.6 % were classified into the lower and upper quartile. All subjects were assigned into the same or adjacent quartiles. The FFQ distributed the omega-3 FA intake correctly into the same quartiles and adjacent quartiles compared to the FR.

DISCUSSION

In this study, FFQ showed a higher total omega-3 PUFAs intake compared to FR, possibly due to the underestimation of the exact intake among the participants using the PETRA (Portable Electronic Tape Recorded Automatic). This is probably due to seafood intake such as squid, shrimp and anchovies that are usually added to food to enhance the flavour of the dishes and reported in the FFQ but not in the diet record. Even though complete instructions have been given to the participants, they might not be able to memorise the specific ingredients added due to their limited knowledge of food preparation.

It is obvious that the level of dietary omega-3 PUFAs intake in the present study of 0.67 g/d was lower compared to the recommended level for 9 to 13-year-old children suggested by Food and Nutrition Board (IOM, 2002), i.e., 1.2 g/day. Similar results were reported among 93 children aged 5 to 12 years in Australia with 0.74±0.28 g/day for total omega-3 dietary intake. Besides, a study among children aged 8 to 11 years also shows low estimated dietary DHA intake (0.06±0.05 g/day) with a large standard deviation (Meyer *et al.,* 2003). The total intake was below the suggested Australian and international target dietary intake for children. Australian recommendations advocate 12 to 20 g/day or 6 to 10% energy from total omega-6 and 0.16 to 0.43 g/day or 0.08 to 0.22% energy from omega-3 fatty acids. Low DHA intake among children in this study is likely to be influenced by the dietary habit practised by the caregivers or family. There is a significant correlation between the parents' and children's dietary habit, especially in food preference (Scaglioni, Salvioni & Galimberti, 2008).

Local studies conducted to investigate omega-3 PUFAs intake among Malay and Chinese elderly (Lee *et al.,* 2013), pregnant mothers and lactation mothers (Ho *et al.,* 2011; Wong, Chee & Cheryl, 2011) and among adults (Ng, 2006) also reported intakes that were lower than the recommendation. The World Health

Table 2. DHA intake estimated by the FFQ and 3-day FR

Fatty acid	*FFQ (mg/d)*				*FR (mg/d)*				*Mean difference (FFQ-FR)/FR*	*Wilcoxon signed rank test p value*
	Mean	SD	Median (IQR)	Range	Mean	SD	Median (IQR)	Range		
DHA	474	343	392	65 – 1495	391	279	339	43 - 1107	0.21	0.11
DPA	66.5	50	55	6 – 229	32.5	16	32	6 – 72	0.51	0.10

FFQ: food frequency questionnaire; FR: food record; DHA: docosahexaenoic acid; SD: standard deviation.

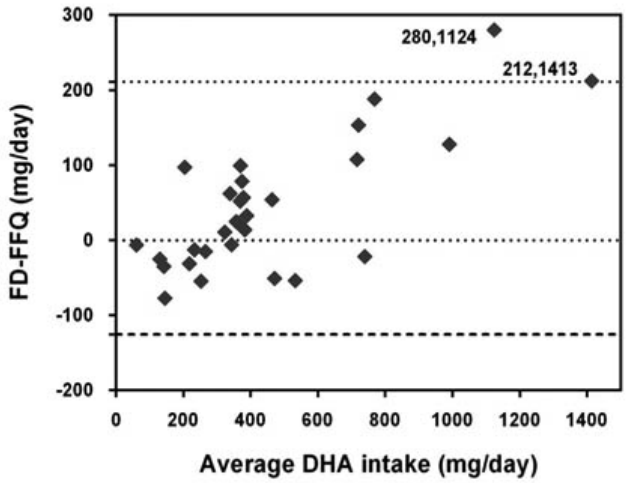

Figure 1. Agreement analysis between DHA intake estimated from FFQ plotted against the average DHA intake as assessed by the Bland-Altman technique

Table 3. Agreement analysis between FFQ and FR for DHA intake

Quartile	*Participants in the same quartile*		*Participants in the adjacent quartile*	
	N	*%*	*n*	*%*
25th quartile	6	85.7	1 (above)	14.3
50th quartile	4	50.0	4(1below, 3above)	50.0
75th quartile	5	62.5	3 (below)	37.5
100th quartile	7	100.0	0	00.0

Data is presented using quartile's cross-classification in number and percentage

Organization recommends 1.0-2.0% of energy intake for adults (WHO, 2003) while the National Coordinating Committee on Food and Nutrition suggests 0.3 - 1.2% of omega-3 fatty acids from 2000 kcal energy intake (NCCFN, 2005). Generally, the consumption of total omega-3 PUFAs is low among the Malaysian community with fish and seafood constituting the major proportion (Ng, 2006). Ho *et al.* (2011) reported that the average daily intake of the fish group, either ocean fish or freshwater fish, is 31 grams per day compared to the 70 grams per day recommended by the Ministry of Health (MOH, 2006).

Unadjusted correlation coefficients for DHA was 0.812(p<0.001), which suggests that the correlation within the two tools is relatively high. This indicates that the newly developed FFQ has high concurrent validity. Nevertheless, it should be noted that this correlation is not adjusted for confounding variables. After adjusting for confounders including household income, parental education level, and BMI of subjects using multiple regression R^2 for omega 3, a DHA of 0.73 was obtained.

The present results showed good correlation with the previous studies for DHA (r =0.870-0.875) intake (Woods *et*

al., 2002). On the other hand, weaker correlation (r = 0.290 - 0.450) has been shown for DHA intake in a study conducted by Wennberg, Vessby & Johansson (2009) among adults aged 30 to 60 years old. Wennberg *et al.*(2009) reported that the weaker value correlates with a high proportion of under-reporting with evidence that 42.7% and 49.5% (male and female respectively) under-reported based on the FFQ and 55.7% and 51.5% based on the 24-h diet recall.

Data from the present study have shown that the newly-developed FFQ was able to estimate DHA intake and its results are consistent with previous studies. Additionally, it is among the very few tools to specifically estimate the intake of omega-3 PUFAs among children, thereby bridging the research gap in the pool of currently available FFQ. Though this FFQ is an adequate dietary assessment tool for direct extrapolation to a younger population, it could be further developed with larger and more representative samples to confirm its use. Its reproducibility has to be further tested to reflect the consistency of the designed FFQ.

CONCLUSION

The present research explored the versatility of population-focused FFQ for estimating the dietary intakes of omega-3 PUFAs among children and capture sufficient dietary coding. The newly developed 30-item FFQ is valid for estimating intake of DHA specifically. However, further analyses validating regional variations and biological markers of PUFAs from a larger sample size should be considered in future studies.

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Appendix 1. DHA food frequency questionnaire: sample page for fish and other seafood categoies

Kumpulan makanan (food group)	*Saiz sajian (Serving Size)*			*Kekerapan makanan diambil (Frequency)*					
Ikan dan makanan laut	*Kecil (small)*	*Sederhana (medium)*	*Besar (large)*	*Lebih 1 kali/ sehari (once a day or more)*	*4 hingga 6 kali/* seminggu *(4-6 times a week)*	*1 hingga 3 kali/ seminggu (1 – 3 times a week)*	*2 hingga* 3 kali/ *sebulan (2 – 3 times a month)*	Kurang dari sekali/ sebulan *(once a month)*	Tidak pernah (never)
Ikanbilis (Anchovy)									
Ikan tilapia (African beam)									
Ketam (Crab)									
Ikanharuan (Snakehead fish)									
Belut (Eel)									
Ikansiakap (Sea bass)									
Ikansardin (sardine)									
Ikan tuna/tongkol (Tuna)									
Ikan salmon (salmon)									
Ikan jelawat (Sultan fish)									
Ikan tenggiri (Spanish mackerel)									