A Comparison of Omega-3 Fatty Acids Intakes from Three Dietary Screening Tools

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Abstract

Background: A newly developed omega-3 questionnaire (O3Q) designed to capture habitual intakes of omega-3 fatty acids was previously validated based on the whole blood levels of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and Omega-3 Index, but lacks comparison with existing "gold standard" dietary tools.

Objective: To compare the estimated habitual intakes of omega-3 fatty acids from three dietary assessment tools including O3Q and two other commonly used methods (24-hour recall and Diet History Questionnaire).

Methods: A correlational study of estimated omega-3 intakes from the O3Q, multiple 24-hour recalls, and Diet History Questionnaire collected from an observational study (n = 49) were compared to corresponding whole blood levels of EPA, DHA, and Omega-3 Index.The 49 participants included in this study completed the O3Q, Diet History Questionnaire, and at least one 24-hour recall during the observational study. Any incomplete data sets (missing one of the three kinds of dietary assessments) were excluded. Spearman's correlation evaluated the relationship between estimated omega-3 intake from each diet assessment method and biomarkers. Stepwise multiple linear regression examined associations of estimated dietary intakes of EPA+DHA from the three intake methods and the Omega-3 Index level.

Results: The estimated intakes from the O3Q had higher correlation coefficients with the corresponding blood biomarkers (EPA, rs=0.75; DHA, rs=0.74; Omega-3 Index, rs=0.77; $p<0.001$ for all) compared to the DHQ (EPA, rs=0.53; DHA, rs=0.41; Omega-3 Index, rs=0.45; $p<0.001$ for all) and 24

hr diet recall (EPA, rs=0.61; DHA, rs=0.45; Omega-3 Index, rs=0.55; $p<0.001$ for all). The regression analysis only demonstrated the O3Q as the significant dietary assessment predictor of the Omega 3-Index level ($\beta = 0.66$, $p<0.001$).

Conclusions: The O3Q is the preferred tool to evaluate habitual Omega-3 fatty acids intake and estimate Omega-3 index and outperforms using multiple 24-hour recalls or the Diet History Questionnaire for these specific dietary variables.

Keywords: habitual omega-3 fatty acids intake, omega-3 fatty acids questionnaire, 24 dietary recall, DHQ, diet assessments

1.INTRODUCTION

Two of the most common omega-3 polyunsaturated fatty acids, eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids, are specifically identified for their roles in protective cardiovascular effects.[1] These fatty acids are naturally found in fatty fish such as salmon, mackerel, and tuna. According to the American Heart Association, the general public should aim to consume two servings of fatty fish per week, equal to 0.5 grams per day, to reduce the risk of developing coronary heart disease.[2] Recommendations are increased to one gram per day for those diagnosed with coronary heart disease.[2]

Dietary consumption of EPA and DHA is positively associated with the percent of long chain fatty acids found in the erythrocytes, known as the Omega-3 Index.[3–5] The Omega-3 Index has been validated for use as a risk factor for cardiovascular health, specifically mortality risk from cardiac arrhythmias.[5] According to Harris,[6] the Omega-3 Index can be utilized as a risk factor, an indicator of intake of omega-3 fatty acids, and a goal for nutrition interventions related to coronary heart disease. Harris et al.[7] suggested an Omega-3 Index of 8% or greater is most correlated with a lower risk of developing coronary heart disease.

While blood biomarkers are the ideal measurements to be used in clinical studies, dietary assessments are also routinely used to as a convenient tool to evaluate determinants of health. Instruments such as the 24-hour diet recall and food-frequency questionnaires are widely used in research to estimate overall diet quality[8,9] and health status.[9,10] However, both tools often lack specific measurements on individual nutrients, such as omega-3 fatty acids. For example, Archer et al.[11] discovered that the 24-hour diet recall utilized during the NHANES produced significant under-reporting of American macronutrient intakes.The Diet History Questionnaire (DHQ) was originally developed by the Risk Factor Assessment Branch[12] within the National Cancer Institute's Division of Cancer Control and Population Sciences. DHQ II, U.S. Version was released in 2010 and its nutrient database is based on the NHANES results from 2001-2002, 2003-2004, and 2005-2006. The DHQ is considered an accurate way to measure regular macronutrient intakes in large populations.[13,14] Subar et al.[13] compared the DHQ to Block[15] and Willett[16] food frequency questionnaires and found the DHQ as having only a slight increase in correlation of polyunsaturated fatty acids compared to the two other questionnaires, but the correlation was still less than 0.6.

The O3Q was designed to assess the habitual intake of omega-3 fatty acids and has been validated based on blood indices.[17] However, the O3Q was never compared with other popularly used dietary analysis tools. Therefore, this study aimed to compare the previously validated O3Q[17] to the 24-hour diet recall and DHQ for correlations with corresponding omega-3 fatty acid biomarkers. One must note that this comparison is not entirely equivalent since the O3Q measures usual consumption, whereas the DHQ and 24-hour recall method episodic intakes. The O3Q is designed to be more representative of long-term intake, evaluated by the Omega-3 Index biomarker.

2.METHODS

Recruitment

Data was collected from an observational study conducted by the U.S. Environmental Protection Agency at the Human Studies Facility in Chapel Hill, North Carolina, known as PISCES (Clinicaltrials.gov registration number: NCT02921048).This study was designed to recruit participants with low (dietary intake of EPA and DHA less than 0.5 g per week and Omega-3 Index ≤ 4%) and high EPA and DHA intakes (dietary intake of EPA and DHA greater than 3 g per week and Omega-3 Index ≥ 5.5%).

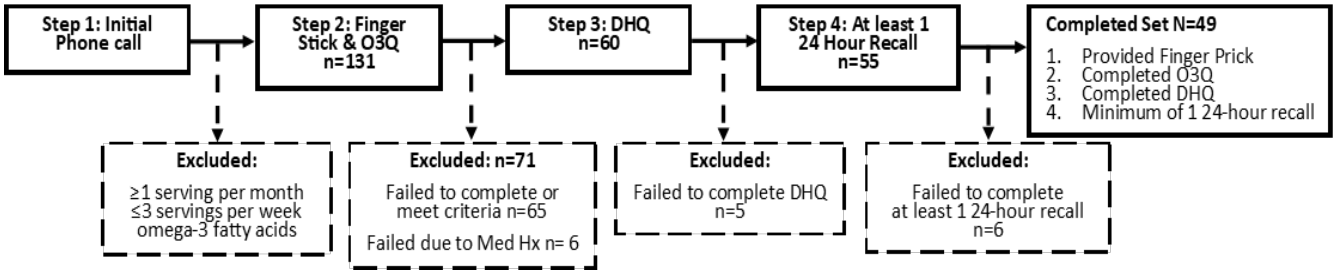
The 49 participants used in this study were recruited from the Research Triangle region in North Carolina from September 2016 to December 2019. At the initial study screening, participants were also asked about habitual intake of foods rich in omega-3 fatty acids, including seafood and fish oil supplements. Dry blood samples were collected using a finger prick for measuring Omega-3 Index. Healthy participants between 25 to 55 years of age with the Omega-3 Index of 4% or lower, or 5.5% or higher were invited to the main observation study which consisted of five visits. DHQ instructions were provided at the first visit and asked to be completed independently before the second visit. A 24-hour dietary recall was collected at each of the following visits (visit two to visit five, for a maximum of four 24-hour recalls). The process of data collection is shown in Figure 1.

The observational study was approved by the University of North Carolina at Chapel Hill Biomedical Institutional Review Board and the U.S. Environmental Protection Agency. All study participants provided informed consent and received monetary compensation for their participation. This study was deemed exempt by the Bowling Green State University Institutional Review Board.

Omega-3 Questionnaire

The O3Q, developed to estimate habitual dietary intakes of EPA and DHA, was previously published and validated based on blood indices.[17] Briefly, this assessment tool estimates the intake of omega-3 from seafood, fortified foods, and omega-3 supplements. Total EPA and DHA were

Figure 1. *Study design*



estimated based on serving sizes and dietary sources. Interviewers were either a dietitian or researcher, trained to probe intake questions and help participants identify portion sizes. Levels of EPA and DHA found in 29 seafood sources were individually calculated using the United States Department of Agriculture food products database.[18] EPA and DHA amounts for every source were calculated to estimate daily EPA, DHA, and EPA+DHA intakes. (EPA from source one added to EPA from source two equals total EPA. Total EPA plus total DHA equals total EPA+DHA).

Diet History Questionnaire

Participants were asked to complete the online DHQ to determine their nutrient intakes before coming to the second study visit. The DHQ is similar to a food frequency questionnaire, in which 142 questions probe participants to consider, within the past 12 months, how often they consumed certain types of food groups, (e.g., fruit, vegetables, grains), specific types of foods (e.g., broccoli, tangerines, tuna), and how it was usually cooked (e.g., breaded, fried, canned). A dietitian provided images of food samples to participants for more accurate and consistent portion size estimates, however, the questionnaire is semi-quantitative. The DHQ asked specifically about tuna, shellfish, fish sticks, and salmon consumption. Supplement usage was considered, however specific intake or EPA and DHA amounts were not recorded. Intake levels of semi qualitative DHQ data were inputted into Diet*Calc (version 1.5.0 released October 2012)[19] to calculate average past year nutrient intakes.

24-Hour Recall

One 24-hour recall was conducted during each of the following study visits, for a maximum total of four dietary recalls. Participants were asked to list everything they consumed including food, liquid, and supplements. Brand names and portion sizes consumed were collected. Information from each 24-hour recall was entered into Nutritionist Pro[20] (version 6.2) to calculate a nutrient analysis. Completed 24-hour recalls were averaged for a daily estimate of each participant's usual nutrient intake. The number of 24-hour recalls obtained per each participant depended on how many study visits they completed (minimum of one, maximum of four). Each participant completed an average of 3.77± 0.62 24-hour recalls.

Blood Sampling

During the Omega-3 Index screening, blood samples were collected from participants via a finger prick to test their omega-3 blood indices. The blood levels of omega-3 were tested with an Omega-3 Index measurement toolkit purchased from OmegaQuant Inc.[21]

Statistical Methods

Descriptive statistics were performed to summarize participant demographics. The Omega-3 Index was compared to calculated dietary EPA + DHA (g/day) from each dietary intake method. Spearman's correlation test was used to analyze the relationship between the dietary estimated intakes and the corresponding blood markers. Further, Williams' Test was used to test the equality of two dependent correlations.[22] Stepwise multiple linear regression

was calculated to evaluate the relationship between estimated dietary intakes of EPA+DHA from the three intake methods and the Omega-3 Index levels. Covariates considered in the regression included: gender, race, age, body mass index (BMI), marital status, and highest education level. Spearman's correlation and multiple linear regression were computed using SPSS Version 26.0 (2-sided, $p < .05$ was considered significant). Williams' Test was performed using the "psych" package in R version 4.1.0. Collinearity was assessed using the variance inflation factor (VIF); VIF values were acceptable (less than 1.5 in the final model).

3.RESULTS

The final sample consisted of 49 participants, 18 males and 31 females with a mean age of 37.8 years (± 9.1 years). The average BMI was 24.5. Thirty-three of 49 of the sample identified as white, non-Hispanic (67%) and 46 of 49 (94%) participants stated as having either a college or graduate level of education (Table 1).

Table 1 *Anthropometrics and demographic characteristics from the sample (n=49)*

Anthropometrics	Mean ± SD
Age (years)	37.8 ± 9.1
Height (cm)	169.10 ± 8.6
Weight (kg)	70.20 ± 12.0
BMI (kg/m^2)	24.5 ± 3.4
Demographics	**n (%)**
Sex	
Male	18 (37%)
Female	31 (63%)
Race/ Ethnicity	
Non-Hispanic White	33 (67%)
African American	10 (20%)
Asian	4 (8%)
White/ Hispanic	2 (5%)
Education level	
High School	1 (2%)
Trade School	2 (4%)
College	26 (53%)
Graduate School	20 (41%)
Marital Status	
Single	21 (43%)
Married	24 (49%)
Divorced/ separated	4 (8%)

While the bivariate correlation analysis (Table 2) illustrates that all dietary assessment methods were significantly associated with whole blood biomarkers, the O3Q derived dietary intakes of EPA+DHA, EPA, and DHA were strongly correlated with corresponding blood markers. The estimated intake values from the DHQ had the weakest correlation with corresponding biomarkers. Additionally, for the Omega-3 Index, Williams' Tests showed that the correlation between O3Q and blood marker is significantly higher than the correlations between 24-hr recall and blood marker, as well as between DHQ and blood marker, t=2.94, $p<.01$ and t=3.33, $p<.01$, respectively. Similarly, for the EPA, the correlation between O3Q and blood marker is significantly higher than the correlation between DHQ and blood marker, t=2.08, $p<.05$. Lastly, for the DHA, the correlation between O3Q and blood marker is significantly higher than the correlations

Table 2 *Spearman Correlations among omega-3 fatty acids estimated intakes from three dietary screening tools and whole blood biomarker levels (n=49)*[a]

Biomarkers	Dietary Assessment Tools		
	O3Q[b]	24-Hour Recall	DHQ[c]
EPA[d]	0.75***	0.61***	0.53***
DHA[e]	0.74***	0.45***	0.41***
EPA+DHA	0.77***	0.55***	0.45***

***=$p<0.001$

a=The sample size for individual measurements of EPA and DHA is n = 45. This is because the supplement information from 4 participants did not provide individual EPA and DHA amounts. Therefore, information from these 4 participants was excluded from the individual EPA and DHA analyses. The sample size for EPA and DHA combined (EPA +DHA) is n = 49.

b=Omega-3 Questionnaire

c=Diet History Questionnaire

d=Eicosapentaenoic acid

e=Docosahexaenoic acid

between 24-hr recall and blood marker, as well as between DHQ and blood marker, t=3.35, $p<.001$ and t=3.18, $p<.001$, respectively (Table 3).

The results from the stepwise multiple linear regression are presented in Table 4. Three significant variables, O3Q (β=0.66, $p<0.001$), education level (β=0.35, p=0.001), and age (β =0.22, p=0.024) were retained in the final model $F(3, 44) = 23.07$, $p<0.001$. When the diet assessment variables were entered into the model, O3Q was the only method retained and the coefficient of determination (r^2) increased from 0.184 to 0.611; meaning that 42.7% of the variance in Omega-3 Index can be attributed from the estimated intake of EPA+DHA from the O3Q alone.The regression was able to discriminate among the three dietary assessment tools.

Table 3 *Comparisons of correlation coefficients between the Omega-3 Questionnaire, 24-hour recall, and Diet History Questionnaire*

	t	*p*
Total Omega-3		
O3Q[a] vs. 24HR[b]	0.82	0.42
O3Q vs. DHQ	2.94	0.0051**
24HR vs. DHQ[c]	3.33	0.0017**
EPA		
O3Q vs. 24HR	1.81	0.078
O3Q vs. DHQ	2.08	0.0044**
24HR vs. DHQ	0.69	0.49
DHA		
O3Q vs. 24HR	3.35	0.0017**
O3Q vs. DHQ	3.18	0.0028**
24HR vs. DHQ	0.21	0.83

**=$p<0.01$

a= Omega-3 Questionnaire
b=24-Hour Recall
c=Diet History Questionnaire

Table 4 *Linear regression results for demographics, estimated intakes of EPA+DHA and blood Omega-3 Index*

Characteristics	β	*p*	*t*	95% CI
O3Q	0.66	<0.001***	6.95	1.06-1.92
Education	0.38	0.001**	3.69	0.39-1.34
Age	0.22	0.024*	2.34	0.01-0.07

[a]R^2=61.1%
CI= Confidence Interval
*Statistically significant $p<0.05$
**Statistically significant $p<0.01$
***Statistically significant $p<0.001$

4.DISCUSSION

The estimated dietary intakes of omega-3 fatty acids from the O3Q were consistently correlated to corresponding biomarkers (whole blood EPA, DHA, and omega-3 indices), whereas the estimated intakes from the DHQ had lower levels of association to the three biomarkers. The estimated intakes of EPA, DHA, and EPA+DHA

derived from the O3Q had a higher level of association with whole blood EPA, DHA, and Omega-3 Index, opposed to the DHQ (rs=0.53, 0.41, 0.45, $p<0.001$ for all, respectively) and 24-hour recall (rs=0.61, 0.45, 0.55, $p<0.001$ for all, respectively). The regression results reiterated the association of the O3Q with the Omega 3-Index. These findings indicate the O3Q is the best habitual predictor of the omega-3 index compared to the DHQ and 24-hour recall assessments.

It is important to note that although the bivariate tests demonstrated association between the DHQ or 24-hour recall with the biomarkers, neither the DHQ nor 24-hour recall method were retained in the regression analysis. These methods are regularly preferred for identifying nutrient intakes and relating them to disease risks. As previously mentioned, omega-3 fatty acids are stored in the body, therefore an assessment that focuses on habitual intake is preferred. The DHQ and 24-hour recalls do not consider the length of intake of omega-3 sources. While all three methods are relatively quick to complete, the 24-hour intake method relies more heavily on participant memory and single days may not be representative of a normal diet, especially over time.[23] Similar to food frequency questionnaires, the DHQ would appear to consider more regular intake of specific nutrients, however studies have evidenced inaccurate reporting from participants[11] and underrepresentation of energy intake.[23] Further studies evaluated the accuracy of food frequency questionnaires but few have compared the estimated nutrient intake to their corresponding biomarkers.[13,23] In addition, there were only a few questions that targeted omega-3 fatty acid intake within the DHQ. The O3Q, however, does account for the length of intake (suggesting habitual consumption) which could more accurately represent the red blood cell concentration of omega-3 polyunsaturated fatty acids. It is possible that these differences may have led to the observations described in this study. While these findings are supportive of using the O3Q over episodic dietary tools in relation to identifying omega-3 biomarkers, further studies to evaluate the validation of the tool in a larger, more diverse participant pool would be necessary.

There are several limitations on this study. First, the sampling frame used for this study does not represent the population as a whole, as the majority of the participants were white, non-Hispanic women with a mean age of 37.8 years. The study does not include population with omega-3 intakes between 0.5 grams per week and 3 grams per week, or Omega-3 Index between 4% and 5.5% based on the study design of the observational study, which needs to be addressed in follow up studies. Results of the NHANES 2003-2014 survey suggests the average EPA+DHA intake in the United States (survey included Mexican Americans, Non-Hispanic Whites, Other Hispanic, Non-Hispanic Black, and races listed as other) following a 2,000 calorie diet is about 0.52 grams per day or 3.63 grams per week.[24] However, Mexican Americans, Non-Hispanic Whites, Other Hispanic, and Non-Hispanic Black participant intakes were significantly lower than those participants who identified under the Other races category. Secondly, while the total sample of this

study included 49 participants' dietary intakes, four participants' estimates were not able to be calculated for total EPA or DHA with the O3Q. This occurred because the supplements provided in the participants' 24-hour recalls did not distinguish individual values for EPA or DHA. Total EPA+DHA was however provided by the supplement label, allowing for an estimate of total EPA+DHA to be calculated for those four participants. Further research could expand on the comparisons noted in this study to include a larger sample size with more diversity.

The estimated omega-3 fatty acids intake from the O3Q, multiple 24-hour recalls, and DHQ were all associated with corresponding omega-3 fatty acids biomarkers. Compared to the DHQ and multiple 24-hour recalls, the O3Q had higher levels of association with corresponding whole blood EPA, DHA, and Omega-3 Index. This screening tool could provide better insight to an individual's omega-3 fatty acid intake. Precise estimates can allow clinicians to identify risks of cardiovascular disease and implement more appropriate dietary interventions. Applications for future use can include instances where blood tests may not be readily available, such as medical screens, clinical trials, and community health clinics.

5.CONCLUSION

In this study, estimated omega-3 fatty acids intakes from 49 healthy individuals were compared among three diet assessment instruments. The estimated intake of EPA, DHA, and EPA+DHA from the newly developed omega-3 questionnaire was better associated with the corresponding biomarkers as compared to other tools including 24-hour diet recalls and DHQ.

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AUTHOR CONTRIBUTIONS

ML, HT, and WS designed the study. HT, HC, and WS collected the data. ML, KLK and WS analyzed the data. ML wrote the first draft with contributions from WS and KLK. All authors reviewed and commented on subsequent drafts of the manuscript.