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Comparison between omega-3 and omega-6 polyunsaturated fatty acid intakes as assessed by a food frequency questionnaire and erythrocyte membrane fatty acid composition in young children

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Abstract

Objective—We conducted a dietary validation study in youth aged 1 to 11 years by comparing dietary intake of omega-3 and omega-6 polyunsaturated fatty acids (PUFA) as assessed by a parent-completed semi-quantitative food frequency questionnaire (FFQ) over time to erythrocyte membrane composition of the same fatty acids.

Design—The study population included youth aged 1 to 11 years who were participants in the Diabetes Autoimmunity Study in the Young (DAISY), a longitudinal study in Denver, Colorado that is following a cohort of youth at risk for developing Type I diabetes. Four hundred four children who had erythrocyte membrane fatty acid data matched to an FFQ corresponding to the same time frame for a total of 917 visits (matches) were included. PUFA intake was expressed as both g/day (adjusted for total energy) and as percent of total fat intake. We used mixed models to test the association and calculate the correlation between the erythrocyte membrane estimates and PUFA intake using all records of data for each youth.

Results—Intakes of total omega-3 fatty acids (β=0.52, $p<0.0001$, ρ=0.23) and marine PUFAs (β=1.62, $p<0.0001$, ρ=0.42), as a percent of total fat in the diet, were associated with percent of omega-3 and marine PUFAs in the erythrocyte membrane. Intakes of omega-6 PUFAs (β=0.04, p=0.418, ρ=0.05) and arachidonic acid (β=0.31, p=0.774, ρ=0.01) were not associated.

Conclusions—In these young children, a FFQ using parental report provided estimates of average long-term intakes of marine PUFAs that correlated well with their erythrocyte cell membrane fatty acid status.

Introduction

Omega-6 and omega-3 fatty acids, two families of polyunsaturated fatty acids (PUFAs), are necessary for complete health. Dietary intake of these PUFAs is crucial because they cannot be produced endogenously by humans (Bezard *et al.*, 1994; Arab, 2003), Linoleic acid (LA; 18:2n-6) and alpha-linolenic acid (ALA; 18:3n-3) are the parent fatty acids of the omega-6 and omega-3 families, respectively. Arachidonic acid (AA; 20:4n-6) and docosahexaenoic acid (DHA; 22:6n-3) are considered the most important derivatives of LA and ALA, respectively,

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and play important roles in neural functioning (Innis, 1991; Youdim *et al.*, 2000). Children with type 1 diabetes have reduced availability of long-chain PUFAs (Decsi *et al.*, 2002), suggesting that an enhanced dietary supply of long-chain PUFAs may be beneficial. Finally, cod liver oil intake is associated with lower risk of type 1 diabetes (Stene *et al.*, 2003), suggesting that either the marine-derived PUFAS (e.g. DHA and eicosapentaenoic acid (EPA; 20:5n-3)), or the vitamin D that is in cod liver oil, or both, may be protective. Accurate measures of PUFA intake in children are necessary to further explore these hypotheses.

Intake of PUFAs can be measured through diet surveys, such as food frequency questionnaires (FFQs) and diet records; however, the ability of these self-reported data to adequately measure PUFA intake has been questioned. Both observational studies (Feunekes *et al.*, 1993; Bingham *et al.*, 1997; Arab, 2003) and clinical trials (Glatz *et al.*, 1989; Poppitt *et al.*, 2005) have shown that fatty acid levels in the body are known to change as a result of changes in dietary intake of fatty acids. Such results support the use of biomarkers such as erythrocyte membrane and adipose tissue to estimate dietary fatty acid intake. The fatty acid composition of erythrocyte membranes has been shown to be a good indicator of fatty acid intake over a 4–6 week period in infants (Baur *et al.*, 2000).

Several studies in adults have compared fatty acid intake as determined by dietary survey with that of a biomarker, such as fatty acid content of the erythrocyte membrane (Feunekes *et al.*, 1993; Romon *et al.*, 1995; Parra *et al.*, 2002), of serum phospholipids (Kobayashi *et al.*, 2003), of platelet phospholipids (Li *et al.*, 2001) or of adipose tissue (Hunter *et al.*, 1992; Feunekes *et al.*, 1993; Tjonneland *et al.*, 1993; Lemaitre *et al.*, 1998; Knutsen *et al.*, 2003; Cantwell *et al.*, 2005). In general, significant correlations were found between PUFA intake and these biomarkers. However, no studies to date have examined the ability of an FFQ to measure PUFA intake in children after infancy by comparing it with the aforementioned biomarkers. Unique issues regarding the measurement of childhood diet include the need to use parental report to complete the FFQ, and the rapidly changing diet of a child. Using estimates of fatty acid composition of erythrocyte membranes as the “gold standard”, we conducted a dietary validation study by comparing omega-3 and omega-6 fatty acid intake as assessed by a parent completed FFQ over time to biomarker data assessing the same.

Materials and Methods

Study Population

The sample for this study was drawn from participants in the Diabetes Autoimmunity Study (DAISY), a longitudinal study in Denver, Colorado that is following over 2400 children at risk for developing Type I diabetes (Rewers *et al.*, 1996; Norris *et al.*, 2003). These youth were identified as at-risk and recruited into DAISY in two ways: (1) through a program that screened newborns at St. Joseph’s Hospital in Denver, Colorado by testing their umbilical cord blood for diabetes-susceptibility alleles, and (2) by identifying children who had a first-degree relative with type 1 diabetes through one of the following sources: the Colorado type 1 diabetes registry; the Barbara Davis Center in Denver, Colorado; the Children’s Hospital of Denver, Colorado; or through media publicity. The St. Josephs Hospital newborn population is representative of the general population of the Denver Metropolitan Area. Eighty-six percent of the families approached gave informed consent to the genetic screening. HLA screening has been completed on over 31,000 newborns; and the details of the newborn screening have been published elsewhere (Rewers *et al.*, 1996). The parents of all DAISY participants have been made aware of their child’s increased risk for type 1 diabetes.

As part of DAISY, food frequency questionnaires (FFQs) are used to collect the dietary intake of each participant annually for as long as the children participate in DAISY. In a sample of these participants, we also collect erythrocytes annually for the measurement of cell membrane

fatty acid content. The intent was that each FFQ would be linked with an erythrocyte sample that was drawn during the year for which the FFQ reported dietary intake. A total of 404 youth aged 1 to 11 years who had erythrocyte membrane fatty acid data linked with dietary intake data in this way were included in this study.

Food Frequency Questionnaire

This study used a semi-quantitative FFQ developed by Willett (1998). This FFQ was designed based on American diets and may not be valid in other countries. Although developed for use with adults, it has been minimally altered for and validated in samples of children (Stein *et al.*, 1992; Parrish *et al.*, 2003). The caregivers of all participants were sent the 111-item FFQ asking them to record their child's diet in the previous year. To calculate intakes of nutrients, a commonly used unit or portion size for each food (eg, one egg or one slice of bread) was specified and the participants were asked how often on average during the previous year they had consumed that amount. Nine responses were possible, ranging from “never” to “>= 6 times /day”. The FFQ inquired specifically about the kind of fat usually used for frying, sautéing, and baking (vegetable oil, solid vegetable oil shortening, butter, margarine, lard or none). The questionnaire asked about the frequency of intake of canned tuna, dark-meat fish (mackerel, salmon, sardines, bluefish, and swordfish), other fish (not specified), and shrimp, lobster, and scallops.

FFQ forms were sent to the Channing Laboratory for scanning and analysis. Fatty acid intakes were computed for each child by multiplying the frequency of consumption of each food by the fatty acid composition for the portion size specified. Composition values for fatty acids and other nutrients are based primarily on the US Department of Agriculture data (Agricultural Research Service, 1995) and supplemented by manufacturer information. The calculation of EPA and DHA intake was described in detail elsewhere (Iso *et al.*, 2001).

We previously evaluated the validity of the food-frequency questionnaire in children by parental report by comparing it with nutrient intakes from four 24-hour recalls throughout the year in 68 DAISY children ages 1–3 years. The correlation between energy-adjusted intake of fat measured by 24 hour recalls and by food-frequency questionnaire was 0.39 ($p < 0.05$) (Parrish *et al.*, 2003). Comparisons of PUFA intake were not done in this study.

Erythrocyte Membrane Fatty Acid Composition

Starting in 2000, at each DAISY clinic visit, blood samples were obtained, from which erythrocytes were separated within 30 minutes of blood draw, flash frozen in liquid nitrogen and stored at −70°C until shipment to the University of Florida laboratories of M. Clare-Salzler and N.J. Szabo. Samples of erythrocytes were extracted for lipids following the method developed by Bligh and Dyer (Bligh *et al.*, 1959), and stored at −20° C. The fatty acids present in the lipid isolates were subsequently methylated using the base-catalyzed procedures by Maxwell and Marmer (Maxwell *et al.*, 1983) in preparation for analysis by gas chromatography (Hewlett-Packard 6890) with mass spectral detection (Hewlett-Packard 5973). The samples, separated across a CP-WAX column (Varian, 25 m × 0.25 mm i.d., 0.2 μm film), were identified by comparing the retention times and *m/z* of selected ions from analytes in the samples to those of authentic standards (NuCheckPrep, Supelco). Quantitation was determined against five-point standard curves and reported as a g/ 100 g RBC lipid.

Analyses

This study included 404 youth aged 1 to 11 years (mean age = 5.07, SD = 2.36). The number of visits for each youth ranged from 1 to 6 (mean number of visits = 2.3, median = 2.0), resulting in a total of 917 visits for which biomarker and corresponding FFQ data were available. The analytic dataset was therefore longitudinal with multiple records per participant. Nearly three-

quarters (74.3%) of the youth were non-Hispanic White and slightly more than half (51.7%) were male. During the course of this validation study, 72 children became positive for a diabetes-related autoantibody and 23 of the children developed type 1 diabetes.

Both FFQ and red blood cells provided estimates of the following fatty acids: 18:2n-6 (linoleic acid (LA)), 20:4n-6 (arachidonic acid (AA)), 18:3n-6 (γ-linolenic acid (γ-LA)), 18:3n-3 (α-linolenic acid (α -LA)), 20:5n-3 (eicosapentaenoic acid (EPA), 22:6n-3 (docosahexaenoic acid (DHA)), and 22:5n-3 (docosapentaenoic acid (DPA)). EPA and DHA were combined to estimate total marine PUFA (aka, fish oil PUFA); α-LA, DHA, EPA and DPA were combined to estimate total omega-3 fatty acid intake; and LA, AA and γ-LA were combined to estimate total omega-6 fatty acid intake. While measures of erythrocyte membrane fatty acids were expressed as a percentage of total RBC lipid (g fatty acid/100 g total RBC lipid), intake estimates from the FFQs were expressed both in percent of total fat, and in grams per day. The FFQ variables expressed in grams per day were energy-adjusted using the residual method (Willett *et al.*, 1997; Willett, 1998).

In order to examine the association between the percent of fatty acid in the erythrocyte membrane and the FFQ estimate of intake of the same fatty acid, mixed models were developed with the erythrocyte membrane estimates as the dependent variables and the energy-adjusted FFQ estimates as the independent variables. These mixed models included a random subject effect in order to account for the correlation between measurements on the same youth and were adjusted for age (in years, at time of clinic visit), gender and ethnicity (non-Hispanic White versus others). Mixed models including both within and between subject correlations were also used to estimate Pearson correlation coefficients between the FFQ and erythrocyte membrane variables utilizing all records (Lam *et al.*, 1999). The variation within person depended on the measure of interest. The largest within person variation was seen for erythrocyte membrane omega-6 (average variance over all subjects was 1.9 and ranged from 0.02 to 7.6). The smallest within person variation was seen for erythrocyte membrane marine fatty acids, with an average variance over all subjects of 0.30 and range of 0.003 to 1.52. Overall, the magnitude of the variation within subjects was not large. All analyses were done using SAS 9.1 (SAS Institute Inc., 2004). Although the distributions of the measurements from both erythrocyte membranes and the FFQ were skewed, log transformation did not help normalize the distributions. Given the large sample size, non-transformed values were used in all analyses.

Results

The mean intake and erythrocyte fatty acid composition of each PUFA are presented for the sample in Table 1. For those participants with multiple records of data available, only one record was chosen randomly, so that each participant contributed only one data point to the means presented.

Results of the mixed models are presented in Table 2. Energy-adjusted intakes of omega-3 fatty acids, and in particular, marine PUFA were significantly and positively associated with percent of omega-3 fatty acids and marine PUFA, respectively, in the erythrocyte membrane. This was also seen when omega-3 fatty acid and marine PUFA intakes were expressed as a percent of total fat. Energy-adjusted FFQ estimates of total omega-6 PUFA estimates were also positively and significantly associated with the erythrocyte membrane omega-6 fatty acid content. This was not seen when omega-6 fatty acid intake was expressed as a percent of total fat. Correlations reported in Table 2 were consistent with these mixed model results. Correlations between either measurement of FFQ and erythrocyte membrane composition was higher for total omega-3 and marine PUFAs.

We stratified our cohort into two age groups (age 1–5 years and 6–11 years) to examine whether agreement the FFQ estimates and the biomarker differed by age (Table 2). While agreement appeared to be slightly stronger in the 6–11 year olds, significant associations were seen in both age groups for omega-3 and marine PUFAs in particular.

Discussion and Conclusions

While several studies have examined the validity of using an FFQ to estimate dietary PUFA intake by comparing the FFQ to biomarkers such as adipose tissue and erythrocyte membranes in adults, no studies have considered a population of children where data are collected via FFQ using parental report. It is useful to examine the utility of an FFQ in children regarding this question because they may have low or infrequent intakes of fish, in particular, which may not be adequately picked up by 24-hour recall or diet record that are collected only periodically for a small number of days.

We compared intake of fatty acids as measured by multiple FFQ with fatty acid content of erythrocyte membranes over the same time periods for a cohort of healthy children aged 1 to 11 years, at increased risk for type 1 diabetes. Intakes of omega-3 fatty acids and, in particular, marine PUFAs were strongly associated with levels of these PUFAs in the erythrocyte membranes.

Our results are consistent with other studies that compared PUFA intake as assessed by an FFQ to biomarkers among adults. One such study compared fatty acid composition of adipose tissue to dietary intake (as a percentage of total fat) as estimated from an FFQ for 86 adults and reported correlations of 0.42 for DHA and 0.55 for EPA (Tjonneland *et al.*, 1993). Similar to our study, they found that non-marine omega-3 fatty acids and omega-6 fatty acids were less correlated. Fish intake, as assessed by FFQ, was significantly associated with EPA and DHA in platelet phospholipids in adult males (Li *et al.*, 2001). High correlations were found in a study comparing intake of PUFAs (as a percent of total fat) assessed by an FFQ to serum phospholipids levels in adults in a high fish-consuming population, for EPA ($\rho = 0.59$) and DHA ($\rho = 0.49$) (Kobayashi *et al.*, 2003).

These results all suggest that the Willett FFQ is good at picking up omega-3 fatty acids, especially marine PUFAs. Our Pearson correlation estimates indicated that the FFQ is not as good at detecting omega-6 fatty acids, and in particular arachidonic acid. One possible explanation is that there may be unknown upper limits to omega-3 and omega-6 fatty acid levels in erythrocyte membranes. Such an upper limit could result in a lower membrane fatty acid level than expected given fatty acid exposure; thereby underestimating the correlation of the membrane content with levels measured using FFQ.

Another possible explanation is that the FFQ is adequately measuring omega-6 fatty acid intake, but that this intake would not be reflected in the erythrocyte membrane content because of the preferential incorporation of omega-3 fatty acids into the membranes (Willett, 1998). Membrane fatty acid composition is determined by the interplay between available fatty acids from a dietary source and further metabolism of these fatty acids, such as the competition between n-6 and n-3 fatty acids. To look at this issue, we estimated Pearson correlation using the mixed model methods described above for omega-6 total fatty acid by quartiles of marine fatty acid (lower two quartiles versus upper two quartiles) and found that the correlation between intake and membrane levels was higher among the lower two marine PUFA quartiles ($\rho = 0.26$) than among the highest two quartiles ($\rho = 0.07$), suggesting that this may be the case. Romon et al. (1995) found a negative correlation between fish intake and RBC arachidonic acid. In experimental studies, increased n-3 fatty acid intake decreased arachidonic acid content of membrane phospholipids (Wander *et al.*, 1991).

We observed slightly stronger correlations between erythrocyte membrane fatty acid content and intake in 6–11 year old children compared with 1–5 year olds. This may reflect a better ability of the FFQ to record more adult-like diets compared with early childhood diets. It is not known whether there could also be a biological reason for the differences in correlation by age group

This study fills a gap in the literature by validating the FFQ among a cohort of children. Also, the methods used strengthen the conclusions because multiple measures were used for each child while accounting for within subject variation over time. One limitation of this study was that the cohort included children who were all at higher risk for type I diabetes compared to the general population. These children and their families all knew they were at higher risk for diabetes and therefore, their diets may not have been representative of children the same age in the general population. However, very few of these children were autoimmune (n=72), of whom 23 went on to develop diabetes over the course of the study. Moreover, when these children were removed from the analyses, we saw similar results as those presented herein (data not shown).

In conclusion, our validation study finds that the FFQ performs well in measuring intake of marine PUFAs among children.

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Table 1

Mean (SD) FFQ intake and erythrocyte membrane fatty acid composition of sample (404 children)

	Mean	SD
FFQ (grams per day):		
Omega-6 fatty acids [1]	10.61	4.77
Arachidonic acid (20:4n-6)	0.14	0.07
Omega-3 fatty acids [2]	1.18	0.58
Total marine PUFA [3]	0.15	0.15
FFQ (% total fat):		
Omega-6 fatty acids [1]	13.29	2.97
Arachidonic acid (20:4n-6)	0.17	0.07
Omega-3 fatty acids [2]	1.48	0.45
Total marine PUFA [3]	0.19	0.19
Erythrocyte membrane (% total lipids):		
Omega-6 fatty acids [1]	24.93	4.39
Arachidonic acid (20:4n-6)	11.12	2.23
Omega-3 fatty acids [2]	3.81	1.32
Total marine PUFA [3]	2.34	0.89

Abbreviations:
FFQ = food frequency questionnaire

[1]Total omega-6 fatty acid = 18:2n-6, 20:4n-6 and 18:3w6

[2]Total omega-3 fatty acid = 18:3n-3, 20:5n-3, 22:6n-3, 22:5n-3

[3]Total marine PUFA = 20:5n-3 and 22:6n-3

Table 2

Linear mixed models predicting fatty acid content of erythrocyte membranes with FFQ estimates (n=404 children with 917 visits)[1]

	All ages (0 – 11 years)				Age 0 – 5 years				Age 6 – 11 years			
	β	(95% CI)	p-value	r^2 [5]	β	(95% CI)	p-value	r^2 [5]	β	(95% CI)	p-value	r^2 [5]
Energy-adjusted FFQ:												
Omega-6 fatty acids [2]	0.27	(0.12, 0.41)	0.0004	0.16	0.20	(−0.04, 0.45)	0.099	0.07	0.33	(0.14, 0.51)	0.0005	0.23
Arachidonic acid (20:4n-6)	2.32	(−0.58, 5.22)	0.117	0.07	1.96	(−3.17, 7.09)	0.451	0.07	2.70	(−0.73, 6.13)	0.122	0.02
Omega-3 fatty acids [3]	0.60	(0.24, 0.95)	0.0011	0.25	0.70	(0.19, 1.21)	0.0075	0.05	0.53	(0.02, 1.03)	0.042	0.10
Total marine PUFA [4]	1.67	(1.14, 1.93)	<0.0001	0.38	2.35	(1.50, 3.21)	<0.0001	0.21	1.62	(0.87, 1.89)	<0.0001	0.22
Percent total fat FFQ:												
Omega-6 fatty acids [2]	0.04	(−0.06, 0.14)	0.418	0.05	−0.01	(−0.15, 0.14)	0.942	−0.02	0.10	(−0.03, 0.23)	0.141	0.17
Arachidonic acid (20:4n-6)	0.31	(−1.81, 2.44)	0.774	0.01	0.17	(−3.01, 3.34)	0.918	−0.03	0.73	(−2.16, 3.61)	0.619	−0.08
Omega-3 fatty acids [3]	0.52	(0.35, 0.69)	<0.0001	0.23	0.39	(0.18, 0.60)	0.0004	0.09	0.75	(0.48, 1.02)	<0.0001	0.26
Total marine PUFA [4]	1.62	(1.33, 1.90)	<0.0001	0.42	1.30	(0.91, 1.69)	<0.0001	0.27	2.15	(1.75, 2.56)	<0.0001	0.41

[1] Adjusting for age, gender and race/ethnicity (NHW vs other).

[2] Total omega-6 fatty acid = 18:2n-6, 20:4n-6 and 18:3w6

[3] Total omega-3 fatty acid = 18:3n-3, 20:5n-3, 22:6n-3, 22:5n-3

[4] Total marine PUFA = 20:5n-3 and 22:6n-3

[5] Estimated from mixed model using all records