PLASMA OMEGA-3 FATTY ACID REPONSE TO A FISH OIL SUPPLEMENT IN THE HEALTHY ELDERLY

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Running Title: Plasma response to fish oil in the elderly

Abstract

Little information is available concerning whether incorporation of dietary omega-3 fatty acids into plasma lipids changes during healthy aging. Elderly (74 ± 4 y old) and young (24 ± 2 y old) adults were given a fish oil supplement for 3 wk that provided 680 mg/d of docosahexaenoic acid and 320 mg/d of eicosapentaenoic acid, followed by a 2 wk wash-out period. Compliance was monitored by spiking the capsules with carbon-13 glucose, the excretion of which was measured in breath CO_2. In response to the supplement, plasma docosahexaenoic acid rose 42% more in the elderly but eicosapentaenoic responded similarly in both groups. Despite raising docosahexaenoic acid intake by 5-10 fold, the supplement did not raise plasma free docosahexaenoic acid (% or mg/dl) in either group. We conclude that healthy aging is accompanied by subtle but significant changes in DHA incorporation into plasma lipids.

Key words:

Fish oil, compliance, omega-3 fatty acids, ω3 polyunsaturated fatty acids, elderly, arachidonic acid, docosahexaenoic acid, eicosapentaenoic acid.

Abbreviations:

ARA - arachidonic acid, DHA - docosahexaenoic acid, EPA - eicosapentaenoic acid

INTRODUCTION

Studies using both epidemiological (1,2) and other approaches (3) are broadly supportive of a protective role of fish ω3 fatty acids against Alzheimer's disease, the most common form of declining cognition in the elderly. We sought to address one background question that has received little attention but which may have an impact on why the elderly may be vulnerable to cognitive decline: is healthy aging associated with physiological (obligatory) changes in metabolism in ω3 fatty acid metabolism? More specifically, we asked - do plasma ω3 fatty acid levels respond similarly to a fish oil supplement in healthy young and elderly subjects? Given the vulnerability of the elderly to cognitive decline and the role of docosahexaenoic acid (22:6ω3; DHA) in cognitive function, we hypothesized that aging would dampen the plasma DHA response to an ω3 fatty supplement.

In order to be sure of a possible age-related difference in ω3 metabolism, compliance had to be verified independently of reported capsule counts or changes in plasma ω3 fatty acids. Since our young and elderly participants were free-living, we could not readily supervise in person the consumption of the ω3 fatty acid supplement, so a new method of assessing compliance was developed and validated prior to doing the metabolic assessment.

The data reported here focus on plasma values for the two principal ω3 fatty acids in the supplement provided - eicosapentaenoic acid (20:5ω3; EPA) and DHA, as well as the principal 'competing' ω6 fatty acid - arachidonic acid (20:4ω6; ARA) (4). Because the level of 'free' (non-esterified) plasma DHA and ARA is thought to be important for their uptake by the brain (5), those data are provided as both percentages and concentrations.

METHODS

All procedures reported here were approved by the Human Ethics Research Committee of the Health and Social Sciences Center – Sherbrooke University Geriatrics Institute, which is the committee mandated to oversee human experimentation at our institution.

Compliance Methodology: In addition to capsule counts and a checklist completed by the subjects, we independently verified compliance by adding a small amount of carbon-13-labeled glucose (^{13}C; Cambridge Isotopes Limited, Andover, USA) to the ω3 fatty acid capsules used in the metabolic study. This was done only during the third week of the study. With 10 mg/capsule of ^{13}C-glucose, we established in preliminary tests that if breath ^{13}C value during the supplementation period did not exceed 2.5 times the baseline ^{13}C value, the subject was non-compliant on that day. Non-compliance on more than one day/week led to exclusion of the subject's data from the final analysis.

ω3 Fatty Acid Supplementation: Following informed written consent, the participants' medical histories were taken by a registered nurse and 12 h fasting blood chemistries were assessed. Inclusion criteria were: 18-29 y old or 70-79 y old, thyroid stimulating hormone (0.35-7.0 mIU/L), glucose (3.3-6.1 mmol/L), cholesterol (3.3-4.6 mmol/L in the young and 3.3-6.2 mmol/L in the elderly), triglycerides (0.6-2.3 mmol/L), and hemoglobin A_{1c} (4.0-6.0 mmol/L). Exclusion criteria were: smokers, pregnancy/lactation, <18 y old or between 30-70 y old or >70 y old, or use of medication for diabetes, liver disease, renal disease, hypertension, anemia or low serum albumin or other evidence of malnutrition. An upper limit of 3.0% DHA in plasma total lipids was set to exclude those individuals who were probably already consuming ω3 fatty acid supplements or relatively high amounts of fish.

A dose of ω3 fatty acids (EPA and DHA) was provided that was calculated to be 4-5 times the average intake in Quebec of ω3 fatty acids from fish or seafood (6). One lot of an encapsulated commercially available brand of fish oil was purchased in sufficient quantity to supply all the participants for the duration of the study (Genuine Health, O3mega+ Think, Toronto, Canada). According to our measurements, two capsules/d provided 1070 mg of ω3 fatty acids: 3 mg α-linolenic acid (18:3ω3), 64 mg docosapentaenoic acid (22:5ω3), 323 mg of eicosapentaenoic acid (20:5ω3; EPA) and 680 mg of DHA (Table 1). Participants consumed the two capsules each morning for 3 wk, followed by a 2 wk wash-out during which no capsules were consumed. Participants were told to take the capsules in the morning at breakfast and to take duplicate breath samples not less than 2 and not more than 4 h later. Fasting blood samples were collected once weekly between 7-8 a.m. They were anti-coagulated in EDTA and the plasma removed by centrifugation and stored at -20^{o} C.

Analytical Methodology: For fatty acid analysis, total lipids from 0.5 ml were extracted into chloroform-methanol, internal standards added, the lipid classes separated by neutral lipid thin layer chromatography, and fatty acid methyl; esters prepared and analyzed as previously described (7). Glucose, triglycerides, total cholesterol, and free fatty acids were measured by an automated analyzer (Xpand, Dade-Behring, Mississauga, ON).

Statistics: Using DHA values in plasma total lipids analyzed in our laboratory as a benchmark, power analysis showed that 9-10 subjects/group would be sufficient to demonstrate a significant effect of the supplement. Data are shown as mean±SD. Fatty acid values were not normally distributed so the non-parametric Mann-Whitney test was used (SPSS; Chicago, Illinois, USA) with the cut-off for statistical

significance set at $p<0.05$. Freidman's test was used to verify whether the young and elderly groups differed when there was no effect of the supplement.

RESULTS

Compliance: Based on the rise in ^{13}C in breath CO_2 from the ^{13}C-glucose on the capsules, we observed that one young participant was non-compliant on two occasions and another was non-compliant on six of the seven days tested, leaving n=9 in the young group. Lack of change in plasma ω3 fatty acids in the least compliant participant supported the ^{13}C compliance data. All 10 elderly participants who started the supplementation study were included in the final analyses.

ω3 Fatty Acid Supplementation: DHA in plasma total lipids was not different between the two groups at baseline. After 3 wk supplementation, in the elderly, plasma DHA stabilized 46% higher than baseline ($p<0.05$), a rise that was 42% higher than in the young group. During the wash-out, plasma DHA declined to a similar value in both groups (Figure 1). After the supplementation, EPA in plasma total lipids rose similarly in both groups. In both groups, whereas % DHA rose in plasma phospholipids during the supplementation period, EPA was largely unaffected in phospholipids, and neither changed significantly in plasma triglycerides (data not shown). Other ω3 fatty acids (α-linolenic acid, ω3 docosapentaenoic acid) remained statistically unchanged during supplementation and did not differ between the two groups.

Expressed as a % of the plasma free (non-esterified) fatty acid pool, free DHA did not differ between the two groups and also did not change significantly during either the supplementation or the wash-out phase of the study. The plasma *concentration* of free DHA also did not change during fish oil supplementation, but owing to the higher concentration of plasma total free fatty acids in the elderly, the

plasma concentration of free DHA averaged over both phases of the study was 82% higher in the elderly (data not shown). EPA was largely undetectable in plasma free fatty acids so the few values that were recorded could not be compiled and are not reported here.

In the elderly, plasma total ARA started and remained about 20% higher throughout the supplementation and wash-out periods ($p<0.00004$; Figure 1). The higher % of ARA in plasma of the elderly seen both at baseline and throughout the study was a function of higher ARA in both phospholipids and triglycerides but not free fatty acids (data not shown). When expressed as a % of the total free fatty acid pool, free ARA did not differ between the two groups at any time point. When expressed as a concentration (mg/dL), free ARA differed between the two groups at a couple of time points but not overall (data not shown).

There was no treatment effect on the plasma concentration of the main plasma lipid classes during the supplementation period or during the wash-out. Hence, during the overall study period, the elderly averaged 9% higher phospholipids, 19% higher triglycerides, and 64% higher free fatty acids; all $p<0.05$). The significant difference in plasma total cholesterol between the two groups seen at baseline was unaffected during the supplementation and wash-out periods (data not shown).

DISCUSSION

This study shows that compared to young adults, healthy, free-living elderly from our region of Québec have a somewhat higher response of plasma DHA to a short-term daily fish oil supplement. This result contradicts our initial hypothesis that the plasma response to a fish oil supplement would be significantly dampened in the

healthy elderly. Higher baseline plasma EPA in our elderly group (Table 1) confirms observations reported previously for plasma EPA (8,9) and red cell ω3 PUFA (10,11). In 30 year old women, plasma EPA tends to rise more quickly than DHA during short term supplementation with fish oil and fall more quickly after the supplementation period (12).

Despite raising DHA intake by 5-10 fold compared to average intake in Quebec (6) and approximately doubling plasma total DHA during the supplementation period, in neither group did plasma *free* DHA rise significantly during the supplementation period nor fall during the wash-out (whether expressed as % or as mg/dL). The higher concentration of free plasma DHA in our elderly subjects was clearly a function of a larger plasma free fatty acid pool and not due to higher % DHA in plasma free fatty acids. No change in % DHA in plasma free fatty acids has previously been reported during a 3 wk supplementation with 750 mg/d of DHA but no EPA (13). However, in that study, plasma free DHA did rise significantly after the 6^{th} week of supplementation or when a 1500 mg daily dose of DHA was given. We focused on plasma free DHA because of its purported role as a precursor to brain DHA (5). Given the emerging though still controversial link between low DHA status and increased risk of cognitive decline in the elderly (3), it was therefore not anticipated that the pool of plasma free DHA would be higher in the elderly than in our young subjects. If, as proposed by Lagarde's group, a lysophosphatidylcholine form of DHA is the carrier of DHA to the brain (14), then the larger pool of plasma free DHA in the elderly would have no direct bearing on their susceptibility to cognitive decline.

In all plasma lipid classes we evaluated, ARA was statistically unaffected by an intake of about 1000 mg/d of ω3 fatty acids, given mostly as EPA and DHA.

Higher baseline ARA in our elderly group contrasts somewhat with the results of Rees et al (9) but their elderly group was 15 y younger than ours. Rees et al (9) also used a higher dose of EPA and DHA and for a longer period but otherwise both our studies agree that raising EPA and DHA intake by up to 10 fold does not seem to significantly affect plasma ARA.

Incomplete compliance is one potential confounder that was not a factor in the results of the present study. Our stable isotope-based method was relatively non-invasive and well tolerated. It was a good independent measure of compliance but because of cost, labor-intensiveness and the need for specialized equipment to analyze ^{13}C in breath CO_2, it perhaps not widely applicable.

We conclude that during short-term supplementation with fish oil capsules, healthy aging is associated with significantly higher DHA incorporation into plasma lipids. This result contributes to the emerging literature suggesting that subtle but potentially important changes in ω3 PUFA metabolism occur during healthy aging. The possible relevance of these aging-related changes in ω3 PUFA metabolism to risk of chronic disease, particularly cognitive decline, remains to be established.

Acknowledgements

Financial assistance was provided by the Canadian Institutes for Health Research, Natural Sciences and Engineering Research Council of Canada, the Canada Research Chairs (SCC), the Canadian Foundation for Innovation, and FORMSAV (MV). Excellent technical assistance was provided by Mary Ann Ryan, Julie Desgagné and Martine Fisch.

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Figure legends

Figure 1

Docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (ARA) in plasma total lipids (%) during 3 weeks supplementation and 2 weeks follow up in 9 young and 10 elderly subjects. Black squares - young adults, White squares – elderly adults: mean ± SD, * $p<0.05$.

Table 1

Baseline characteristics of the two groups.

Group	Young (n=9)	Elderly (n=10)
Age	24 ± 2	74 ± 4 *
Male/Female	5/4	5/5
Weight (kg)	71.1 ± 18.1	75.0 ± 13.4
Body Mass Index (kg/m^2)	24.5 ± 4.1	28.0 ± 4.3
Free Fatty Acids (mmol/L)	0.48 ± 0.21	0.62 ± 0.24
Phospholipids (mmol/L)	0.72 ± 0.21	0.84 ± 0.17
Triglycerides (mmol/L)	1.0 ± 0.4	1.3 ± 0.4
Cholesterol (mmol/L)	4.4 ± 0.6	5.5 ± 0.9 *
Fatty acids** (%)		
Sum Saturates	28.9 ± 2.5	29.5 ± 2.5
Sum Monounsaturates	24.7 ± 3.8	27.6 ± 2.6 *
Linoleate	34.3 ± 3.1	28.8 ± 3.2 *
Arachidonate	6.6 ± 0.9	7.5 ± 1.0 *
α-Linolenate	1.0 ± 0.5	0.8 ± 0.3
Eicosapentaenoate	0.6 ± 0.3	1.0 ± 0.3 *
Docosapentaenoate	0.4 ± 0.2	0.5 ± 0.1
Docosahexaenoate	1.5 ± 0.5	1.6 ± 0.6

Mean ± SD

*p<0,05, Mann-Whitney

**Plasma total lipids