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HIGHER PLASMA N-3 FATTY ACID STATUS IN THE MODERATELY HEALTHY ELDERLY IN SOUTHERN QUÉBEC: HIGHER FISH INTAKE OR AGING-RELATED CHANGE IN N-3 FATTY ACID METABOLISM?

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ABSTRACT

The elderly reportedly have a significantly higher % of eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids in plasma and red cell lipids. However, these results are from a few small studies and the health status of the elderly in these studies is for the most part unclear. Since the elderly are susceptible to cardiovascular and neurological illnesses that seem to be related in part to lower intake of n-3 fatty acids it seems paradoxical that their blood levels of EPA and DHA would be higher than in young adults. We report here plasma fatty acid profiles and their response to supplementation with two types of fish oils from several of our recent studies in the moderately healthy elderly. We define the moderately healthy elderly as those who were in good physical condition, had no cognitive decline and, if present, in whom hypothyroidism, hyperlipidemia and/or hypertension were well-controlled. As shown previously, we confirm the higher % EPA and % total n-3 fatty acids (but not DHA) in fasting plasma and extend these findings to include higher plasma concentrations (mg/L) of n-3 fatty acids as well. The EPA-predominant supplement raised DHA only in the young, whereas the DHA-predominant supplement raised EPA more in young than in the elderly. The moderately healthy elderly clearly have higher plasma n-3 fatty acids but whether this reflects differences in intake versus aging-related changes in n-3 fatty acid metabolism remains to be elucidated.

Key words: aging, n-3 fatty acids, eicosapentaenoic acid, docosahexaenoic acid,

Abbreviations: EPA (eicosapentaenoic acid), DHA (docosahexaenoic acid)

INTRODUCTION

N-3 fatty acids from fish are now generally recognized as beneficial for optimal function of the cardiovascular system in adults, whether consumed as fish or as fish oil supplements. Long term fish intake is also associated with a lower risk of cognitive decline and Alzheimer's disease during aging [1, 2], but it is not yet clear that n-3 fatty acid or fish oil supplements can replace long term fish intake for this purpose [3].

Because of the increasing risk of deteriorating health of the cardiovascular system and brain with age, we and others have sought to establish whether healthy aging is associated with changes in plasma n-3 fatty acid content or response to n-3 fatty acid supplementation that would leave the elderly with poorer n-3 fatty acid status that predisposes them to these degenerative diseases. In fact, perhaps surprisingly, in both plasma and red blood cells, several papers show that the content of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) rises significantly from the second to the seventh decade of life [4-9]. Nevertheless, these studies all had significant limitations, including small numbers of subjects. Furthermore, although 65 years old is frequently used as reference, there is no official or widely accepted definition of *elderly*, so the cut-off is often as low as 50 y old. Furthermore, information about the health status, blood chemistry, cognitive function or physical activity of elderly research subjects is rarely given in sufficient detail to establish whether or not the data reported are for the *healthy* elderly.

Our aim here is to report more detailed information on anthropometry, blood chemistry, as well as fasting plasma fatty acid profiles and responses to two types of n-3 fatty acid supplementation of young adults and elderly living in or around the city of Sherbrooke in southern Québec whom we classify as *«moderately healthy»*. We base this definition on their active lifestyle, normal cognition and relatively good physical condition. Nevertheless, because most of our subjects used prescription medications to control their

blood pressure and blood lipids in particular, without which they would not have been within accepted limits for these parameters, we define them here as «moderately healthy». The data reported here are pooled from our published [8-10] and unpublished studies.

METHODS

Young adults were defined as between 18-30 y, while the elderly group was ≥65 y old. There were 24-28 participants in each age group (except as noted in the Tables). They were recruited by public announcements and word of mouth. All were pre-screened for medical history, and excluded if they had diabetes, evidence of overt hepatic or renal disease, or untreated hypertension, dyslipidemia or thyroid disease. Fasting glucose and hemoglobin A_{1c} were used to rule out the presence of diabetes or probable glucose intolerance. All subjects had a score of at least 28/30 on the Mini-Mental State Exam. None were smokers and none in the young age group were on prescribed medications.

Unmedicated subjects were preferred but those on medications for elevated blood pressure, hyperlipidemia or inflammatory joint pain were accepted into the study if their blood pressure and blood lipids were within normal limits for at least 6 months. In the elderly group, 29% were medication free, 18% were taking one prescribed medication, 32% were taking two different medications and 21% took 3-7 different medications. By type of medication, 25% of the elderly were on hypolipidemics, 43% on antihypertensives, 25% on a non-steroidal anti-inflammatory drug, 21% on thyroid hormone replacement, and 25% on inhibitors of bone resorption. Other medications were also consumed but less commonly, including beta-blockers, antidepressants, sedatives, conjugated estrogen, proton pump inhibitors and autacoids, each of which was used by no more than one subject each.

Approval for all studies was obtained from the Research Ethics Committee of the Health and Social Services Center – Sherbrooke University Geriatrics Institute, which oversees all human research done at the Research Center on Aging. All subjects gave informed, written consent before participating.

Plasma from venous forearm blood samples obtained after a 10-12 h overnight fast was kept at -20°C until analyzed. Triglycerides, cholesterol, glucose, free fatty acids, and

β-hydroxybutyrate were analyzed in our lab by clinical chemistry analyzer (Dimension Xpand Plus (Siemans Healthcare Diagnostics, Deerfield, IL, USA). Others plasma metabolites and the complete blood cell count were done by the clinical laboratory of our affiliated hospital (CHUS, Sherbrooke, QC). Plasma insulin was analyzed by enzyme-linked immunosorbent assay (Alpco, Salem, NH) with a Victor X4 multilabel plate reader (Perkin Elmer, Woodbridge, ON).

Fatty acid profiles of plasma total lipids were analyzed by capillary gas chromatography as previously described [9]. Some subjects were then given an n-3 supplement enriched in DHA (680 mg DHA/d plus 323 mg EPA/d) for 3 wk, or a supplement enriched in EPA (1480 mg EPA/d plus 250 mg DHA/d) for 6 wk, and fatty acid profiles periodically analyzed in plasma samples (see Figures 1, 2).

Mann-Whitney non-parametric tests were used to compare data between the young and elderly groups. The paired sample Wilcoxen signed Rank test was used to assess the effect of the n-3 fatty acid supplements. A p value < 0.05 was considered statistically significant.

RESULTS

The mean age of the elderly was 74 y old and they were 51 years older than the young subjects. The elderly were 0.07 m shorter (5%; $p<0.01$) and 0.8 kg heavier (1.1%, n.s.), resulting in a body mass index 12% higher ($p<0.05$) than the young subjects (Table 1). Fasting plasma triglycerides, total cholesterol, LDL cholesterol, glucose, thyroid stimulating hormone, and hemoglobin A_{1c} were 10-40% higher in the elderly group ($p<0.01$), but HDL cholesterol, free fatty acids, β-hydroxybutyrate, insulin, and C-reactive protein were not different. Red cell count, albumin, alanine aminotransferase and lymphocyte count were 5-23% lower in the elderly (Table 1).

Expressed as a percentage of total extracted fatty acids, fatty acid profiles of fasting plasma total lipids from the elderly group had 11% higher total monounsaturates and 32% higher total n-3 polyunsaturates, no difference in individual or total saturates, and 7% lower total n-6 polyunsaturates (all $p<0.05$; Table 2). Amongst the n-6 polyunsaturates, only 18:2n-6 (linoleic acid) was significantly lower in the elderly (-12%; $p<0.01$), while amongst the n-3 fatty acids, only EPA and 22:5n-3 (n-3 docosapentaenoic acid) were higher in the elderly (+100% and +25%, respectively, $p<0.05$-0.01). Expressed as concentrations (mg/L), total plasma fatty acids were 29% higher in the elderly ($p<0.01$), with the difference being much more for the n-3 polyunsaturates (+74%) than for the saturates, monounsaturates or n-6 polyunsaturated fatty acids (+22 to +41%; Table 3). Individually, concentrations of all the n-3 polyunsaturates were significantly higher in the elderly by +40% (18:3n-3), +63% (DHA), +85% (22:5n-3) and +142% (EPA).

After the supplementation period, both the EPA- and DHA-predominant supplements raised plasma EPA in the young and elderly. The EPA-predominant supplement raised plasma DHA only in the young subjects and to a level equivalent to that in the elderly. The DHA-predominant supplement raised plasma DHA equally in the young

and elderly (+70%) but raised plasma EPA more in young than in the elderly (126% vs. 59%, respectively; Figures 1,2).

DISCUSSION

We report here plasma long chain fatty acid profiles and response to two types of n-3 fatty acid supplementation in moderately healthy elderly and young adults in southern Québec. As reported elsewhere [7, 11] and compared to our young adults, the moderately healthy elderly in this study had a higher proportion of total n-3 fatty acids in plasma total lipids. This higher proportion of n-3 fatty acids in plasma was statistically significant only for EPA and 22:5n-3 (Table 2). Expressed as concentrations, all the n-3 fatty acids were 40-142% higher in plasma total lipids; this difference was in part due to the 29% higher total plasma fatty acids in the elderly, but % composition of the n-3 fatty acids was still higher than for other fatty acids (Table 3). We are not aware of any previous reports of higher n-3 *concentration* in plasma total lipids in the healthy elderly. Our present data confirm previously published reports suggesting that the n-3 fatty acid status of red blood cells [4-6] and plasma [7-9, 11] is higher in moderately healthy elderly compared to younger adults. Although most of our elderly subjects were on one or more prescription medications, otherwise, they were in relatively good physical and cognitive health. Hence, on the face of it and whether expressed as % composition or concentration, the moderately healthy elderly have higher fasting plasma n-3 fatty acid status than young adults.

Higher plasma (and red cell) plasma n-3 fatty acid status in the elderly would seem to be due to higher fish and/or seafood intake. This is principally because higher plasma EPA and DHA is usually a direct reflection of higher EPA and DHA intake, the principal sources of which are fish and seafood. Furthermore, the parent n-3 fatty acid, 18:3n-3 (α-linolenic acid), cannot be synthesized *de novo* in humans and since there is little conversion of 18:3n-3 to EPA and DHA in humans [12], plasma EPA and DHA originate mostly from their intake.

Nevertheless, there are several reasons why these data need to be interpreted cautiously: First, we did not record their dietary habits so we cannot confirm whether our elderly subjects actually consumed more fish. Second, the elderly are more susceptible to cognitive decline [1, 3, 13], but risk of cognitive decline is lower in those consuming more fish or n-3 fatty acids, so this increased susceptibility seems paradoxical if indeed the plasma n-3 fatty acid status is higher in the elderly. Third, a single blood measurement of EPA and DHA correlates well with fish intake or fish oil supplementation [14] but tells little about the n-3 fatty acid status to the brain or other organs at risk of n-3 fatty acid insufficiency during aging. Fourth, apolipoprotein E4 significantly raises fasting plasma EPA and DHA [15,16] and reduces the plasma EPA and DHA response to a fish oil supplement [16], but whether these effects influences apparent n-3 fatty acid status of the elderly is not yet known. Fifth, our studies with two types of n-3 fatty acid supplements [8, 9; Figures 1,2] indicate that subtle differences exist in the way the healthy elderly process n-3 fatty acids through the blood. Thus, we believe it is premature to conclude that higher fasting blood EPA and DHA in the elderly is necessarily a result of higher fish intake.

A significant proportion of the elderly participants in this study was on medications and had plasma metabolite profiles significantly different from the young group (Table 1). Despite the use of medications for blood pressure and blood lipid control in particular, our moderately healthy elderly still had higher plasma cholesterol and triglycerides. They also had higher plasma thyroid stimulating hormone and glucose, as well as signs of mild glucose intolerance. Nevertheless, their fasting plasma free fatty acids, ketones, insulin and C-reactive protein were not different from those of the young subjects. Hence, we defined them as «moderately healthy» on the basis of stable and effective pharmaceutical control of blood pressure and blood lipids, normal or near normal plasma metabolites and blood chemistry, hormones, and enzymes, absence of cognitive decline, and relatively good physical condition.

Higher blood n-3 fatty acid status than is commonly observed in North America is desirable at all ages and is most readily achieved by higher fish intake. Nevertheless, in the elderly, higher fasting plasma or red cell n-3 fatty acids may in part be due to higher fish intake but may also be due to aging-related changes in n-3 fatty acid metabolism. This uncertainty requires further investigation so as to correctly assess n-3 fatty acid status of the elderly. We are presently investigating these aging-related changes in more detail using ^{13}C-labeled DHA.

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TABLE 1

Anthropometric and fasting plasma measurements in moderately healthy elderly compared to young adults. Where significant differences were present between the young and elderly, the % difference is also shown (% Δ)[#].

	Young	Elderly	% Δ
Age (y)	23.7 ± 3.2	74.4 ± 3.9 *	
Height (m)	1.73 ± 0.10	1.65 ± 0.09 *	-5
Weight (kg)	71.2 ± 18.0	72.0 ± 12.7	
Body mass index (kg/m^2)	23.8 ± 5.4	26.6 ± 4.1 *	+12
Triglycerides (mmol/L)	1.0 ± 0.4	1.4 ± 0.5 *	+40
Total Cholesterol (mmol/L)	4.1 ± 0.7	5.7 ± 1.2 *	+37
Glucose (mmol/L)	5.1 ± 0.6	5.8 ± 0.7 *	+14
Free fatty acids (μmol/L)	508 ± 207	605 ± 218	
β-Hydroxybutyrate (µmol/L)	69 ± 75	75 ± 52	
Insulin (mIU/L)	5.8 ± 4.4	5.3 ± 3.3	
Hemoglobin A_{1c} (%)	5.0 ± 0.3	5.5 ± 0.4 *	+10
HDL cholesterol (mmol/L)	1.3 ± 0.4	1.4 ± 0.3	
LDL cholesterol (mmol/L)	2.4 ± 0.6	3.1 ± 1.0 *	+29
Thyroid stimulating hormone (mIU/L)	2.0 ± 0.8	2.7 ± 1.2 *	+35
Serum aspartate aminotransferase (U/L)	30 ± 10	28 ± 7	
Serum alanine aminotransferase (U/L)	40 ± 17	31 ± 9 *	-23
Albumen (g/L)	45 ± 4	42 ± 3 *	-8
Creatinine (µmol/L)	86 ± 16	86 ± 11	
C-reactive protein (mg/L)	3.8 ± 2.3	4.8 ± 3.2	

[#] mean±SD for n = 24-28, except insulin (n=11 in the young; n=13 in the elderly).

* $p<0.05$

[##] NOTE: Hemoglobin (g/L), hematocrit (%), platelet, monocyte and leucocyte counts did not differ between groups, but red cell (4.7±0.3 vs. 4.9±0.4 X10^{12}/L; $p<0.05$) and lymphocyte (0.6±0.5 vs. 0.5±0.5 X10^3/μL; $p<0.05$) counts were lower in the elderly.

TABLE 2

Fasting fatty acid profiles (%, mean±SD) in plasma total lipids of moderately healthy young and moderately elderly subjects. Where significant differences were present between the young and elderly, the % difference is also shown (% Δ).

	Young (n=26)	Elderly (n=25)	% Δ
14:0	1.0 ± 0.4	1.0 ± 0.3	
16:0	23.9 ± 2.9	23.3 ± 2.7	
18:0	7.9 ± 2.4	7.4 ± 1.7	
Sum Saturates	32.7 ± 4.8	31.8 ± 3.9	
16:1n-7	2.0 ± 0.9	2.7 ± 1.6 *	+32
18:1n-9	21.3 ± 2.2	23.3 ± 2.6 *	+9
18:1n-7	1.7 ± 0.3	1.9 ± 0.3 *	+15
Sum Monounsaturates	25.0 ± 2.7	27.8 ± 3.3 *	+11
18:2n-6	31.7 ± 4.3	28.0 ± 6.7 *	-12
20:3n-6	1.5 ± 0.3	1.5 ± 0.3	
20:4n-6	6.0 ± 1.0	6.8 ± 1.8	
Sum n-6 Polyunsaturates	39.3 ± 4.4	36.4 ± 6.1 *	-7
18:3n-3	0.8 ± 0.5	0.8 ± 0.3	
20:5n-3	0.5 ± 0.3	1.0 ± 0.5 *	+84
22:5n-3	0.4 ± 0.2	0.5 ± 0.2 *	+40
22:6n-3	1.4 ± 0.5	1.7 ± 0.8	
Sum n-3 Polyunsaturates	3.1 ± 1.0	4.0 ± 1.4 *	+32

* $p<0.05$

TABLE 3

Fasting fatty acid profiles (mg/L, mean±SD) in plasma total lipids of healthy young and moderately elderly subjects. Where significant differences were present between the young and elderly, the % difference is also shown (% Δ).

	Young (n=26)	Elderly (n=25)	% Δ
14:0	27 ± 16	38 ± 16 *	+42
16:0	665 ± 286	826 ± 209 *	+24
18:0	216 ± 103	265 ± 98 *	+23
Sum Saturates	907 ± 385	1,128 ± 302 *	+24
16:1n-7	59 ± 40	98 ± 69 *	+65
18:1n-9	594 ± 254	823 ± 210 *	+39
18:1n-7	47 ± 22	67 ± 18 *	+43
Sum Monounsaturates	700 ± 306	988 ± 266 *	+41
18:2n-6	858 ± 314	1,016 ± 374 *	+18
20:3n-6	42 ± 20	52 ± 14 *	+24
20:4n-6	167 ± 74	235 ± 61 *	+41
Sum n-6 Polyunsaturates	1,067 ± 394	1,304 ± 402 *	+22
18:3n-3	22 ± 15	30 ± 15 *	+40
20:5n-3	15 ± 9	36 ± 24 *	+142
22:5n-3	10 ± 5	18 ± 8 *	+85
22:6n-3	37 ± 20	60 ± 35 *	+63
Sum n-3 Polyunsaturates	83 ± 37	145 ± 77 *	+74
TOTAL	2,758 ± 1057	3,564 ± 919 *	+29

* $p<0.05$

FIGURE LEGENDS

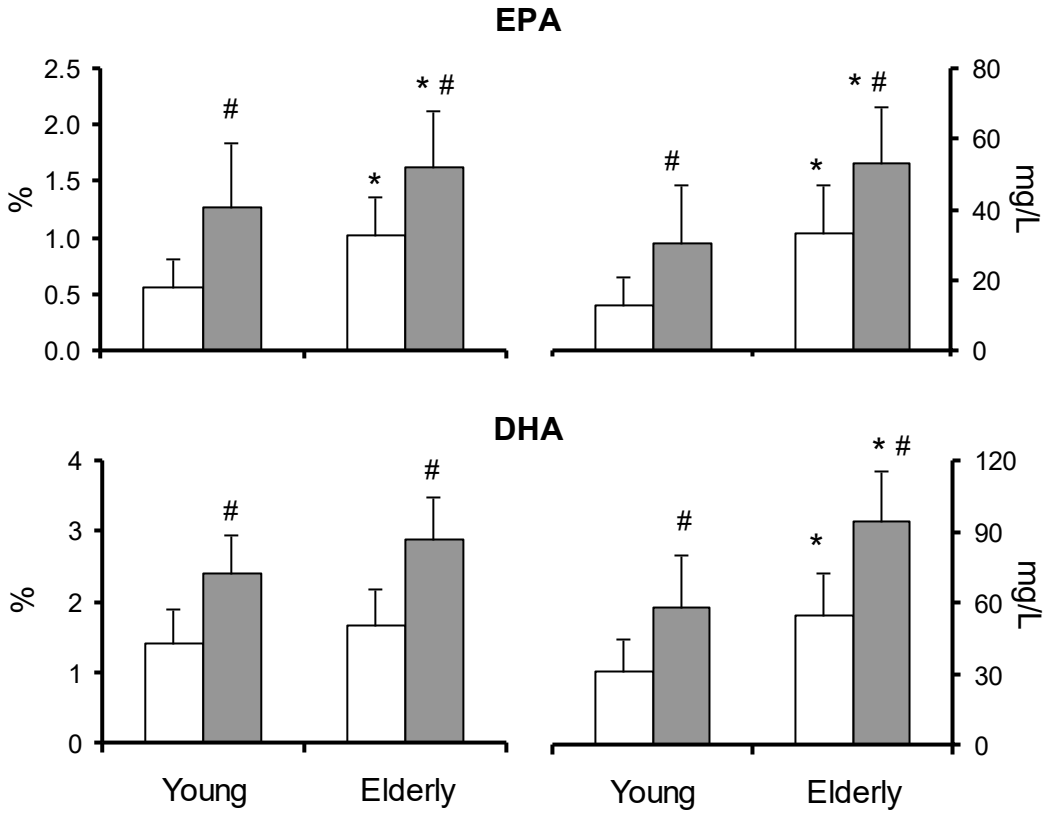
Figure 1

EPA and DHA in plasma total lipids in healthy young (n= 14) and elderly humans (n = 13), before (white) and after (gray) a DHA-predominant omega 3 supplementation (680 mg DHA/d, 323 mg EPA/d) given for 3 wk. * significant difference between young and elderly, # significant difference after supplementation.

Figure 2

EPA and DHA in plasma total lipids in healthy young (n=10) and elderly humans (n =10) before (white) and after (gray) an EPA-predominant omega 3 supplementation (1480 mg EPA/d; 250 mg DHA/d) for 6 wk. Modified from [9].* significant difference between young and elderly, # significant difference after supplementation.

Figure 1


EPA
%
mg/L
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2.0
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1.0
0.5
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60
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20
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DHA
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3
2
1
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120
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60
30
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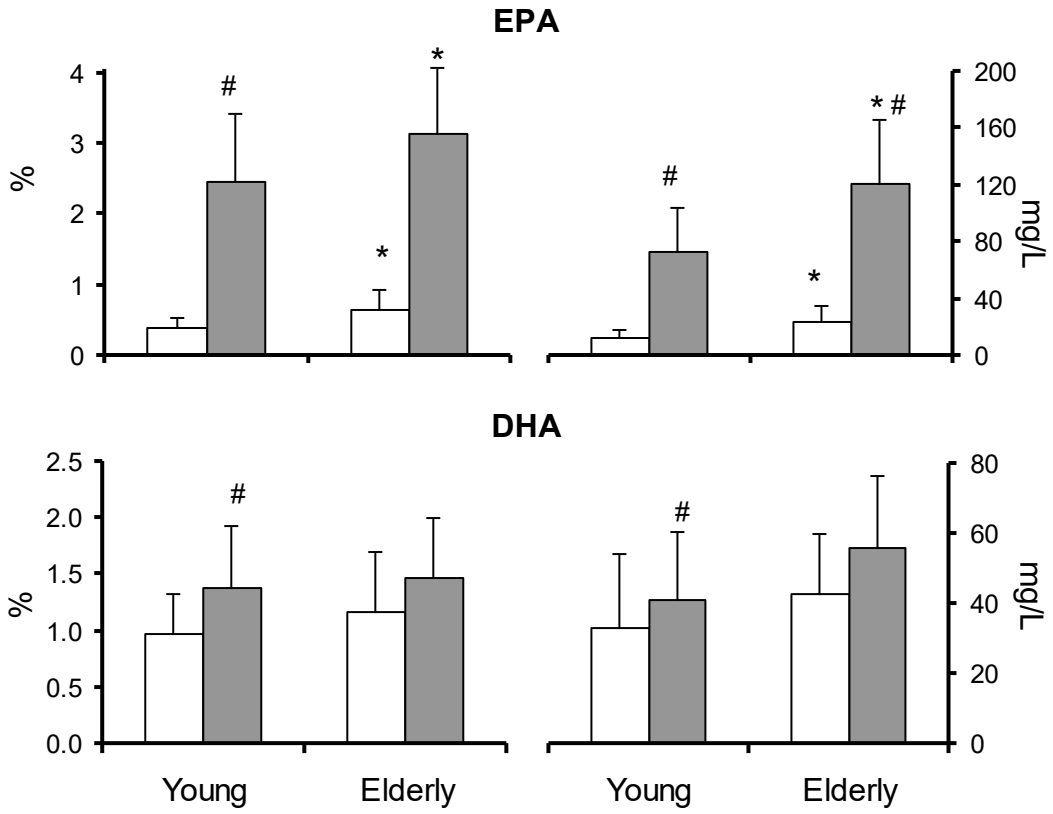
Young
Elderly
Young
Elderly

Figure 2


EPA
%
mg/L
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DHA
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Young
Elderly
Young
Elderly