Lipids (2013) 48:719–727
DOI 10.1007/s11745-013-3763-9

ORIGINAL ARTICLE

Higher Serum EPA or DHA, and Lower ARA Compositions with Age Independent Fatty Acid Intake in Japanese Aged 40 to 79

Rei Otsuka · Yuki Kato · Tomoko Imai · Fujiko Ando · Hiroshi Shimokata

Received: 3 September 2012 / Accepted: 15 January 2013 / Published online: 7 February 2013

Abstract Docosahexaenoic acid (DHA) and arachidonic acid (ARA) are the predominant long-chain polyunsaturated fatty acids (PUFA) among membrane phospholipids in the mammalian brain and neural tissues. This cross-sectional study examined age effects on serum eicosapentaenoic acid (EPA), DHA, and ARA compositions assessed with reference to dietary intakes among 1,014 Japanese men and 1,028 Japanese women aged 40–79 years. Venous blood was collected early in the morning after at least 12-h fasting. Serum fatty acid (FA) compositions were expressed as molar percentages of the total FA (mol% of total). Diet was assessed using a 3-day dietary record that included photographs. Participants were categorized into groups by sex and age (40–49, 50–59, 60–69, and 70–79 years). Intakes of fish, EPA, and DHA tended to increase with age. Significant positive correlations between serum FA composition and the corresponding weight percentage of total FA intake were observed for EPA and DHA in all sex and age groups, and for ARA among females in their 40s. Serum EPA and DHA compositions were higher, while ARA decreased with age, and these associations remained consistent even after adjusting for corresponding FA intake. These results suggest potential effects of age on differences in blood EPA, DHA, and ARA compositions, independent of corresponding FA intake among community-dwelling Japanese men and women.

Keywords Cross-sectional study · Serum fatty acid · Japanese · Docosahexaenoic acid · Eicosapentaenoic acid · Arachidonic acid · Age groups

Abbreviations

ALA	Alpha-linolenic acid
ARA	Arachidonic acid
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FA	Fatty acid(s)
LNA	Linoleic acid(s)
NILS-LSA	National Institute for Longevity Sciences Longitudinal Study of Aging
PUFA	Polyunsaturated fatty acid(s)

Introduction

Docosahexaenoic acid (DHA) and eicosapentaenoic (EPA) of the n-3 polyunsaturated fatty acids (PUFA) and arachidonic acid (ARA) of the n-6 PUFA are the predominant long-chain PUFA of membrane phospholipids in mammalian brain and neural tissues [1, 2]. These PUFA have been shown to partake in numerous cellular functions affecting membrane fluidity, membrane enzyme activities, and eicosanoid synthesis [3]. Several studies have shown that n-3 fatty acid (FA) levels in blood differ significantly between individuals with normal cognitive functioning and those with cognitive impairment [4, 5], and cognitive

R. Otsuka (✉) · Y. Kato · T. Imai · F. Ando · H. Shimokata
Department for Development of Preventive Medicine, National Institute for Longevity Sciences, National Center for Geriatrics and Gerontology, 36-3 Gengo, Morioka-cho, Obu, Aichi 474-8511, Japan
e-mail: otsuka@ncgg.go.jp

T. Imai
Faculty of Human Life and Science, Doshisha Women's College of Liberal Arts, Kyoto, Japan

F. Ando
Faculty of Health and Medical Sciences, Aichi Shukutoku University, Nagakute, Japan

impairment might be partly due to age-dependent decreases in membrane n-6 PUFA levels, particularly as ARA is abundant in hippocampal neurons [6–8].

Conversely, age-related changes in blood FA compositions have been reported, as increases in EPA and DHA and decreases in ARA with age [9–12]. However, blood levels of these FA, particularly the n-3 PUFA EPA and DHA, depend on dietary intake [13], and high blood levels of n-3 PUFA have been reported among Norwegian [14], Japanese [15], and Chinese populations [16], where fish consumption is high compared to American or Hispanic populations.

The Japanese have a long history of eating seafood rich in n-3 PUFA. Nevertheless, fish consumption among Japanese individuals has decreased markedly in the last 50–60 years [17]. In addition, younger and middle-aged individuals consume less seafood than the elderly [17], so PUFA intake and blood FA compositions in Japan might differ by age and generation. To clarify age effects on blood FA composition among Japanese groups, age-related dietary intake should be considered. Kawabata et al. [18] reported that ARA content in erythrocytes and plasma phospholipids in the elderly (22 men aged 60–75 years, and 32 women aged 56–73 years) was lower than that in 20-year-old men and women, even though ARA intake was nearly identical. However, they compared FA levels between only two age strata (20 s and 50–70 s), and age-dependent differences in blood EPA, DHA, and ARA compositions among middle-aged and elderly individuals facing an increased risk of cognitive impairment remain unclear.

The present study therefore examined age effects on serum EPA, DHA, and ARA compositions assessed with reference to dietary intakes among community-dwelling Japanese men and women aged 40–79 years.

Materials and Methods

Study Subjects

Data for this survey were collected as part of the National Institute for Longevity Sciences Longitudinal Study of Aging (NILS-LSA). In this project, the normal aging process has been assessed over time using detailed questionnaires and medical checkups, anthropometric measurements, physical fitness tests, and nutritional examinations. Participants in the NILS-LSA included randomly selected age- and sex-stratified individuals from the pool of residents in the NILS neighborhood areas of Obu City and Higashiura Town in Aichi Prefecture. Details of the NILS-LSA study have been reported elsewhere [19].

Participants in the fifth wave of the NILS-LSA were 1,200 men and 1,219 women between 40 and 88 years old from July 2006 to July 2008. We excluded from analysis those participants who were ≥80 years old ($n = 162$), who fasted <12 h, who were unable to supply a sufficient volume of blood ($n = 54$), or who did not complete either the nutritional assessments ($n = 159$) or the self-reported questionnaire ($n = 2$). A total of 2,042 Japanese (1,014 men, 1,028 women) between 40 and 79 years old were available for analysis.

The study protocol was approved by the Committee of Ethics of Human Research of the National Center for Geriatrics and Gerontology (No. 369-2). Written informed consent was obtained from all subjects.

Blood Sampling and FA Analysis

Upon enrolment in the survey, venous blood was collected early in the morning after at least 12-h fasting. Blood samples were centrifuged at $3{,}500 \times g$ for 15 min. Serum was separated and frozen at −80 °C before analysis for FA content by a single technician. Serum FA composition was measured by gas–liquid chromatography at a clinical laboratory (SRL, Tokyo, Japan). Briefly, total lipids in the serum were extracted using the Folch procedure and FA were then methylated with BF_3/methanol. Transesterified FA were then analyzed using a gas chromatograph (GC-17A; Shimadzu, Kyoto, Japan) with a capillary column Omegawax 250 (Supelco, Bellefonte, PA, USA). Weight of each FA (μg/mL) as FA concentration were identified by comparison with known standards, and the molar percentage of each FA among total FA (mol% total) was quantified by the total moles for all FA. Intra- and inter-assay precision and accuracy values [coefficient of variation (CV)] were 3.2 and 5.8 CV % for ARA, 2.7 and 6.9 CV % for EPA, and 1.9 and 6.9 CV % for DHA, respectively.

We examined 24 serum FA: lauric (12:0), myristic (14:0), palmitic (16:0), stearic (18:0), arachidic (20:0), behenic (22:0), lignoceric (24:0), myristoleic (14:1n-5), palmitoleic (16:1n-7), oleic (18:1n-9), eicosenoic (20:1n-9), erucic (22:1n-9), nervonic (24:1n-9), alpha-linoleic (18:3n-3), eicosapentaenoic (20:5n-3), docosapentaenoic (22:5n-3), docosahexaenoic (22:6n-3), linoleic (18:2n-6), gamma-linoleic (18:3n-6), eicosadienoic (20:2n-6), dihomo-γ-linoleic (20: 3n-6), arachidonic (20:4n-6), docosatetraenoic (22:4n-6), and 5-8-11 eicosatrienoic (20:3n-9) acids. For grouped FA, we defined: saturated FA as the sum of (12:0), (14:0), (16:0), (18:0), (20:0), (22:0), and (24:0); monounsaturated FA as the sum of (14:1n-5), (16:1n-7), (18:1n-9), (20:1n-9), (22:1n-9), and (24:1n-9); n-3 series polyunsaturated FA as the sum of (18:3n-3), (20:5n-3), (22:5n-3), and (22:6n-3); n-6 series polyunsaturated FA as the sum of (18:2n-6), (18:3n-6), (20:2n-6), (20: 3n-6), (20:4n-6), and (22:4n-6); and polyunsaturated FA as the

sum of n-3 and n-6 series polyunsaturated FA and (20:3n-9).

Nutritional Assessments

Nutritional intakes were assessed using a 3-day dietary record after participation in the survey. The dietary record was completed over 3 continuous days (both weekend days and 1 weekday) [20], and most subjects completed it at home and returned records within 1 month. Food was weighed separately on a scale (1-kg kitchen scales; Sekisui Jushi, Tokyo, Japan) before being cooked or portion sizes were estimated. Subjects used a disposable camera (27 shots; Fuji Film, Tokyo, Japan) to take photos of meals before and after eating. Dietitians used these photos to complete missing data, and telephoned subjects to resolve any discrepancies or obtain further information when necessary. Averages for 3-day food and nutrient intakes were calculated according to the fifth edition of the Standard Tables of Foods Composition in Japan and other sources [20]. Alcohol intake in the previous year was assessed using a food frequency questionnaire; trained dieticians interviewed subjects using this questionnaire.

Other Measurements

Medical history of heart disease, hypertension, hyperlipidemia and diabetes (past and current), education ($\leq$9, 10–12, or $\geq$13 years of school), and smoking status (yes/no) were collected using questionnaires. Body mass index was calculated as weight in kilograms divided by the square of height in meters. Serum triacylglycerol levels were measured using enzymatic methods, total and high-density lipoprotein-cholesterol levels were measured using the dehydrogenase method and direct method, respectively, at a clinical laboratory (SRL, Tokyo, Japan).

Statistical Analyses

All statistical analyses were conducted using Statistical Analysis System software version 9.1.3 (SAS Institute, Cary, NC, USA). Serum FA concentrations were determined in moles per liter and serum FA compositions were expressed as molar percentages of the total FA (mol% of total). Participants were categorized into four age groups (40–49, 50–59, 60–69, or 70–79 years). Linear regression models were constructed using the PROC GLM procedure to examine associations between age groups and dietary indices/serum FA (mmol/L). Comparisons between dietary indices/serum FA (mmol/L) according to age group were performed by one-way analysis of variance and the trend test. Relationships between serum FA composition (mol% of total) and the corresponding FA intake (wt%) as the weight percentage of total FA intake (g/day) were examined using Pearson's correlation coefficients according to age group by sex, because interactions existed between some kinds of dietary FA intake and corresponding FA composition by age group in both men and women. Mean serum FA compositions (mol% of total) according to age groups were calculated using the PROC GLM procedure, and to eliminate the effects of FA intake on serum FA compositions, a subsequent model included the corresponding FA intake (wt%) into covariates. All reported P values were two-sided, and values of $P < 0.05$ were considered significant.

Results

The characteristics of the participants are presented in Table 1. Mean (standard deviation) age and BMI were 59.4 (11.4) years and 23.2 (2.7) kg/m^2 for men and 59.1 (11.6) years and 22.4 (3.3) kg/m^2 for women, respectively. Current smokers comprised 25.9 % of men and 5.0 % of women. Prevalences of hypertension and hyperlipidemia were 29.4 and 18.8 % in men and 25.7 and 20.2 % in women, respectively.

Table 2 shows the mean daily food and nutrient intakes according to age group and sex. In both men and women, intakes of fish and seaweed increased and meat intake decreased with increases in age. Energy and fat intake

Table 1 Characteristics of participants

	Men (n = 1,014)	Women (n = 1,028)
	Mean ± SD	Mean ± SD
Age (years)	59.4 ± 11.4	59.1 ± 11.6
Alcohol (g/day)	0.4 ± 2.9	0.3 ± 2.1
Body mass index (kg/m^2)	23.3 ± 2.7	22.4 ± 3.3
Triacylglycerol (mg/dl)	125.3 ± 80.9	98.3 ± 67.2
Total cholesterol (mg/dl)	208.3 ± 32.7	220.9 ± 32.9
HDL-cholesterol (mg/dl)	57.2 ± 14.5	67.5 ± 15.4
	n, %	n, %
Education		
$\leq$9 years	166, 16.4	200, 19.5
10–12 years	358, 35.3	460, 44.8
$\geq$13 years	490, 48.3	368, 35.8
Smoking status		
Current	263, 25.9	51, 5.0
Former/never	751, 74.1	977, 95.0
Clinical history		
Heart disease	160, 15.8	99, 9.6
Hypertension	298, 29.4	264, 25.7
Hyperlipidemia	191, 18.8	208, 20.2
Diabetes	86, 8.5	61, 5.9

Table 2 Mean daily food and nutrient intakes according to age groups by sex

	Men (n = 1,014)				ANOVA[a]	P for trend
	40–49 (n = 241)	50–59 (n = 268)	60–69 (n = 262)	70–79 (n = 243)		
Fish (g)	75.0 ± 45.3	108.5 ± 59.3	116.1 ± 57.2	107.4 ± 50.7	<0.0001	<0.0001
Seaweed (g)	16.3 ± 22.2	17.3 ± 20.1	20.3 ± 21.7	24.2 ± 32.4	0.001	<0.0001
Meat (g)	108.6 ± 52.5	80.7 ± 40.9	72.6 ± 39.9	56.3 ± 39.8	<0.0001	<0.0001
Egg (g)	43.1 ± 28.0	49.3 ± 27.9	46.4 ± 25.9	44.2 ± 26.5	0.050	0.940
Energy (kcal)	2,304.5 ± 416.3	2,267.8 ± 386.4	2,268.9 ± 342.6	2,123.8 ± 378.1	<0.0001	<0.0001
Protein (g)	81.8 ± 16.4	84.1 ± 16.2	86.5 ± 15.9	81.4 ± 17.4	0.002	0.822
Fat (g)	67.4 ± 19.3	62.9 ± 16.7	58.5 ± 15.4	52.3 ± 15.3	<0.0001	<0.0001
Carbohydrate (g)	307.8 ± 65.1	301.2 ± 62.9	310.8 ± 55.4	305.2 ± 62.3	0.322	0.928
Saturated fatty acids (g)	19.1 ± 6.3	17.2 ± 5.6	16.0 ± 5.4	14.5 ± 5.2	<0.0001	<0.0001
Monounsaturated fatty acids (g)	24.7 ± 8.4	22.5 ± 6.8	20.4 ± 6.3	17.7 ± 6.1	<0.0001	<0.0001
Polyunsaturated fatty acids (g)	14.8 ± 4.4	14.5 ± 4.0	13.7 ± 3.4	12.3 ± 3.6	<0.0001	<0.0001
n-6 series polyunsaturated fatty acids (g)	12.3 ± 3.8	11.6 ± 3.4	10.8 ± 2.9	9.6 ± 2.9	<0.0001	<0.0001
LNA (mg)	11,999.9 ± 3,820.9	11,315.1 ± 3,475.1	10,526.5 ± 2,886.9	1,344.3 ± 481.4	<0.0001	<0.0001
ARA (mg)	178.6 ± 68.9	184.5 ± 64.2	182.1 ± 63.0	171.4 ± 64.4	0.120	0.200
n-3 series polyunsaturated fatty acids (g)	2.5 ± 0.9	2.9 ± 1.1	2.9 ± 1.0	2.7 ± 1.1	0.0001	0.153
ALA (mg)	1,699.5 ± 643.9	1,598.3 ± 514.1	1,479.5 ± 483.6	6.0 ± 8.4	<0.0001	<0.0001
EPA (mg)	232.6 ± 211.1	367.8 ± 295.7	403.0 ± 263.3	389.8 ± 257.1	<0.0001	<0.0001
DHA (mg)	437.0 ± 331.0	662.1 ± 475.8	717.7 ± 422.2	691.8 ± 437.1	<0.0001	<0.0001
	Women (n = 1,028)				ANOVA[a]	P for trend
	40–49 (n = 263)	50–59 (n = 259)	60–69 (n = 261)	70–79 (n = 245)		
Fish (g)	69.4 ± 41.0	81.1 ± 40.2	83.8 ± 43.3	87.8 ± 44.7	<0.0001	<0.0001
Seaweed (g)	15.4 ± 19.9	15.6 ± 18.8	19.8 ± 26.4	19.8 ± 21.2	0.014	0.004
Meat (g)	68.8 ± 38.5	59.1 ± 32.6	51.0 ± 31.3	44.9 ± 27.8	<0.0001	<0.0001
Egg (g)	38.9 ± 22.7	37.2 ± 23.0	38.1 ± 25.3	40.1 ± 24.6	0.573	0.508
Energy (kcal)	1,862.1 ± 317.1	1,857.7 ± 305.4	1,814.5 ± 293.4	1,766.8 ± 276.4	0.0009	0.0001
Protein (g)	68.7 ± 13.6	70.7 ± 13.0	71.5 ± 13.2	70.0 ± 13.6	0.103	0.205
Fat (g)	59.8 ± 15.1	55.4 ± 14.6	50.0 ± 12.9	46.9 ± 13.3	<0.0001	<0.0001
Carbohydrate (g)	250.4 ± 45.8	258.4 ± 49.1	264.3 ± 49.8	260.5 ± 43.5	0.008	0.007
Saturated fatty acids (g)	17.9 ± 5.6	16.2 ± 5.9	14.1 ± 4.5	13.2 ± 4.6	<0.0001	<0.0001
Monounsaturated fatty acids (g)	21.4 ± 5.9	19.4 ± 5.5	17.1 ± 5.2	15.8 ± 5.0	<0.0001	<0.0001
Polyunsaturated fatty acids (g)	12.5 ± 3.5	12.1 ± 3.3	11.5 ± 3.4	11.1 ± 3.3	<0.0001	<0.0001
n-6 series polyunsaturated fatty acids (g)	10.3 ± 3.0	9.8 ± 2.8	9.2 ± 2.8	8.7 ± 2.7	<0.0001	<0.0001
LNA (mg)	10,079.2 ± 2,949.7	9,601.6 ± 2,744.0	8,935.5 ± 2,742.9	8,523.6 ± 2,614.5	<0.0001	<0.0001
ARA (mg)	153.5 ± 51.5	147.9 ± 50.9	149.1 ± 52.9	144.4 ± 55.0	0.279	0.079
n-3 series polyunsaturated fatty acids (g)	2.2 ± 0.8	2.3 ± 0.8	2.3 ± 0.9	2.3 ± 0.9	0.524	0.279
ALA (mg)	1,410.2 ± 486.5	1,332.1 ± 457.3	1,243.8 ± 474.2	1,256.8 ± 450.2	<0.0001	<0.0001
EPA (mg)	216.6 ± 184.6	267.5 ± 202.2	300.4 ± 196.4	300.4 ± 219.6	<0.0001	<0.0001
DHA (mg)	414.4 ± 305.1	486.8 ± 321.8	532.0 ± 311.5	524.5 ± 340.1	<0.0001	<0.0001

All values are expressed as means ± SD

LNA linoleic acid, *ARA* arachidonic acid, *ALA* alpha-linolenic acid, *DHA* docosahexaenoic acid, *EPA* eicosapentaenoic acid

[a] ANOVA one-way analysis of variance

decreased in both men and women, and carbohydrate intake increased in women with age.

Lower intakes of saturated, monounsaturated, PUFA, n-6 PUFA including linoleic acid (LNA), and alpha-linolenic acid (ALA) were seen in older age groups in both men and women. In contrast, EPA and DHA intakes tended to increase in older age groups in both sexes.

Table 3 shows Pearson's correlation coefficients between serum FA composition (mol% of total) and corresponding FA intake (wt%) according to age group and sex. In all age

Table 3 Pearson's correlation coefficients between serum FA composition (mol% of total) and corresponding FA intake (wt%) according to age groups by sex

	Men (n = 1,014)				Women (n = 1,028)			
	40–49 (n = 241)	50–59 (n = 268)	60–69 (n = 262)	70–79 (n = 243)	40–49 (n = 263)	50–59 (n = 259)	60–69 (n = 261)	70–79 (n = 245)
Saturated fatty acids	0.10	0.03	−0.10	−0.02	−0.01	−0.01	0.12*	0.02
Monounsaturated fatty acids	0.10	0.03	0.09	0.22*	0.15*	0.11	0.06	0.07
Polyunsaturated fatty acids	0.21*	−0.12	−0.01	0.06	0.12	−0.01	0.09	0.02
n-6 series polyunsaturated fatty acids	0.20*	−0.04	−0.05	0.11	0.09	−0.09	0.12	−0.03
LNA	0.20*	0.02	−0.02	0.11	0.11	−0.05	0.10	−0.02
ARA	0.12	0.06	0.07	0.04	0.15*	0.06	−0.005	0.002
n-3 series polyunsaturated fatty acids	0.39*	0.34*	0.39*	0.27*	0.22*	0.35*	0.33*	0.42*
ALA	0.26*	0.23*	0.19*	0.18*	0.14*	0.24*	0.11	0.07
EPA	0.34*	0.35*	0.37*	0.25*	0.17*	0.30*	0.33*	0.40*
DHA	0.38*	0.38*	0.32*	0.26*	0.21*	0.33*	0.31*	0.37*

Pearson's correlation coefficients are expressed, and * means $P < 0.05$. FA (wt%): weight percentage of total fatty acid intake (g/day)
LNA linoleic acid, *ARA* arachidonic acid, *ALA* alpha-linolenic acid, *DHA* docosahexaenoic acid, *EPA* eicosapentaenoic acid

groups, significant positive correlations between serum FA composition and the corresponding FA intake were observed for n-3 PUFA, EPA and DHA in both men and women. For ARA, a positive correlation was only seen among women in their 40 s.

Table 4 shows serum FA concentration (mmol/L) according to age group and sex. Serum FA concentration of n-6 PUFA including LNA and ARA decreased in men; in contrast, n-3 PUFA concentration including EPA, and DHA increased in older age groups in both sexes.

Serum FA composition (mol% of total) of ARA was decreased in older age groups in both sexes: 5.44, 5.04, 5.02, and 4.76 mol% in men; and 5.67, 5.41, 5.22, and 5.07 mol% in women, respectively. In contrast, EPA and DHA increased in older age groups in both sexes: EPA, 1.80, 2.37, 2.91, 2.58 mol% in men, 1.67, 2.35, 2.47, 2.55 mol% in women; and DHA, 3.99, 4.58, 5.19, 5.02 mol% in men, 4.04, 4.61, 5.01, 5.20 mol% in women, respectively (ANOVA $P < 0.05$, trend $P < 0.05$). To account for the effects of FA intake on serum FA compositions, a subsequent model included the corresponding FA intakes (wt%) as covariates. Figure 1 shows serum FA composition (mol% of total) adjusted for corresponding FA intake (wt%) according to age group and sex. Even after adjusting for dietary FA intake, serum n-3 PUFA compositions including EPA and DHA were higher, whereas serum n-6 PUFA compositions including ARA were lower in older age groups for both men and women.

Discussion

Our findings suggest that serum n-3 PUFA compositions, including EPA and DHA, increased, while serum n-6 PUFA compositions, including ARA, decreased with age. These associations were consistent even after adjusting for the corresponding FA intake. To the best of our knowledge, this represents the first observational study to examine age-related effects on serum EPA, DHA, and ARA compositions assessed with reference to dietary intakes among middle-aged and elderly Japanese individuals.

Consistent with previous studies [9–12, 21, 22], blood EPA, DHA, and n-3 PUFA compositions increased and LNA, ARA, and n-6 PUFA compositions decreased with age in both men and women. In addition, FA or fish intake-adjusted EPA or DHA compositions were also positively associated with age group [23, 24], while ARA composition was negatively associated with age group [18]. Three possibilities for these findings can be considered.

First, FA metabolism may change with age. Previous epidemiological studies have indicated that older men show a greater capacity to incorporate dietary EPA into plasma phospholipids than younger men [25], and the capacity to convert ALA acid to EPA and DPA appears to decline with age, since this conversion is down-regulated by a high EPA and DHA diet [26]. An alternative explanation is that aging is associated with increased utilization of n-6 PUFA. Several studies have indicated age-dependent

Table 4 Serum FA concentration (mmol/L) according to age groups by sex

	Men (n = 1,014)				ANOVA[a]	P for trend
	40–49 (n = 241)	50–59 (n = 268)	60–69 (n = 262)	70–79 (n = 243)		
Saturated fatty acids (mmol/L)	3,866 ± 1,164	4,052 ± 1,482	3,821 ± 1,063	3,794 ± 916	0.055	0.185
Monounsaturated fatty acids (mmol/L)	2,622 ± 970	2,737 ± 1,317	2,511 ± 921	2,535 ± 754	0.048	0.097
Polyunsaturated fatty acids (mmol/L)	5,008 ± 962	5,156 ± 1,124	4,903 ± 921	4,827 ± 917	0.001	0.005
n-6 series polyunsaturated fatty acids (mmol/L)	4,185 ± 804	4,151 ± 909	3,825 ± 729	3,794 ± 744	<0.0001	<0.0001
LNA (mmol/L)	3,363 ± 681	3,371 ± 786	3,089 ± 636	3,095 ± 669	<0.0001	<0.0001
ARA (mmol/L)	614 ± 159	583 ± 141	555 ± 129	521 ± 126	<0.0001	<0.0001
n-3 series polyunsaturated fatty acids (mmol/L)	817 ± 291	999 ± 395	1,073 ± 394	1,028 ± 369	<0.0001	<0.0001
ALA (mmol/L)	97 ± 47	110 ± 62	97 ± 41	105 ± 50	0.007	0.395
EPA (mmol/L)	203 ± 110	274 ± 153	319 ± 168	286 ± 154	<0.0001	<0.0001
DHA (mmol/L)	454 ± 154	542 ± 207	581 ± 213	561 ± 189	<0.0001	<0.0001
	Women (n = 1,028)				ANOVA[a]	P for trend
	40–49 (n = 263)	50–59 (n = 259)	60–69 (n = 261)	70–79 (n = 245)		
Saturated fatty acids (mmol/L)	3,338 ± 647	3,706 ± 837	3,853 ± 1,041	3,912 ± 851	<0.0001	<0.0001
Monounsaturated fatty acids (mmol/L)	2,107 ± 516	2,363 ± 688	2,524 ± 1,077	2,631 ± 819	<0.0001	<0.0001
Polyunsaturated fatty acids (mmol/L)	4,682 ± 699	5,122 ± 816	5,191 ± 1,033	5,095 ± 813	<0.0001	<0.0001
n-6 series polyunsaturated fatty acids (mmol/L)	3,975 ± 602	4,186 ± 689	4,149 ± 838	4,015 ± 703	0.001	0.680
LNA (mmol/L)	3,233 ± 526	3,399 ± 620	3,352 ± 743	3,228 ± 620	0.003	0.719
ARA (mmol/L)	569 ± 112	597 ± 116	595 ± 130	581 ± 116	0.027	0.303
n-3 series polyunsaturated fatty acids (mmol/L)	701 ± 223	931 ± 292	1,036 ± 330	1,074 ± 307	<0.0001	<0.0001
ALA (mmol/L)	76 ± 26	92 ± 35	111 ± 148	107 ± 41	<0.0001	<0.0001
EPA (mmol/L)	164 ± 98	259 ± 140	278 ± 125	290 ± 145	<0.0001	<0.0001
DHA (mmol/L)	408 ± 115	513 ± 142	573 ± 145	600 ± 155	<0.0001	<0.0001

All values are expressed as means ± SD

LNA linoleic acid, *ARA* arachidonic acid, *ALA* alpha-linolenic acid, *DHA* docosahexaenoic acid, *EPA* eicosapentaenoic acid

[a] ANOVA, one-way analysis of variance

decreases in membrane levels of n-6 PUFA, particularly for ARA, which is abundant in the hippocampal neurons [6, 7], and consumption of ARA was higher in patients with Alzheimer's disease compared with age-matched healthy volunteers [27, 28]. In addition, the ARA cascade is thought to be altered in a reciprocal fashion to the DHA cascade [28], and Kawabata et al. [29] demonstrated that dietary EPA and/or DHA intakes affect blood ARA levels among Japanese individuals. They indicated the possibility of the displacement and inhibition of ARA incorporation by dietary EPA and DHA in blood phospholipid in elderly subjects. As a result, higher serum n-3 PUFA and lower serum n-6 PUFA were shown among elderly individuals with high dietary intakes of EPA and DHA.

DHA and EPA are important for the prevention of cardiovascular disease [30]. EPA has a more pronounced effects on eicosanoid production [31], whereas DHA has particular effects on membrane properties and cell signaling [32], and ARA is known as a precursor for eicosanoid production, and thus might be involved in inflammation or immunological diseases [28]. The cross-sectional nature of the study did not permit the assessment of causality, and the high levels of DHA and EPA and low levels of ARA might be attributable to increased utilization of ARA under chronic inflammatory conditions among the elderly, and/or an increased capacity to incorporate dietary DHA and EPA into serum lipids to prevent cardiovascular disease through the beneficial effect of n-3 PUFA to cardiovascular health, including lowering blood pressure and improving endothelial health [30]. In fact, weakly positive correlations between serum ARA composition and ARA intake were observed only among females in their 40 s ($r = 0.15$, $P < 0.05$). This result suggests two hypotheses. The first is that utilization of serum ARA might be increased independent of the ARA intake in older age groups compared with females in their 40 s. Second, the effect of displacement and inhibition of ARA incorporation by dietary EPA and DHA was small among females in their 40 s, since n-3 PUFA intake/serum n-3 PUFA concentration, including EPA and DHA, was lower than that in older females.

Potential confounding variables might have attenuated associations between serum FA composition and aging. To

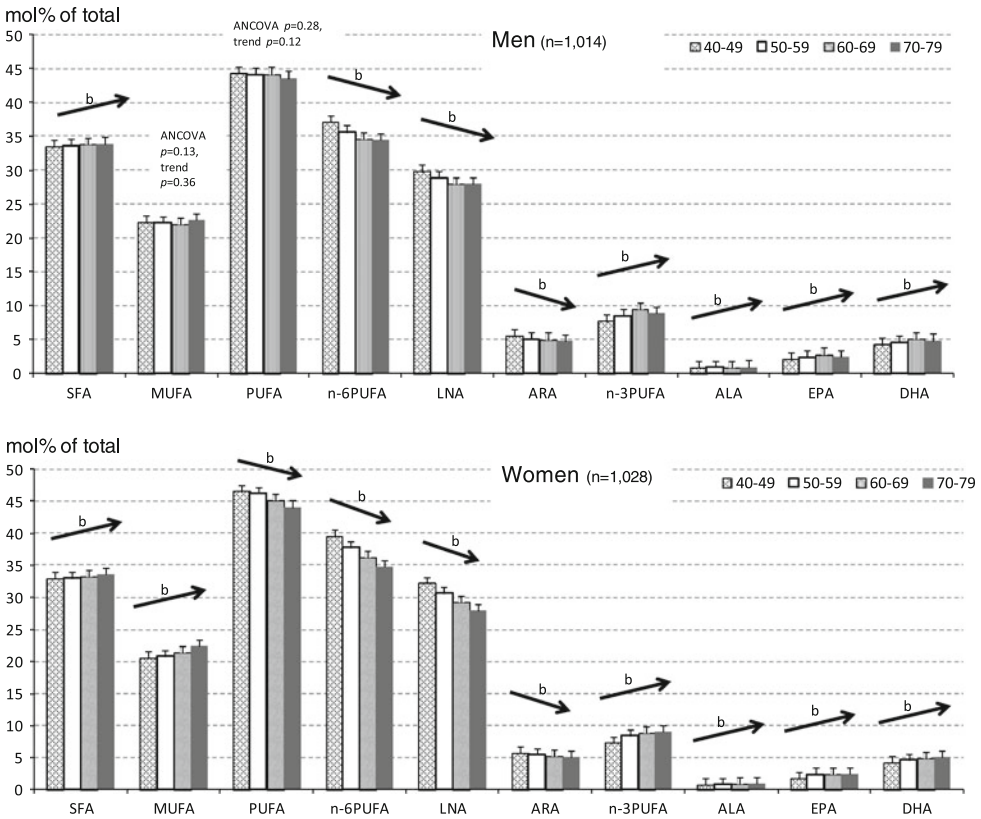

Fig. 1 Serum FA composition (mol% of total) adjusted for corresponding FA intake (wt%)[a] by sex. All values are expressed as means ± SE. [a]FA intake (wt%): weight percentage of total fatty acid intake (g/day). [b]ANCOVA (one-way analysis of covariance) $P < 0.05$ and trend $P < 0.05$. *FA* fatty acids, *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *n-6PUFA* n-6 series polyunsaturated fatty acids, *LNA* linoleic acid, *ARA* arachidonic acid, *n-3PUFA* n-3 series polyunsaturated fatty acids, *ALA* alpha-linolenic acid, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid

exclude the effects of several potential variables, we estimated serum FA composition by age group in sub-analysis after adjusting for education, smoking status, alcohol intake, BMI, and clinical history (heart disease, hypertension, hyperlipidemia, diabetes). No changes in study findings were identified from this sub-analysis (data not shown).

Third, older Japanese tended to eat more a traditional Japanese diet and consume more fish than younger individuals [17]. In fact, marine-derived DHA and EPA consumption among our subjects increased with age. One could speculate that serum FA composition depended, at least in part, on long-term EPA or DHA intake. However, serum or plasma FA composition is considered a reliable index of dietary FA intake over a relatively short duration, such as several weeks or months [21]. In addition, age was an independent predictor of n-3 PUFA levels in red blood cells, even after adjusting for FA intake among American individuals [23] who show low n-3 PUFA intake. Factors other than long-term FA intake could thus be affecting serum FA composition.

In this study, serum FA compositions were expressed as molar percentages of the total FA (mol% of total). However, percentages of n-3 or n-6 PUFA (mol% of total) depend on the absolute quantity of other PUFA (mol/L); that is, they only reflect the relative amount of FA and do not provide a measure of absolute FA concentrations [33]. Therefore, if one of the absolute FA concentrations is higher, the other percentages of FA composition will be reduced. In sub-analyses, we examined age effects on absolute concentrations (mmol/L) of each FA after adjusting for dietary FA intakes (g/day). Even after adjusting for dietary FA intakes, absolute DHA and EPA levels were increased in both men and women, and absolute ARA was decreased in men (data not shown). This means that absolute concentrations of each FA were also dependent on age group.

The strength of this study derives from the use of biomarkers and middle and aged samples from community-dwelling Japanese with high-level n-3 PUFA intake. To the best of our knowledge, no other large-scale epidemiological data have been accumulated to assess dietary intake using 3-day dietary records for middle-aged and elderly individuals aged 40–79 years. Intakes and serum levels of n-3 PUFA, including DHA and EPA, were comparable to those reported in other Japanese populations [9, 29], and higher than those in Europeans and Americans [16]. However, positive or negative associations between serum EPA, DHA or ARA compositions and age groups were seen independently with FA intakes in our sample, despite high n-3 PUFA intake. The present results may therefore also be applicable to American populations with lower consumption of seafood and n-3 PUFA, compared to Japanese or Norwegian populations.

Several limitations to the present study warrant consideration. First, nutritional intakes were assessed by 3-day

dietary records. We did not take supplementation of n-3 PUFA and other FA or medications into account. In addition, it is unclear whether short-term records adequately reflect long-term dietary intake [34], because individual food intakes vary greatly from day to day [20]. On the basis of this limitation, we preliminarily decided on 3 continuous days (both weekend days and 1 weekday) to avoid events or special days such as trips, long vacations, or out-of-the-ordinary events and thus minimize food variations. As a result, average dietary intake in the present study was similar to the National Nutrition Survey in Japan [17]. Although the 3-day dietary record is not the best way to assess long-term dietary intake, it can be considered to have a certain level of accuracy that reflects typical nutrient intakes.

Second, 3-day dietary records were conducted after blood sampling and most subjects returned the records within 1 month, but a time-lag still existed between dietary assessment and the blood sampling. In addition, serum FA concentrations were assessed from a single blood sampling. However, Kobayashi et al. [35] examined correlations between serum phospholipid FA levels collected twice and FA intake assessed from 7-day weighed dietary records among 87 Japanese males, and reported single measurement of serum phospholipids as a useful biomarker of n-3 PUFA. While they used serum phospholipids, Ogura et al. reported PUFA in plasma and erythrocyte phospholipids were nearly identical among 75 Japanese patients admitted for non-malignant diseases [21].

In summary, the present study demonstrated that higher serum EPA and DHA and lower serum ARA compositions were shown among middle-aged and elderly Japanese, and these associations were consistent even after adjusting for the corresponding FA intake. While various measurement errors associated with FA intakes might have been present and the results need to be interpreted with caution, the present findings suggest an effect of age on differences in blood EPA, DHA, and ARA compositions independent of the corresponding FA intake among community-dwelling Japanese men and women.

Acknowledgments We wish to express our sincere appreciation to study participants and colleagues in the NILS-LSA for completing the survey for this study. This work was supported in part by grants from the Japanese Ministry of Education, Culture, Sports, Science and Technology (22790584 to R.O).

Conflict of interest The authors declare that there are no conflicts of interest.

References

1. Wijendran V, Huang MC, Diau GY, Boehm G, Nathanielsz PW, Brenna JT (2002) Efficacy of dietary arachidonic acid provided as triglyceride or phospholipid as substrates for brain arachidonic acid accretion in baboon neonates. Pediatr Res 51:265–272
2. Whelan J (2008) (n-6) and (n-3) Polyunsaturated fatty acids and the aging brain: food for thought. J Nutr 138:2521–2522
3. Youdim KA, Martin A, Joseph JA (2000) Essential fatty acids and the brain: possible health implications. Int J Dev Neurosci 18:383–399
4. Heude B, Ducimetiere P, Berr C (2003) Cognitive decline and fatty acid composition of erythrocyte membranes–The EVA Study. Am J Clin Nutr 77:803–808
5. Conquer JA, Tierney MC, Zecevic J, Bettger WJ, Fisher RH (2000) Fatty acid analysis of blood plasma of patients with Alzheimer's disease, other types of dementia, and cognitive impairment. Lipids 35:1305–1312
6. McGahon B, Clements MP, Lynch MA (1997) The ability of aged rats to sustain long-term potentiation is restored when the age-related decrease in membrane arachidonic acid concentration is reversed. Neuroscience 81:9–16
7. Soderberg M, Edlund C, Kristensson K, Dallner G (1991) Fatty acid composition of brain phospholipids in aging and in Alzheimer's disease. Lipids 26:421–425
8. Lynch MA, Voss KL, Gower AJ (1994) Impaired spatial memory in aged rats is associated with alterations in inositol phospholipid metabolism. NeuroReport 5:1493–1497
9. Kuriki K, Nagaya T, Imaeda N, Tokudome Y, Fujiwara N, Sato J, Ikeda M, Maki S, Tokudome S (2002) Discrepancies in dietary intakes and plasma concentrations of fatty acids according to age among Japanese female dietitians. Eur J Clin Nutr 56:524–531
10. Crowe FL, Skeaff CM, Green TJ, Gray AR (2008) Serum n-3 long-chain PUFA differ by sex and age in a population-based survey of New Zealand adolescents and adults. Br J Nutr 99:168–174
11. Rizzo AM, Montorfano G, Negroni M, Adorni L, Berselli P, Corsetto P, Wahle K, Berra B (2010) A rapid method for determining arachidonic:eicosapentaenoic acid ratios in whole blood lipids: correlation with erythrocyte membrane ratios and validation in a large Italian population of various ages and pathologies. Lipids Health Dis 9:7. doi:10.1186/1476-511X-9-7
12. Saga LC, Liland KH, Leistad RB, Reimers A, Rukke EO (2012) Relating fatty acid composition in human fingertip blood to age, gender, nationality and n-3 supplementation in the Scandinavian population. Int J Food Sci Nutr 1–6. doi:10.3109/09637486.2012.681631
13. Wakai K, Ito Y, Kojima M, Tokudome S, Ozasa K, Inaba Y, Yagyu K, Tamakoshi A (2005) Intake frequency of fish and serum levels of long-chain n-3 fatty acids: a cross-sectional study within the Japan Collaborative Cohort Study. J Epidemiol 15:211–218
14. Hjartaker A, Lund E, Bjerve KS (1997) Serum phospholipid fatty acid composition and habitual intake of marine foods registered by a semi-quantitative food frequency questionnaire. Eur J Clin Nutr 51:736–742
15. Kobayashi M, Sasaki S, Kawabata T, Hasegawa K, Tsugane S (2003) Validity of a self-administered food frequency questionnaire used in the 5-year follow-up survey of the JPHC Study Cohort I to assess fatty acid intake: comparison with dietary records and serum phospholipid level. J Epidemiol 13:S64–S81
16. Steffen BT, Steffen LM, Tracy R, Siscovick D, Jacobs D, Liu K, He K, Hanson NQ, Nettleton JA, Tsai MY (2012) Ethnicity, plasma phospholipid fatty acid composition and inflammatory/endothelial activation biomarkers in the Multi-Ethnic Study of Atherosclerosis (MESA). Eur J Clin Nutr 66:600–605. doi:10.1038/ejcn.2011.215
17. Annual Report of the National Nutrition Survey in 2009, Ministry of Health, Labor and Welfare of Japan, Editor 2012, Daiichi Publishing Co., Tokyo, Japan (in Japanese)

18. Kawabata T, Hirota S, Hirayama T, Adachi N, Hagiwara C, Iwama N, Kamachi K, Araki E, Kawashima H, Kiso Y (2011) Age-related changes of dietary intake and blood eicosapentaenoic acid, docosahexaenoic acid, and arachidonic acid levels in Japanese men and women. Prostaglandins Leukot Essent Fatty Acids 84:131–137
19. Shimokata H, Ando F, Niino N (2000) A new comprehensive study on aging–the National Institute for Longevity Sciences, Longitudinal Study of Aging (NILS-LSA). J Epidemiol 10:S1–S9
20. Imai T, Sakai S, Mori K, Ando F, Niino N, Shimokata H (2000) Nutritional assessments of 3-day dietary records in National Institute for Longevity Sciences–Longitudinal Study of Aging (NILS-LSA). J Epidemiol 10:S70–S76
21. Ogura T, Takada H, Okuno M, Kitade H, Matsuura T, Kwon M, Arita S, Hamazaki K, Itomura M, Hamazaki T (2010) Fatty acid composition of plasma, erythrocytes and adipose: their correlations and effects of age and sex. Lipids 45:137–144. doi:10.1007/s11745-010-3386-3
22. McNamara RK, Liu Y, Jandacek R, Rider T, Tso P (2008) The aging human orbitofrontal cortex: decreasing polyunsaturated fatty acid composition and associated increases in lipogenic gene expression and stearoyl-CoA desaturase activity. Prostaglandins Leukot Essent Fatty Acids 78:293–304. doi:10.1016/j.plefa.2008.04.001
23. Sands SA, Reid KJ, Windsor SL, Harris WS (2005) The impact of age, body mass index, and fish intake on the EPA and DHA content of human erythrocytes. Lipids 40:343–347
24. Itomura M, Fujioka S, Hamazaki K, Kobayashi K, Nagasawa T, Sawazaki S, Kirihara Y, Hamazaki T (2008) Factors influencing EPA + DHA levels in red blood cells in Japan. In Vivo 22:131–135
25. Rees D, Miles EA, Banerjee T, Wells SJ, Roynette CE, Wahle KW, Calder PC (2006) Dose-related effects of eicosapentaenoic acid on innate immune function in healthy humans: a comparison of young and older men. Am J Clin Nutr 83:331–342
26. Burdge GC, Finnegan YE, Minihane AM, Williams CM, Wootton SA (2003) Effect of altered dietary n-3 fatty acid intake upon plasma lipid fatty acid composition, conversion of [13C]alpha-linolenic acid to longer-chain fatty acids and partitioning towards beta-oxidation in older men. Br J Nutr 90:311–321
27. Esposito G, Giovacchini G, Liow JS, Bhattacharjee AK, Greenstein D, Schapiro M, Hallett M, Herscovitch P, Eckelman WC, Carson RE, Rapoport SI (2008) Imaging neuroinflammation in Alzheimer's disease with radiolabeled arachidonic acid and PET. J Nucl Med 49:1414–1421
28. Rapoport SI (2008) Arachidonic acid and the brain. J Nutr 138:2515–2520
29. Kawabata T, Hirota S, Hirayama T, Adachi N, Kaneko Y, Iwama N, Kamachi K, Araki E, Kawashima H, Kiso Y (2011) Associations between dietary n-6 and n-3 fatty acids and arachidonic acid compositions in plasma and erythrocytes in young and elderly Japanese volunteers. Lipids Health Dis 10:138
30. Harris WS, Poston WC, Haddock CK (2007) Tissue n-3 and n-6 fatty acids and risk for coronary heart disease events. Atherosclerosis 193:1–10
31. Bakewell L, Burdge GC, Calder PC (2006) Polyunsaturated fatty acid concentrations in young men and women consuming their habitual diets. Br J Nutr 96:93–99
32. Salem N Jr, Litman B, Kim HY, Gawrisch K (2001) Mechanisms of action of docosahexaenoic acid in the nervous system. Lipids 36:945–959
33. Baylin A, Campos H (2006) The use of fatty acid biomarkers to reflect dietary intake. Curr Opin Lipidol 17:22–27
34. Hu FB (2008) Dietary assessment methods: obesity epidemiology, Oxford University Press, New York
35. Kobayashi M, Sasaki S, Kawabata T, Hasegawa K, Akabane M, Tsugane S (2001) Single measurement of serum phospholipid fatty acid as a biomarker of specific fatty acid intake in middle-aged Japanese men. Eur J Clin Nutr 55:643–650