

# Direct Diet Quantification Indicates Low Intakes of (n-3) Fatty Acids in Children 4 to 8 Years Old<sup>1,2</sup>

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## Abstract

Estimates of essential fatty acid intakes, including (n-3) PUFA, are available in pediatric populations based on limited indirect approaches. Furthermore, recommended intakes for short- and long-chain (LC) (n-3) PUFA have emerged for this population. This study provides direct quantification of fatty acid intakes in children aged 4–8 y. Identical portions of all food and natural health products consumed over 3 d were collected. Duplicate samples were analyzed for energy, macronutrients, and fatty acids, including  $\alpha$ -linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) by high performance capillary GLC. The results for 41 children [25 females, 16 males; 5.8  $\pm$  0.2 y (mean age  $\pm$  SEM)] showed daily energy intakes of 5879  $\pm$  211 kJ (mean  $\pm$  SEM) and (n-3) PUFA intakes in mg/d as follows: ALA, 1161  $\pm$  108; EPA, 38.4  $\pm$  9.3; DPA, 26.3  $\pm$  3.9; and DHA, 54.1  $\pm$  11.4. Based on the Dietary Reference Intakes from the Institute of Medicine, 61% of the children met the adequate intake for ALA and 22% met the suggested adequate intake for DHA+EPA (10% of the adequate intake for ALA). These intakes were also compared with the recent Australia/New Zealand recommendations for children, where only 51% met the recommended intake for EPA+DPA+DHA. These results demonstrate a moderate shortfall in ALA intake in Canadian children and a nutrient gap for the LC (n-3) PUFA, including DHA, when comparing intakes for this population to suggested and recommended intakes. *J. Nutr.* 139: 528–532, 2009.

## Introduction

The scientific literature has provided substantial evidence demonstrating that (n-3) PUFA are beneficial with respect to the maintenance, prevention, and treatment of a wide range of health conditions, including mental and visual health, cardiovascular disease, and inflammatory and other chronic disorders (1,2). Furthermore, crucial structure-function roles for long-chain (LC)<sup>3</sup> (n-3) PUFA, specifically docosahexaenoic acid [DHA; 22:6 (n-3)], in fetal and infant development have been documented (3–5). DHA accrues at a high rate during development in the nervous tissues, particularly the brain and retina, until its accretion plateaus around 2–3 y of age (6). This has resulted in commercial infant formulas being supplemented with DHA over the past few years, in addition to the  $\alpha$ -linolenic acid [ALA; 18:3 (n-3)] present as vegetable oils, as recently recommended by the American Dietetic Association (ADA) and

Dietitians of Canada (DC) (7). It has been estimated that the human brain turns over DHA at a rate of  $\sim$ 4 mg/d (8); whole body rates of DHA loss would be expectedly higher due to the wide presence of DHA in the membranes of all cells and tissues. A recent review indicated promising but not yet conclusive evidence (9) for dietary LC (n-3) PUFA in support of visual and cognitive performance in healthy children older than 2 y of age. Recent studies have supported considerable beneficial effects when children with chronic disorders, including developmental coordination disorder (10) and depression (11), were supplemented with DHA plus eicosapentaenoic acid [EPA; 20:5 (n-3)].

Recommended intakes for ALA and LC (n-3) PUFA, including DHA, EPA, and docosapentaenoic acid (DPA), to support optimal neuronal functioning and overall health in children and adults have been established by various internationally recognized organizations (7,12–16). Estimations of (n-3) PUFA intakes in children have been reported from various countries using 24-h to 3-d recalls and FFQ or estimates of past mean intakes (16–20). Such estimates have numerous limitations (21–25). Directly quantitated (n-3) PUFA intakes for pregnant Canadian women have been reported from our laboratory (26).

The primary purpose of the present study was to directly quantify and assess daily intakes of (n-3) PUFA in a population of Canadian children aged 4–8 y with an emphasis on LC (n-3) PUFA to compare such intakes with recent recommendations.

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<sup>3</sup> Abbreviations used: AA, arachidonic acid; ADA, American Dietetic Association; ALA,  $\alpha$ -linolenic acid; DC, Dietitians of Canada; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; DRI, Dietary Reference Intake; EPA, eicosapentaenoic acid; LA, linoleic acid; LC, long-chain; MUFA, monounsaturated fatty acid.

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## Materials and Methods

**Ethics/recruitment.** This study was approved by the University of Guelph Research Ethics Board. The parent(s)/guardian(s) of 41 children aged 4–8 y (16 males and 25 females) were recruited in southwestern Ontario, Canada, by various means such as advertisements on public billboards, in newspapers, and by campus e-mail. The interested parent(s)/guardian(s) contacted the researchers by phone or e-mail. At this occasion, the researchers explained the study protocol. If the parent(s)/guardian(s) were interested, an initial meeting time was arranged at a location of their choice: either the Human Nutraceutical Research Unit, which is located at the University of Guelph, or at the participants' respective homes.

**Initial meeting with participants.** During the initial meeting period, the parent(s)/guardian(s) of the children provided written consent to participate in this study. The parent(s)/guardian(s) were informed that the researchers were quantifying macronutrient intakes and were interested in specific nutrients; however, the parent(s)/guardian(s) were not informed of these nutrients or of (n-3) fatty acids. The parent(s)/guardian(s) had the option of obtaining their data upon completion of the study. At the initial meeting time, the weight (in kg) and height (in cm) of all participants were recorded. Finally, the participants were provided with an 11.3-L Rubbermaid Latchable container to store the duplicate food samples under cold conditions. All participants were between the ages of 4 and 8 y during the food collection period.

**3-D food collection.** The parent(s)/guardian(s) was asked to duplicate in quantity and quality all food and natural health products consumed by their child or children over a period of 3 d, with the exception of water, pharmaceutical, and homeopathic agents. The parent(s)/guardian(s) had to have complete control over their children's food consumption over the 3-d food collection period. If the participants were attending school, the parent(s)/guardian(s) were asked to make a duplicate lunch and to collect what was consumed. All parents confirmed that their children were not allowed to trade or share food in their lunches. Furthermore, children were instructed to bring home any uneaten portions of their lunch so that parents could ensure accurate duplicate portion collections. The parent(s)/guardian(s) also took an active role in monitoring the food consumption and collections. In addition, the parent(s)/guardian(s) was asked to include at least 1 weekday and weekend day in the collections to provide a more representative sample of their children's eating habits. Furthermore, the food duplications did not have to be collected on consecutive days to allow the participants some flexibility during this period. Finally, the parent(s)/guardian(s) was informed of the importance of maintaining their child's current dietary habits and not to attempt to change these habits to "impress" the investigators.

**Compliance and payment.** All parent(s)/guardian(s) were provided an honorarium payment for their participation and cooperation. The parent(s)/guardian(s) (in the presence of their children) were clearly advised on providing complete collections and regular follow-up by phone during the collection period was maintained. No participants withdrew or were asked to withdraw from this study and all participants confirmed compliance with the food duplication collections. Compliance of food duplication/collections was also assessed by comparing actual energy intakes with the expected Dietary Reference Intakes (DRI) from the Institute of Medicine (Washington, DC).

**Proximate analysis.** The 3-d duplicated diet samples were collected and frozen at  $-10^{\circ}\text{C}$  until blended. Before blending, the samples were thawed, weighed, and homogenized into a homogenous slurry using a high volume Waring industrial blender (VWR Scientific). Sample aliquots were taken in duplicate for all analyses. The 82 sample aliquots were frozen at  $-10^{\circ}\text{C}$  before analysis. Agri-Food Laboratories analyzed the samples for total macronutrient contents. The homogenate was measured for percent moisture (135°C, 2 h), percent ash, percent crude fiber, percent carbohydrate (calculated), percent protein (nitrogen  $\times$  6.25), and percent fat (acid hydrolysis) of each individual diet in duplicate. The reference numbers for these procedures can be found in the Official Methods of Analysis of AOAC International (27): percent moisture, AOAC #930.15;

percent ash, AOAC #942.05; percent crude fiber, AOAC #962.09; percent carbohydrate was calculated by difference using the formula  $100\% - (\% \text{moisture} + \% \text{ash} + \% \text{protein} + \% \text{fat})$ ; percent protein, AOAC #990.03 and percent fat, AOAC #954.02. With these percentages, total energy intake (kcal/d)<sup>4</sup> was determined using the formula  $E = [(\text{CHO} \times F \times 4 \text{ kcal/g}) + (\text{Pr} \times F \times 4 \text{ kcal/g}) + (\text{fat} \times F \times 9 \text{ kcal/g})]$ , where E is energy in kcal/d, CHO is carbohydrate as % of food, F is food in g/d, Pr is protein as % food and fat is fat as % of food.

**Fatty acid analysis.** Fatty acid analyses were performed by Lipid Analytical Laboratories (University of Guelph Research Park, Guelph, Canada) based on a previous published study on pregnant women from our laboratory (26). A fixed weight of the diet homogenate sample was subjected to lipid extraction in the presence of a known amount of tritridecanoic (triaclyglycerol containing 13:0) added as an internal standard (Nu-Chek Prep) for quantification of the fatty acid amounts following GLC. An aliquot of the lower chloroform phase was taken for transmethylation and formation of the FAME derivatives (28). Capillary GLC analysis of the FAME was performed on a Varian 3400 gas-liquid chromatograph with a 60-m DB-23 capillary column (0.32-mm i.d.). Routine GLC analyses with an appropriate quantitative fatty acid standard mixture (GLC-461, Nu-Chek Prep) indicated that no detector response correction was required.

**Statistical analysis.** Data were entered into Microsoft Excel for analyses. Daily nutrient, including fatty acid, intakes were calculated by dividing the mean nutrient/fatty acid intakes (from duplicate determinations on the 3-d cumulative collections) by 3. The dietary nutrient intakes from the 41 participants (82 samples comprising energy, macronutrient, and fatty acid data) were calculated as the mean  $\pm$  SEM. In addition, a paired *t* test (significance set at  $P = 0.05$ ) was used to compare the 2 different methods used to quantify total fatty acid intakes from the different procedures.

## Results

**Demographic information.** All 41 children (16 males and 25 females) were 4–8 y old during the time of food collection. The BMI of the children (Table 1) were close to the proposed ideal BMI (in  $\text{kg/m}^2$ ) of 17.28–18.35 for females and 17.55–18.44 for males aged 4–8 y (29). The mean BMI for our female participants was  $15.9 \pm 0.3 \text{ kg/m}^2$  and for male participants was  $17.1 \pm 0.5 \text{ kg/m}^2$ .

**Fatty acid intakes.** The total fat intakes as determined by proximate analyses (acid/hydrolysis) did not differ ( $P < 0.05$ ) via a paired *t* test from those for total fatty acid amounts as quantified by GLC (Table 1). The major fatty acid types contributing to the total daily intakes (mean values) of fatty acids were SFA, monounsaturated fatty acids (MUFA), and PUFA (Table 2). The (n-6) series represented 86% of the total PUFA intake and almost all of the (n-6) PUFA (98%) was consumed as LA with very minor amounts of arachidonic acid (AA). The (n-3) series represented 14% of the total PUFA intake; ALA represented 89% of the (n-3) PUFA consumption. The LC (n-3) PUFA were very minor components of the total PUFA intake such that the sum of EPA, DPA, and DHA contributed only 9% to the total (n-3) PUFA intake. Wide variations among participants were found in both individual LC (n-3) PUFA and summed intakes of EPA, DPA, and DHA (Table 2).

## Discussion

The (n-3) fatty acid, DHA, has become recognized as a physiologically essential nutrient in the brain and retina for optimal

<sup>4</sup> 1 kcal = 4.184 kJ.

**Table 1** Characteristics and daily nutrient intakes of 4- to 8-y-old children<sup>1</sup>

Variable	Mean ± SEM	Range
Age, y	5.8 ± 0.2	4.0–8.0
Weight, kg	23.1 ± 0.8	14.9–44.6
Height, cm	118.0 ± 1.6	99.0–147.4
BMI, kg/m <sup>2</sup>	16.4 ± 0.3	13.5–20.5
Food intake, g/d	3209 ± 132	1464–5814
Carbohydrate intake		
g/d	222.0 ± 7.2	130.3–306.4
% energy	63.7 ± 1.1	48.0–81.1
Protein intake		
g/d	47.7 ± 2.1	26.7–94.7
% of energy	13.3 ± 0.3	8.4–18.2
Fat intake		
g/d	36.3 ± 2.5	9.3–100.8
% energy	22.8 ± 0.9	7.7–36.1
Total fatty acids, <sup>2</sup> g/d	36.8 ± 2.4	5.4–93.4
SFA	13.1 ± 0.7	1.6–25.6
MUFA	14.6 ± 1.1	2.4–43.0
PUFA	9.0 ± 0.8	1.3–26.7
Energy intake, kJ/d	5879 ± 211	3488–10036

<sup>1</sup> Values are means ± SEM, n = 41.

<sup>2</sup> Determined by quantitative capillary GLC.

cognitive and visual functioning, respectively (6). The known metabolic conversion, via desaturation plus elongation reactions, of ALA (from plant sources) via EPA and DPA to DHA in the liver (8,30) led to the establishment of recommended intakes by Health Canada for all sectors of the population in Canada in 1990 (31) and subsequently from the National Academy of Sciences, Institute of Medicine, Food and Nutrition Board, in Washington, DC (13). In vivo evidence that ALA is converted to DHA with a very limited efficiency in humans (8,30) and the recognition of potential health benefits emerging for both EPA and DHA (as found in fish), and possibly also DPA, have led to recommended intakes for DHA alone, DHA+EPA, or DHA+EPA+DPA in addition to ALA from various sources (Table 3) (7,12–16).

The present study provided direct quantification of actual fatty acid intakes with the primary intent to assess individual (n-3) intakes (as ALA, DHA, EPA, and DPA) in children aged 4–8 y. This particular group was targeted for various reasons, including their being in a stage of growth and continued neuronal development (32), evidence that their fish/seafood intakes (as sources of DHA+EPA) have often been reported to be modest (33), and the fact that existing intake data on (n-3) fatty acids has been estimated by indirect methods (16–20). Further, neurodevelopmental outcomes (including developmental, behavioral, and cognitive outcomes) in children up to 8 y of age were suboptimal if exposed to little DHA+EPA earlier in life, particularly via maternal seafood consumption in pregnancy (34). Finally, dietary habits and food consumption patterns during childhood have shown significant predictabilities for such intakes in adulthood (35).

The mean energy intake of our participants was 5879 kJ/d (1405 kcal/d), which was within the Estimated Calorie Requirements from the USDA for female and male children 4–8 y old, where a moderately active child is advised to consume between 1400–1600 kcal/d according to the Institute of Medicine DRI (36). These energy values support the conclusion that the food collection strategies were complete or very close to complete. The total fatty acid (sum) intake was 36.8 g/d and was almost identical to the mean total fat intake (36.3 g/d) as determined by proximate analysis (Table 1). We previously reported that 3-d food record estimates for fat intakes (as percent of energy) in pregnant women yielded values that were higher than those derived by direct quantification (26). A comparison of total dietary fat intakes in 18 adult volunteers via nutritional database analysis compared with chemical analysis of duplicate diets over the same 3-d period reported that the former approach (indirect) overestimated the total fat intake along with extremely wide discrepancies in fat intakes when the indirect approach was compared with direct chemical analysis (37).

The mean daily intake of the plant-based (n-3) fatty acid as ALA was 1161 mg/d as determined by our direct assessments, which can be compared with indirect estimates (16–20) from various countries (Australia, Belgium, Canada, China, and the United States) ranging from 0.34 to 1.72 g/d. The substantial

**TABLE 2** Daily dietary fatty acid intakes of 4- to 8-y-old children as quantified by GLC<sup>1</sup>

Fatty acids	Mean	Median	SD	SEM	Range	% of Energy <sup>2</sup>
SFA, g/d	13.1	13.0	4.6	0.7	1.6–25.6	8.4
MUFA, g/d	14.6	13.2	7.1	1.1	2.4–43.0	9.3
Σ (n-6) (Σ (n-6)) PUFA, g/d	9.0	7.7	5.3	0.8	1.3–26.7	5.8
Σ (n-6) PUFA, <sup>3</sup> mg/d	7741.0	6417.4	4647.7	725.9	1153.6–23353.9	5.0
LA [18:2(n-6)], mg/d	7583.2	6313.2	4569.3	713.6	1131.1–22863.3	4.9
Σ (n-3) AA [20:4(n-6)], mg/d	61.7	58.5	37.2	5.8	5.2–198.4	0.040
Σ (n-3) PUFA, <sup>3</sup> mg/d	1298.0	1141.4	762.2	119.0	154.1–3543.6	0.83
ALA [18:3 (n-3)], mg/d	1160.6	1007.4	690.0	107.8	138.9–3452.8	0.74
EPA [20:5(n-3)], mg/d	38.4	12.0	59.6	9.3	1.3–213.7	0.025
DPA [22:5(n-3)], mg/d	26.3	18.8	25.2	3.9	2.2–131.1	0.017
DHA [22:6(n-3)], mg/d	54.1	21.9	72.7	11.4	1.0–281.4	0.035
EPA+DHA, mg/d	92.5	31.5	129.6	20.2	2.3–466.5	0.059
EPA+DPA+DHA, mg/d	118.8	50.8	150.7	23.6	11.7–519.9	0.076
Total fatty acids, g/d	36.8	36.3	15.6	2.4	5.4–93.3	23.5
Σ (n-6):Σ (n-3) ratio	6.23	6.12	1.76	0.27	2.93–10.17	N/A

<sup>1</sup> Values are reported for 41 children based on duplicate diet analyses for each.

<sup>2</sup> Represents mean for all 41 participants.

<sup>3</sup> Other minor (n-6) and (n-3) fatty acids have been included in the sum values

**TABLE 3** Intakes of (n-3) fatty acid by aged 4- to 8-y-old Canadian children in relation to recommendations from various international sources<sup>1</sup>

Source of recommendation	ALA	DHA	DHA+EPA	DHA+EPA+DPA	% Meeting recommendation			
					ALA	DHA	DHA+EPA	DHA+EPA+DPA
North America	900 mg	N/A <sup>2</sup>	Up to 90 mg	N/A	61%	N/A	22%	N/A
Australia/New Zealand	800 mg	N/A	N/A	55 mg	68%	N/A	N/A	51%
Netherlands	1% of energy	N/A	N/A	0.15–0.20 of energy	20%	N/A	N/A	20%
Belgium	0.45–1.50% of energy	0.10–0.40% of energy	N/A	N/A	83%	15%	N/A	N/A
ADA and DC	0.6–1.2% of energy	N/A	351 mg/d	N/A	68%	N/A	10%	N/A

<sup>1</sup> The North American DRI provides an adequate intake of 900 mg for ALA, whereas 10% (90 mg) may be consumed as DHA+EPA for children aged 4–8 y (13). For the percent of energy recommendations from the various sources (7,12,15), the percent meeting recommendations for (n-3) PUFA was based on those participants who met or surpassed the minimum of recommended percent of energy. The recommended intake for DHA+EPA from the ADA and DC (7) for adults (500 mg/d) was normalized to the energy intake of each participant (e.g. an energy intake of 5879 kJ/d would yield a target minimal intake of 351 mg/d).

<sup>2</sup> N/A, Not applicable.

intake of ALA is not surprising in view of its common occurrence in canola oil, soybean oil, flaxseed, walnuts, and processed foods containing these ingredients as constituents (38,39). The mean group daily intake of DHA was 54.1 mg/child from our direct quantification, which can be compared with indirect estimates of intakes from the aforementioned countries ranging from 0.02 to 1.09 g/d. The mean intake of EPA was 38.4 mg/d, which compares to intakes ranging from 0.01 to 60 mg from indirect estimates. DHA+EPA are consumed primarily in the form of fish/seafood (38,40); they represented only 7.1% of the total (n-3) intake (mean of 1298 mg/d) in the children in this study. The very low intakes of DHA+EPA (averaging 92.5 mg/d) from our direct assessments are not surprising based on a recent study indicating that 16% of U.S. children consume no fish or shellfish during a 12-mo period and that the mean consumption rate among those who ate fish (the remaining 84%) was <1 meal/wk (33). The mean intake of DPA was 26.3 mg/d, which compares with indirect estimates ranging from 10 to 32 mg/d for children in various countries (16–20). The overall ratio of the summed (n-6) fatty acids to the summed (n-3) fatty acids yielded a mean (n-6):(n-3) ratio of 6.23, which was almost identical to that reported (6.20) in a previous study from our laboratory that directly quantified PUFA intakes in pregnant Canadian women (26). The much higher levels of DHA in the breast milk of Japanese women compared with the overall population of Canadian and American women has been attributed to a much higher intake of fish/seafood containing DHA+EPA in the former population (41).

To the best of our knowledge, our study as reported herein is the first to provide direct quantification for intakes of the various (n-3) fatty acids in a pediatric population (specifically, children aged 4–8 y). Previous estimations have been based on indirect assessments, including FFQ (16–20), which have various limitations. These limitations are numerous and include misreporting (types and amounts of foods), inaccuracies and variations from standard food tables in food compositional data (e.g. farmed compared with wild fish species, origin, seasonal variation, cooking method), and exclusion of DHA+EPA intakes from foods enriched in (n-3) PUFA and supplements, etc. (21–25, 42,43). In addition, the estimates for (n-3) fatty acid intakes for the U.S. population of children from the NHANES 1999–2000 (17) provides intakes of the LC (n-3) PUFA in gram quantities (e.g. 0.02 g DHA, etc.). Such imprecise reporting leaves considerable doubt as to specific quantity for a micronutrient such as DHA, because a reported value of 0.02 g could include quantities ranging from 15 to 24 mg (extremely wide range).

The mean AA intake in our children were 62 mg/d (Table 2), which can be compared with the mean intake of 99 mg/d as

reported from our group via direct determination for pregnant women (26). Dietary AA is consumed from various animal-based food sources (44). Physiological levels of AA, as derived metabolically from LA and dietary AA, are particularly high in neuronal membrane phospholipid along with DHA, where they support cognitive functionality, thereby resulting in their inclusion in selected infant formulas (3). Interestingly, the mean intakes of AA (0.17%) and DHA (0.15%) as a percent of total dietary fatty acids in our children were much lower than the corresponding levels (0.47% and 0.32%, respectively) in human breast milk worldwide (41).

In view of the very wide variance in (n-3) fatty acid intakes across individual children (Table 2), and nutritional recommendations from selected international sources on such intakes for health (7, 12–16), it was of interest to determine the proportions of our children that would meet such recommendations based on minimal recommended intakes or ranges of intakes (Table 3). For ALA, 61% of our children met the North American DRI, but only 22% consumed at least 90 mg of DHA+EPA daily. It was determined that 68 and 51%, respectively, of the recommended intakes from Australia/New Zealand for ALA at 800 mg/d and also for DHA+EPA+DPA at 55 mg/d would be fulfilled by these children. The corresponding percentages meeting or exceeding the minimum recommended intakes were 20% for both in the case of the Netherlands. Furthermore, 83% and only 15% of the children met or exceeded the minimum intakes set in Belgium for ALA and DHA, respectively. If the recent recommended daily intakes from the ADA and DC (7) for (n-3) fatty acids for adults consuming 8372 kJ (2000 kcal) were extrapolated to the energy intakes of our individual children, 59% would have met the recommended daily intake for ALA but only 10% for DHA+EPA.

The relevant literature has concluded that, although labor intensive and costly, direct determinations of nutrient consumption represent the most accurate tool for estimating dietary fatty acid intakes and are superior to indirect assessments via estimated dietary/food records and tabular compositional nutritional data (26,37). The direct quantification of (n-3) intakes in our group of children aged 4–8 y as reported herein raises concerns in regards to their low intakes of these fatty acids and particularly the LC (n-3) PUFA (DHA+EPA). There is an apparent need to create greater awareness of the importance of the LC (n-3) PUFA among both health professionals and the general public as well as the existing gap between actual and recommended intakes from various sources. This gap can be readily filled with an increased consumption of fish/seafood containing DHA+EPA, the increased availability of foods (eggs,

dairy products, breads, beverages, and others) that have been nutritionally enriched with various delivery forms of LC (n-3) PUFA (DHA+EPA), and the use of supplementation where necessary.

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