Dietary Arachidonic Acid to EPA and DHA Balance Is Increased among Canadian Pregnant Women with Low Fish Intake

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Abstract

Arachidonic [ARA, 20:4(n-6)], eicosapentaenoic [EPA, 20:5(n-3)], and docosahexaenoic acids [DHA, 22:6(n-3)] occur in the diet in animal tissue lipids, play important roles in human development and health, but have interactive and opposing functions. Meat and poultry have higher ARA and fish are richer in EPA and DHA. National databases were recently revised to include complete data on ARA in foods. We used a validated FFQ and the revised nutrient databases to quantify the distribution of ARA, EPA, and DHA intakes and balance for 204 healthy Canadian pregnant women. We focused on intake distributions because risk of adverse health effects increases at lower nutrient intakes. RBC fatty acids were analyzed concurrently with dietary assessment. The distribution of ARA, EPA, and DHA intakes were skewed (P<0.001), with a median (5–95th percentile) of 107 (41–225), 65 (10–228), and 105 (10–430) mg/d ARA, EPA, and DHA, respectively. Fish provided 66 and 76% of EPA and DHA, respectively, whereas eggs, poultry, and meats provided 81% of ARA. Women consuming ≥101 g fish/wk consumed less EPA and DHA and had markedly elevated median dietary ARA:EPA and ARA:DHA ratios compared with women consuming ≤101 g fish/wk (P<0.001). Relatively small increases in fish intake of 1–2 servings (25–50 g)/wk corrected the distorted dietary (n-6):(n-3) fatty acid balance among women consuming meats, but not fish. Median fish and DHA intakes below the recommended 1–2 servings/wk fish for pregnant women suggest major changes in the availability, cost, or acceptance of fish are needed. J. Nutr. 139: 2344–2350, 2009.

Introduction

Dietary (n-6) and (n-3) fatty acids are important due to their essential roles in normal cell function and in influencing risk of several diseases, including cardiovascular disease (CVD) and neurological and retinal disorders, which are of major public health importance (1–9). During development, docosahexaenoic acid [DHA, 22:6(n-3)] and arachidonic acid [ARA, 20:4(n-6)] are of further importance due to their accretion and essential function in the brain and retina and the roles of ARA and its metabolites in growth and maturation of multiple organs, including the immune system and gastrointestinal tract (10–13).

The (n-3) fatty acids are present in the diet as α-linolenic acid [ALA, 18:3(n-3)] and its longer chain metabolites eicosapentaenoic acid [EPA, 20:5(n-3)] and DHA, whereas the major dietary (n-6) fatty acids are linoleic acid [LA, 18:2(n-6)] and ARA. ALA provides ~90% of the (n-3) fatty acids in Western diets (14) and is found predominately in vegetable oils. EPA and DHA are naturally present in human diets in animal tissue lipids, with the richest source being fish. LA is found mainly in vegetable oils and currently represents 90% of the total PUFA in U.S. diets (14), whereas its metabolite ARA is found in animal tissue lipids, with most consumed in meats, poultry, and eggs. Epidemiological studies have shown that women with higher intakes of fish, and thus also EPA and DHA, during pregnancy have a small increase in gestation length and have children who score higher on tests of neural system development (15–19). However, the difference in distribution of ARA from EPA and DHA in meats, poultry, and fish raises the possibility that individuals with low intakes of fish, and thus also EPA and DHA, during pregnancy may have a small increase in gestation length and have children who score higher on tests of neural system development. The difference in distribution of ARA from EPA and DHA in meats, poultry, and fish raises the possibility that individuals with low intakes of fish, and thus also EPA and DHA, during pregnancy may have a small increase in gestation length and have children who score higher on tests of neural system development. However, until recently, the incomplete data on ARA in databases on the nutrient content of Canadian and U.S. foods (20,21) required that studies on dietary ARA intake rely on food or duplicate portion analysis, which is difficult with large or diverse populations.

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Participants and Methods

Participants and design. The present study involved 204 pregnant women who were 20–40 y old at 36 wk gestation with no known maternal or fetal complications, including metabolic, congenital, or infectious diseases and who were registered to deliver 1 full-term infant at the British Columbia’s Women’s and Children’s Hospital. Each participant was enrolled at 16 wk of gestation and randomized to receive 400 mg/d DHA from single-cell triglycerides or a corn oil/soybean oil placebo (23). The placebo provided a similar LA:ALA balance to the usual diet but at low levels compared with the gram quantities of LA and ALA in the diet. The women were followed prospectively and seen at 36 wk of gestation for collection of dietary information and a venous blood sample. All of the women were part of a larger study that included assessments of their infants’ development. Women with insufficient English language skills to complete the informed signed consent or in-person interviews, including dietary assessments, were not enrolled. Women following a vegan diet or taking fatty acid, fish, or other oil supplements were also ineligible to participate. The protocol and procedures were approved by the Committee for Ethical Review of Research Involving Human Subjects at the University of British Columbia and the British Columbia’s Children’s Hospital. All participants provided written informed consent prior to participation.

Assessment of dietary fatty acid intake. Dietary assessment was conducted at 16 and 36 wk gestation using an interview-administered FFQ specifically designed to collect information on fat and fatty acid intakes (24). We collected information on the frequency with which each food was eaten, including the portion size, brand name, or place of purchase and methods of food preparation. Particular focus was given to all types of margarine, vegetable and animal fats, milk and milk products and their alternates, eggs, meats, and poultry. In addition, because the fat content and composition of fish varies among species and between wild and farmed fish and is influenced by method of preparation, we also collected detailed information on the fish and seafood consumed by species, as wild or farmed, as fresh, frozen, or canned, and method of preparation. Information from the dietary records was entered into a nutrient database (FOOD PROCESSOR 11; ESHA Research) containing the recently updated Canadian Nutrient File and the USDA Nutrient Database (20,21). Further, the FOOD PROCESSOR database allows addition of food items and their nutrients, and is updated by us to include current data on the fatty acid composition of local foods, such as salad dressings, margarines, and shortenings (24).

To determine the contribution of different foods to the total intake of ARA, EPA, and DHA, foods were grouped into 6 categories (poultry, ruminant meats, nonruminant meats, eggs, dairy milk and milk products, and fish) and then the intake of DHA, EPA, and ARA from each food group was determined for each subject. Based on the types of fish and seafood reported in the dietary interviews, 6 categories of fish and seafood were selected (fresh and frozen salmon, lean white fish, canned tuna, canned salmon, all other fish, and crustaceans and shellfish) and the intake of EPA and DHA from each of these categories was determined. The women were then grouped by their average weekly intake of fish plus seafood (fish) as <101, 101–200, 201–300, and >300 g. Smaller categories of fish intake were not used because of imprecision in dietary databases that are unable to reflect variability in the fatty acid composition of individual fish due to specific geographical location, season, or age when fish are caught. Foods of vegetable origin were not included, because, unless supplemented, DHA, EPA, and ARA are present exclusively in animal tissue lipids.

Assessment of RBC glycerophospholipid fatty acids. Fasting venous blood was collected from each participant at 36 wk gestation in the outpatient laboratory of the British Columbia’s Women’s and Children’s Hospital. The RBC were separated from plasma by centrifugation at 2000 × g for 15 min at 4°C; washed twice by resuspension in normal saline, then frozen at –70°C. For analysis, the RBC total lipids were extracted, then the ethanolamine phosphoglycerides (EPG; including diacyl and plasmalogen ethanolamine phosphoglycerides) and phosphatidylcholine (PC) were separated from other lipids and the fatty acids quantified by routine GLC (18,23–26). Because supplemental DHA increases blood lipid levels of DHA (23,25), results to address the relationship between fish intake and RBC lipid EPA, DHA, and ARA are given for only the 120 women in the placebo group.

Statistical analysis. The median, 25th–75th and 5th–95th percentile ranges, and the mean and SD of the intakes of total fat and individual fatty acids were calculated. The Kolmogorov-Smirnov test was used to test for normal distributions of the data. Wilcoxon’s and Mann-Whitney U tests were used to determine potential differences in fatty acid intake among women grouped by fish intake. ANOVA with Bonferroni adjustments were used to compare RBC fatty acids in subgroup analysis. All statistical analysis was performed with the SPSS statistical software package for Windows (version 17). Values in the text are the mean ± SD unless otherwise indicated.

Results

Characteristics of the participants. The age of the 204 women studied was 33.0 ± 3.9 y and 72% were Caucasian, 18% were Chinese, and 10% were from other ethnic groups, and 78% had some form of postsecondary education. From the food records, 80, 57, 29, and 38 women consumed <101, 101–200, 201–300, and >300 g fish/wk, respectively. All of the 22% of women who had no postsecondary education consumed <201 g fish/wk.

Dietary fat and fatty acid intakes. Random assignment to 400 mg/d DHA or placebo did not result in any differences in dietary fat or fatty acid intake; thus, results concerning dietary intake are presented for all participants. The intakes of (n-6) and (n-3) fatty acids, but not total energy, total fat, or saturated or monounsaturated fatty acids, were skewed, with a consistently lower median than mean intake of LA, ARA, EPA, and DHA (Table 1; P < 0.05; n = 204). Fat provided 34 ± 10% of total energy, with a 5–95th percentile range of 18–56% total energy from fat. The intakes of SFA, monounsaturated acids, and PUFA were 11.4 ± 4.2, 12.9 ± 4.8, and 5.4 ± 2.7% total energy, respectively. LA provided a median of 4.5% dietary energy with 25th–75th and 5th–95th percentile ranges of 3.1–5.3% and 1.8–8.7% total energy, respectively. The mean intake of ARA was 107 mg/d, with a 5–95th percentile range of 80–157 mg/d (Table 1). Comparison of the median and 5–95th percentile range of intakes of 6.5–10–228 mg/d for EPA and 105 and 10–430 mg/d for DHA, respectively, shows that interindividual
intakes of EPA and DHA varied widely and were skewed to higher means by high intakes in some individuals. The dietary ARA:EPA, ARA:DHA, and LA:ALA ratios were also skewed ($P < 0.001$) and varied over 40-fold among the participants.

### Dietary sources of long chain (n-6) and (n-3) fatty acids.

Although ARA is found in animal tissue lipids, eggs are a common ingredient in many foods and contributed 30% of the ARA intake in our study. Poultry, ruminant, and nonruminant animal meats provided 28, 14, and 9% ARA, respectively, with 10% ARA from milk and milk products and the remaining 9% from fish. Fish was the major dietary source of EPA and DHA, providing 66 and 76% of EPA and DHA, respectively. A further 15% EPA and 1% DHA were consumed in milk and milk products; eggs contributed 1% EPA and 4% DHA, and 9% EPA and 15% DHA was from poultry, with the remainder from meats.

### Influence of fish intake on dietary intake and balance of (n-6) and (n-3) fatty acids.

The intakes of EPA and DHA increased with increasing fish intake (Fig. 1A,B; $P < 0.05$). The median intakes of EPA were 20, 70, 120, and 170 mg/d with 30, 120, 230, and 315 mg/d DHA among women who consumed <101, 101–200, 201–300, and >300 g/wk fish, respectively. The variability in EPA and DHA intake increases among women grouped by similar fish intake increased with increasing fish intake (Fig. 1A,B), explained by the higher variety of lean white fish and shellfish among women who consumed >201 g/wk fish (data not shown).

Dietary ARA intakes also differed among women with different intakes of fish (Fig. 1C; $P < 0.01$). Rather than having higher intakes of ARA, we found that the median ARA intake of women who consumed <101 g/wk fish was lower than for women who consumed >201 g/wk fish ($P < 0.01$). Regardless, the median dietary ARA:EPA and ARA:DHA ratios decreased with increasing fish intake, with values of 3.9, 1.5, 0.9, and 0.7:1 for ARA:EPA, and 3.4, 0.9, 0.5, and 0.4:1 for ARA:DHA for women consuming <101, 101–200, 201–300, and >301 g/wk fish, respectively (Fig. 1E,F). Subgroup analysis showed that the dietary ARA:EPA and ARA:DHA ratios were higher in women consuming <101 compared with ≥101 g/wk fish ($P < 0.001$) and also higher in women consuming 101–200 compared with 201–300 g/wk fish ($P < 0.001$). The 25–75th percentile of dietary ARA:DHA and EPA:EPA were 1.4–7.4 and 2.5–6.6, respectively, in women consuming <101 g/wk fish, but 0.7–1.3 and 1.2–2.5; 0.4–0.6 and 0.7–1.6; and 0.3–0.5 and 0.5–1.1 among women consuming 101–200, 201–300, and >300 g/wk fish, respectively. These results demonstrate both the extreme heterogeneity in dietary ARA:EPA and DHA balance among women with low intakes of fish and that the dietary ARA:EPA and ARA:DHA ratios were markedly elevated above the 75th percentile of ratios for women consuming ≥101 g/wk fish in 75% of women consuming <101 g/wk fish.

LA contributed 84.2% of total dietary PUFA and 99% of the total (n-6) fatty acids and this did not differ among the women grouped by usual fish intake. Similarly, ALA intake did not differ and thus the dietary LA:ALA ratio did not differ among the women with different intakes of fish (Fig. 1D; $P > 0.05$). However, the contribution of ALA to total (n-3) fatty acid intake decreased from 97 to 74% with increasing fish intake from <101 g to ≥101 g/wk ($P < 0.001$). Due to the increase in EPA and DHA intake, the dietary ratios of LA:EPA + DHA and ALA: EPA + DHA also decreased with increasing fish intake from <101 to >300 g/wk (Fig. 1G,H; $P < 0.05$).

### Fish intake and membrane biomarkers of long chain (n-6) and (n-3) fatty acid status.

The analyses of the RBC PC and EPG fatty acids showed that as fish intake increased, the levels of EPA and DHA increased, whereas the ratio of ARA:EPA + DHA decreased ($P < 0.05$; Table 2).

### Discussion

This study shows the distribution of ARA, EPA, and DHA intakes and dietary ARA:EPA and DHA balance among pregnant women. Although a large number of studies and national surveys have reported information on DHA and EPA intake, information on ARA is limited. Further, in addition to description of average population intakes, knowledge of the distribution of (n-6) and (n-3) fatty acids is crucial, because this provides information on the possible extent of dietary deficiency or imbalance in a population. Our results show a median intake of 107 g/d with a 5–95th percentile range of 41–225 mg/d ARA, with median intakes of 65 and 105 mg/d and 5–95th percentile ranges of intake of 10–228 and 10–430 mg/d for EPA and DHA, respectively, among 204 Canadian pregnant women.

Regardless of how information on dietary intake is collected, analysis of food intake records depends on accurate and complete information on the nutrient content of foods. Until recently, missing data on ARA in some pork, ham, poultry, and beef products in the USDA and Canadian nutrient files (20,21) limited the use of these databases for estimated ARA intakes. Previous studies by us circumvented this problem by analysis of the fatty acid composition of local foods (24), while others have used analysis of duplicate food portions (27,28). The intake of ARA in the present study using the revised food fatty acid composition tables (20,21) was 118 ± 59 mg/d, which agrees closely with our previous studies that found intakes of 121 ± 59 mg/d ARA among Canadian pregnant women based on food analyses (24). Similarly, studies using duplicate portion analysis reported intakes of 99 ± 85 mg/d ARA for 20 Canadian pregnant women.
pregnant women (27). These results support the use of the updated national databases to address the potential significance of differences in ARA intake on human health.

Epidemiological studies have provided evidence that fish intake is inversely associated with risk of several chronic diseases, including CVD and some neurological and retinal diseases (6–9, 29–31), whereas in pregnancy, higher intakes of fish are associated with a small increase in gestation length and lower risk of poor neurocognitive development and allergic and inflammatory disorders in children (15–19, 32). In each of these cases, the beneficial effects of fish are considered to be due to EPA and DHA. However, early studies linking diet to CVD showed disease risk was lower among vegetarians (33, 34). Subsequent reports continue to raise concern that diets high in meat, ARA, or with high (n-6):(n-3) fatty acid ratios contribute to risk of several diseases (35–40). In this regard, recent epidemiological studies involving over one-half a million individuals in the US reported that meat intake was associated with increased total mortality, cancer, and CVD mortality (40). Further, increasing intakes of fatty fish in patients with CVD led to lower ARA:EPA ratios in plasma lipids and decreased mononuclear cell inflammatory gene expression (41). In the present study, we found that the dietary ARA:EPA and ARA:DHA balance is dramatically increased in women who consume >100 g/wk fish and that this was accompanied by increased RBC lipid ARA:EPA+DHA ratios. However, it is important to note that over the range of ARA intakes in the present study, higher intakes of ARA did not lead to higher RBC lipid ARA. Thus, changes in the RBC lipid ARA:EPA+DHA balance among women with different intakes of fish were best explained by changes in EPA and DHA. The absence of any relationship between dietary ARA intake and RBC glycerophospholipid ARA could reflect specificity of acyltransferases involved in acylation and reacylation and the relatively modest range of ARA intakes among the women in the present study. The greatest impact of reducing the dietary ARA:DHA and ARA:EPA ratios was between women consuming <101 g/wk and those consuming 101–200 g/wk of fish, equivalent to 1–2 servings (25–50 g)/wk fish, with little additional benefit of intakes above 201 g/wk fish. Consistent

**FIGURE 1** Dietary intakes of DHA (A), EPA (B), and ARA (C) and the LA:ALA (D), ARA:DHA (E), ARA:EPA (F), LA:EPA+DHA (G), and ALA:EPA+DHA (H) ratios among Canadian pregnant women grouped by intake of fish as <100 (n = 80), 101–200 (n = 57), 201–300 (n = 29), or >301 g/wk (n = 38). The box plots show the median, 25–75th percentile ranges, and the minimum and maximum for each variable. Labeled values without a common letter differ, P < 0.05 (by Wilcoxon’s and Mann-Whitney U tests).
with our results, epidemiological studies have suggested health benefits of relatively low intakes of fish. For example, a follow-up of over 40,000 men in the US found that while fish intake was not associated with risk of total major chronic disease, 1 serving/wk compared with <1 serving/mo fish was associated with about a 15% lower risk of total CVD (42). Similarly, earlier meta-analysis of observational studies also concluded that eating fish one or two times per week might reduce death from coronary heart disease by 15%, with each 20-g/d increment in fish intake possibly lowering coronary heart disease mortality rates by 7% (43). Likewise, a cohort study of 3654 Australians found that 1 serving/wk fish was associated with reduced risk of early aging-related macular degeneration (44). However, our study shows that dietary ARA:EPA and DHA ratios varied widely among women consuming <101 g/wk fish, from 0.2 to >11 compared with ratios of 0.2 to 4 in those consuming ≥101 g/wk fish. Although >50% of women consuming <101 g/wk fish had dietary ARA:EPA and ARA:AHA ratios above the 95th percentile of the ratios for women with higher fish intakes, women who had low intakes of both fish and ARA had low dietary ARA:DHA and ARA:EPA ratios that were within the range of women with higher fish intakes. The possibility that beneficial effects of increasing fish intake are realized through restoration of the distorted dietary (n-6):(n-3) fatty acid balance that occurs when meat and poultry but not fish are consumed has implications for dietary recommendations for (n-6) and (n-3) fatty acids in the general population and those following vegetarian diets (45). Future studies will need to address if the balance of ARA:EPA:DHA or if the absolute intake of EPA and DHA is more important.

Several countries and groups have published recommendations for fish or DHA intakes, generally recommending 1–2 servings of fish/wk or 120 mg/d DHA or more for pregnant women (29,46–48). While specific information on the dietary (n-3) or (n-6): (n-3) fatty acid balance that poses risk of inadequate DHA is difficult to obtain, epidemiological and intervention studies to increase DHA intake in pregnancy that show benefits to gestation length and child visual and neurocognitive development indicate that dietary patterns among some women following western diets do result in functional deficiency of DHA (15–19,23,49–53). For comparison to other studies with pregnant women, the intake of DHA in the present study was 160 ± 169 mg/d (n = 204), while other studies in Canada have reported intakes of 82 ± 115 mg/d and 160 ± 246 mg/d DHA (24,27), with 81 ± 94, 280 ± 190, and 300 ± 300 mg/d DHA among African American women and women in Belgium and Holland, respectively (54–56). The consistently high SD of DHA intake shows the distribution of DHA intakes is wide in all of these populations. Furthermore, >40% of the women in the present study consumed <100 g/wk fish and for these women, the median intake of DHA intake was 40 mg/d, and 55% of the women consumed <200 mg/d EPA + DHA. Given the coastal location of this study, our results suggest major changes in cost or acceptance of fish will be needed if the suggested recommended intakes of 1–2 servings/wk fish (29,46–48) are to be met.

In conclusion, we have shown that the distribution of ARA, EPA, and DHA intakes and the dietary ARA:(n-3) fatty acid balance is wide and skewed. We demonstrated a markedly elevated dietary and RBC lipid ARA:EPA and DHA balance among women consuming <101 g/wk fish that shows the distorted ratio is decreased by a relatively modest increase in fish intake, equivalent to 1–2 servings/wk. We also emphasize that the dietary (n-6):(n-3) fatty acid balance among women with low fish intakes is heterogeneous. Given the health benefits of vegetarian diets, positive effects of relatively small intakes of fish, yet apparent health risks associated with meat, further studies using the updated information on ARA in foods will be useful to understand if the (n-6):(n-3) fatty acid balance in diets affects human health.

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**Literature Cited**


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