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ORIGINAL ARTICLE

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

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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

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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×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 (≤9, 10–12, or ≥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		
≤9 years	166, 16.4	200, 19.5
10–12 years	358, 35.3	460, 44.8
≥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

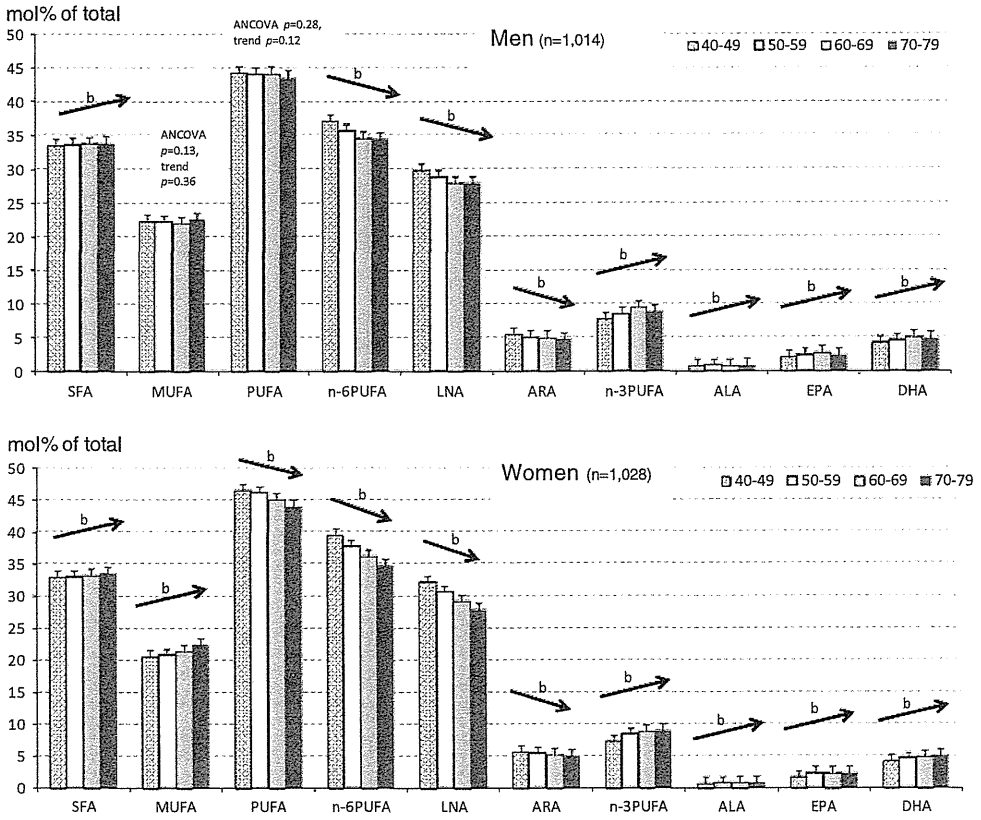

Fig. 1 Serum FA composition (mol% of total) adjusted for corresponding FA intake (wt%)[a] by sex. All values are expressed as means ± SE. [a]FA intake (wt%): weight percentage of total fatty acid intake (g/day). [b]ANCOVA (one-way analysis of covariance) $P < 0.05$ and trend $P < 0.05$. *FA* fatty acids, *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.

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

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中高年者の脳萎縮を抑制する日常歩行量の解明
～地域からの無作為抽出者を対象とした大規模縦断研究～

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The Association between Daily Physical Activity Levels and Brain Atrophy Progression in Middle-aged and Elderly Japanese

by

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ABSTRACT

Brain structural atrophy is associated with impairment in learning function and cognitive function. The purpose of this study was to determine whether daily physical activity prevents age-related brain atrophy progression.

The subjects were 381 males and 393 females who had participated in both the baseline and follow-up examinations (mean duration, 8.2 years). Magnetic resonance imaging of the frontal and temporal lobes was performed at the time of the baseline and follow-up surveys. The number of steps of the subjects was recorded at baseline with uniaxial accelerometry sensors. Multiple logistic regression models were fit to

determine the association between number of steps variables and frontal and temporal lobe atrophy progression while controlling for possible confounders.

In males, the odds ratio of frontal lobe atrophy progression was increased by 1.480 (95% confidence interval [CI], 1.007-2.175)-fold for every 3,000 decrease in the number of steps. The odds ratio of frontal lobe atrophy progression for the fifth quintile compared to the first quintile in the number of steps was 3.651 (95% CI, 1.304-10.219). There were no significant differences between frontal lobe atrophy progression and the number of steps in females. There were also no significant differences between temporal lobe atrophy progression and the number of steps in males and females.

The results indicate that physical activity is significant predictors of frontal lobe atrophy progression over an 8-year period. Promoting participation in activities may be beneficial for attenuating age-related frontal lobe atrophy and for preventing dementia.

要　旨

地域から無作為抽出された中高年者を対象に，日常歩行量が脳萎縮進行に与える影響を検討した．

対象者は「国立長寿医療研究センター・老化に関する長期縦断疫学研究」第2次調査と8年後に実施された第6次調査の両方に参加した，50～79歳の男性381名，女性393名とした．8年間における前頭葉及び側頭葉萎縮の進行状況を，MRI画像より評価した．第2次調査時における歩行量調査を基に，脳萎縮進行を防ぐ歩行量閾値について，ロジスティック回帰分析により検討した．

男性において，歩行量が3,000歩ずつ減少した際の前頭葉萎縮進行のオッズ比は1.480（95%信頼区間，1.007－2.175）であった．また歩行量を5分位とした際の，第5分位に対する第1分位の前頭葉萎縮進行のオッズ比は3.651（95%信頼区間，1.304-10.219）であった．女性では前頭葉萎縮進行と歩行量との間に関連を認めなかった．側頭葉萎縮進行は，男女ともに歩行量との関連を認めなかった．

中高年男性では，前頭葉萎縮進行を予防するために，一日あたり5,800歩以上の歩行量を維持する必要性が示唆された．

緒　言

アルツハイマー病では脳の構造的な萎縮が顕著におこり，認知機能や学習機能に障害をきたす[13]．脳萎縮は加齢によっても進行し，ヒトの脳灰白質量は20歳代から70歳代にかけて，約15%減少することが報告されている[25]．一般高齢者を対象とした6年間の追跡調査では，脳萎縮の進行状態と認知機能レベルは強い関連を示すことが報告されており[20]，脳萎縮を予防することで認知機能の低下や障害の予防に繋がる可能性が示唆されている．

近年では，有酸素運動が神経新生を促進し，脳量の増加，保持に働くことが示されている[8]．高齢者では6ヶ月間の有酸素運動トレーニングによって前頭葉，側頭葉，海馬の脳量が増加したことが報告されている[5]．また，速歩を用いた有酸素性トレーニングは海馬の萎縮を改善するなど[10]，有酸素運動による脳萎縮の予防的効果が示されている．

脳量と有酸素運動の関連性が示される一方で，日常生活における身体活動と脳量の関連については不明な点が多い．中高年者では，身体活動量と有酸素能は相関することが報告されており[1, 4]，日常の身体活動量を高く保つことが脳萎縮の予防へと繋がるものと考えられる．実際に横断研究において，身体活動量と脳量は関連することが報告されているが[3, 11]，日常の身体活動量と脳量及び脳萎縮との関連を検討した縦断研究は見当たらない．近年では，身体活動量の多い高齢者では加齢による認知機能低下のリスクが低いことが，縦断研究によって示されている[21]．日常身体活動による脳萎縮抑制へと繋がる知見が得られれば，身体活動が認知機能低下を抑制することを裏付ける根拠となり，認知機能低下の予防を目的とした身体活動を推奨するためのエビデンスとなると考えられる．

そこで本研究は，無作為抽出された地域在住の中高年者を対象とする約8年間の追跡データを用い，日常歩行量と加齢による脳萎縮進行の関連について検討を行い，脳萎縮進行を抑制する歩行量閾値を解明することで，認知機能低下の予防を目的とした運動処方のエビデンスを作成することを目的とした．

1．方　法

1．1　地域住民におけるデータの収集

本研究は「国立長寿医療研究センター・老化に関する長期縦断疫学研究（NILS-LSA）」の参加者のデータを用いて行われた．NILS-LSAの参加者は長寿医療研究センター周辺の，観察開始時年齢が40歳から79歳までの地域住民約2,300名であり，住民台帳から年齢・性別に層化した無作為抽出によって選定された[22]．選定された者を説明会に招き，調査の目的や方法などを十分に説明し，書面による同意を得た上で調査は実施された．またNILS-LSAは，国立長寿医療研究センター倫理委員会での研究実施の承認を受けた上で実施された．

1．2　対象者

対象者は，ベースラインとしたNILS-LSAの第2次調査（2000年4月から2002年5月まで）を完了した参加者1,545名（男性715名，女性830名）中，その約8年後に実施された第6次調査（2008年7月から2010年7月まで）にも参加した男性514名，女性566名とした．そのうち，脳萎縮の保有率と進行率が他の年代と比較して特に少ない40歳代と，参加人数の少ない80歳代は解析の対象から除外した．また，パーキンソン病既往歴，認知症既往歴，開頭手術歴を有する者についても解析の対象から除外し，最終的な解析の対象は男性381名，女性393名とした．解析対象者のうち，第2次調査時において脳萎縮がグレード4（重度）に該当する者は含まれていなかった．

1．3　頭部核磁気共鳴画像法（MRI）検査

第2次調査時とその8年後に実施された第6次調査時において，頭部MRI検査（Visart 1.5T，東芝）を実施した．頭部MRI検査はRepetition time = 500msec，Echo time = 15msec，Slice thickness = 8mm，Slice gap = 1.5mm，Matrix = 256×256の条件でスキャンを行い，眼窩耳孔線に対し平行となるT1強調画像14枚を得た．

各調査時において得られた頭部MRI画像を基に，前頭葉及び側頭葉についてそれぞれ，萎縮を4段階（1無し；2軽度；3中等度；4重度）に分類した（図1）[16, 23]．さらに第2次調査時と第6次調査時の萎縮グレードを比較し，第6次調査時の萎縮グレードが第2次調査時のものと比較して高い群を「萎縮進行あり群」として，それ以外の場合を「萎縮進行なし群」として分類した．

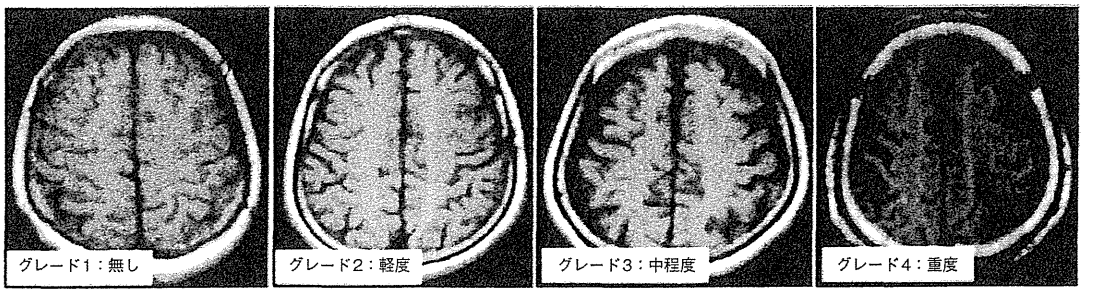

図1　脳萎縮の分類

1．4　歩行量調査

本研究では身体活動量指標として，歩行量を用いた．歩行量調査は，ベースラインに当たる第2次調査時において実施した．歩行量は，加速度計（Lifecorder, スズケン）を対象者の腰部に装着してもらうことで得た．調査期間は，旅行などの特別なイベントの無い7日間とし，入浴時及び就寝時は除外した．得られた7日間の歩行量のうち，最大値と最小値を除外した計5日間分のデータより，一日当たりの平均歩数を算出した．

1．5　対象者の特性に関する検査及び調査

第2次調査時における身長及び体重より，BMIを求めた．体脂肪率は，二重エネルギーX線吸収法による全身スキャンによって算出した（QDR-4500A, Hologic）．一日当たりのアルコール摂取量，現在の喫煙の状況，教育年数は自記式の調査票により得た．脳卒中，虚血性心疾患，糖尿病，高血圧症，脂質異常症の既往歴について，対象者に自記式の調査票への回答を求めるとともに，医師の問診による確認を行った．またNILS-LSAでは，対象者の過去2週間において使用したすべての処方薬及び市販薬について調査を行っている[22]．本研究では，血糖降下薬，降圧薬，脂質降下薬の使用者はそれぞれ，糖尿病，高血圧症，脂質異常症既往歴保有者に分類した．

1．6　統計解析

各変数のデータは，平均値 ± 標準偏差，または標準誤差で示すとともに，t検定または χ^2 検定を用い，群間における差及び分布状況についての比較を行った．対象者の年代と脳萎縮の進行の関係について，Cochran-Mantel-Haenszel 検定を用いて年代上昇による増減傾向を検定した．

ベースラインから8年間の脳萎縮の進行状況と一日平均歩数の関連について，多重ロジスティック回帰分析を用いて検討した．多重ロジスティック回帰分析は，目的変数に萎縮進行の有無を，説明変数として一日平均歩数を投入し，年齢[25]，BMI[11]，教育年数[11]，脳卒中，虚血性心疾患，糖尿病，高血圧症，脂質異常症既往歴の有無[2,7]，現在の喫煙状況[6]，アルコール摂取量で調整した[24]．一日平均歩数は，連続変数，5分位としたカテゴリー変数としてそれぞれ投入し，脳萎縮進行のオッズ比を求めた．解析はStatistical Analysis System ver. 9.3（SAS Institute Inc）を用いて行い，有意水準は5% 未満とした．

2．結　果

2．1　対象者の特性

表1に，ベースライン時における対象者の特性について男女別に示した．平均追跡期間は男女共に8.2± 0.3年であった．年齢，BMI，一日平均歩数は男女間に差を認めなかった．身長及び体重，アルコール摂取量，教育年数は，女性と比較して男性で高値を示した（各 $p<0.0001$）．体脂肪率は，男性と比較して女性で高値を示した（$p<0.0001$）．脳卒中，虚血性心疾患，高血圧症の

表１　対象者の特性

	男性 (n = 381)	女性 (n = 393)	p value
追跡期間（年）	8.2 ± 0.3	8.2 ± 0.3	0.5777
年齢	60.4 ± 7.3	60.8 ± 7.6	0.5421
身長（cm）	164.7 ± 5.4	152.2 ± 5.2	＜0.0001
体重（kg）	62.5 ± 7.1	52.7 ± 7.0	＜0.0001
BMI（kg/m2）	23.0 ± 2.4	22.7 ± 2.9	0.1279
体脂肪率（%）	21.0 ± 4.0	31.3 ± 4.9	＜0.0001
アルコール摂取量（g/day）	16.6 ± 20.9	2.7 ± 6.1	＜0.0001
教育年数（年）	12.3 ± 2.7	11.4 ± 2.3	＜0.0001
一日平均歩数（/day）	7993.2 ± 2588.0	7925.6 ± 2297.1	0.7011
脳卒中既往歴（n）	14(3.7%)	7 (1.8%)	0.105
虚血性心疾患既往歴（n）	13(3.5%)	19 (4.8%)	0.3203
高血圧症既往歴（n）	40(10.5%)	40 (10.2%)	0.8836
脂質異常症既往歴（n）	61(16.0%)	94 (23.9%)	0.006
糖尿病既往歴（n）	32(8.4%)	16 (4.1%)	0.0126
喫煙者（n）	102(26.8%)	27 (6.9%)	＜0.0001

平均値 ± 標準偏差　p 値は t 検定，χ^2 検定による

既往歴保有者の割合は，男女間で差を認めなかった．脂質異常症の既往歴保有者の割合は，男性と比較して女性で高かった（p=0.0060）．糖尿病既往歴保有者，喫煙者割合は，女性と比較して男性で高かった（糖尿病 p=0.0126; 喫煙者 p<0.0001）．

２．２　年代別にみた脳萎縮進行の頻度

表２に，ベースラインから８年間の前頭葉及び側頭葉における萎縮の進行状況について，性，年代別に示した．男性対象者 381 名中 55 名（14.4%），女性対象者 393 名中 35 名（8.9%）に萎縮の進行が認められ，その割合は女性と比較して男性で高かった（χ^2 検定，p = 0.0213）．また，男女とも年代上昇で前頭葉萎縮進行者の割合は増加した（p trend < 0.0001）．側頭葉では，男性対象者 381 名中 100 名（26.3%），女性対象者 393 名中 78 名（19.8%）に萎縮の進行が認められ，その割合は女性と比較して男性で高かった（χ^2 検定，p = 0.0344）．また，男女とも年代上昇で側頭葉萎縮進行者の割合は増加した（p trend < 0.0001）．

２．３　脳萎縮の進行状況と一日平均歩数

図２に，一日平均歩数について，前頭葉及び側頭葉の萎縮進行群別に示した．男性では，前頭葉の萎縮進行なし群と比較して，萎縮進行あり群では一日平均歩数が低値を示した(p = 0.0131)．一方側頭葉では，群間に差を認めなかった．また女性では前頭葉及び側頭葉ともに，一日平均歩数は群間に差を認めなかった．

表２　脳萎縮進行者の年代別分布

		前頭葉萎縮		trend p value	側頭葉萎縮		trend p value
		進行なし	進行あり		進行なし	進行あり	
男性（n）	50-59 歳	176 (95.1%)	9 (4.9%)		156 (84.3%)	29 (15.7%)	
	60-69 歳	112 (79.4%)	29 (20.6%)	＜0.0001	87 (61.7%)	54 (38.3%)	＜0.0001
	70-79 歳	38 (69.1%)	17 (30.9%)		38 (69.1%)	17 (30.9%)	
	計	326 (85.6%)	55 (14.4%)		281 (73.8%)	100 (26.3%)	
女性（n）	50-59 歳	191 (96.0%)	8 (4.0%)		188 (94.5%)	11 (5.5%)	
	60-69 歳	117 (90.0%)	13 (10.0%)	＜0.0001	92 (70.8%)	38 (29.2%)	＜0.0001
	70-79 歳	50 (78.1%)	14 (21.9%)		35 (54.7%)	29 (45.3%)	
	計	358 (91.1%)	35 (8.9%)		315 (80.2%)	78 (19.8%)	

trend p 値は Cochran–Mantel–Haenszel 検定による

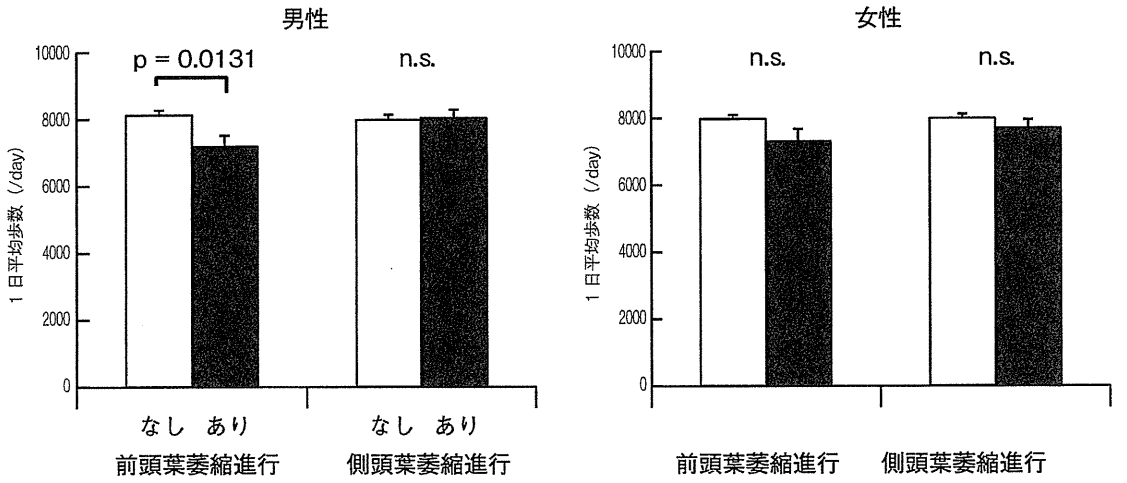

図2　一日平均歩数の比較
平均値±標準誤差　p値はt検定による

2．4　脳萎縮進行のリスクと関連する一日平均歩数

表3及び表4に，前頭葉及び側頭葉における萎縮の進行と，一日平均歩数の関連性について検討したロジスティック回帰分析の結果を示す．男性において，8年後の前頭葉萎縮の進行と一日平均歩数との間に関連性を認めた．一日平均歩数を連続変数（マイナス3,000歩ごと）とした際の前頭葉萎縮進行のオッズ比は，1.480（95%信頼区間，1.007－2.175; p=0.0460）と有意な関連を示した．また一日平均歩数を5分位とし，第5分位を基準した際の各分位の前頭葉萎縮進行のオッズ比を求めたところ，第1分位におけるオッズ比は3.651（95%信頼区間，1.304－10.219; p=0.0072）と有意な関連を示した．一方，側頭葉萎縮進行と一日平均歩数との間に関連を認めなかった．女性では，前頭葉及び側頭葉のいずれも，萎縮の進行と一日平均歩数との間に関連を認め

表3　男性対象者における脳萎縮進行のオッズ比

		n	前頭葉萎縮進行		側頭葉萎縮進行	
			オッズ比（95%信頼区間）	p value	オッズ比（95%信頼区間）	p value
1日平均歩行量	-3000歩ごと	381	1.480（1.007－2.175）	0.046	0.979（0.742－1.290）	0.8787
	Q1: 5736.0歩未満	76	3.651（1.304－10.219）	0.0072	0.938（0.435－2.024）	0.6269
	Q2: 5736.0－6955.0歩未満	76	1.261（0.383－3.863）	0.3108	1.100（0.519－2.330）	0.8715
	Q3: 6955.0－8261.4歩未満	76	1.487（0.471－4.689）	0.6501	1.142（0.538－2.425）	0.7501
	Q4: 8261.4－10407.4歩未満	76	2.403（0.819－7.052）	0.2874	1.123（0.528－2.389）	0.8039
	Q5: 10407.4歩以上	77	1.00（基準）		1.00（基準）	

年齢，BMI，教育年数，脳卒中，虚血性心疾患，糖尿病，高血圧症，脂質異常症既往歴の有無，喫煙状況，アルコール摂取量で調整

表4　女性対象者における脳萎縮進行のオッズ比

		n	前頭葉萎縮進行		側頭葉萎縮進行	
			オッズ比（95%信頼区間）	p value	オッズ比（95%信頼区間）	p value
1日平均歩行量	-3000歩ごと	393	1.298（0.766－2.197）	0.3323	0.961（0.656－1.407）	0.8361
	Q1: 5825.2歩未満	78	1.559（0.420－5.791）	0.78	0.879（0.355－2.178）	0.8452
	Q2: 5825.2－7090.0歩未満	79	2.269（0.627－8.209）	0.1784	0.789（0.311－2.005）	0.5798
	Q3: 7090.0－8374.0歩未満	78	0.826（0.181－3.769）	0.2578	0.825（0.317－2.147）	0.7003
	Q4: 8374.0－9910.4歩未満	79	1.887（0.505－7.053）	0.426	1.206（0.489－2.974）	0.3522
	Q5: 9910.4歩以上	79	1.00（基準）		1.00（基準）	

年齢．BMI，教育年数，脳卒中，虚血性心疾患，糖尿病，高血圧症，脂質異常症既往歴の有無，喫煙状況，アルコール摂取量で調整

なかった.

3．考　察

地域から無作為に抽出された中高年者を対象とし，加齢による脳萎縮進行と関連を示す一日平均歩数について縦断解析を行った結果，男性では前頭葉萎縮進行と一日平均歩数との間に関連を認めた（表4）．一日平均歩数を連続変数とした際の前頭葉萎縮進行のリスクは，歩数が3,000歩ずつ減少するごとに約1.5倍ずつの上昇を示し，日本人の中高年男性では日常の歩行量を高く保つことで，加齢による前頭葉萎縮の進行を抑制する可能性が示唆された．さらに一日平均歩数を5分位とし，歩行量が最も多い群（10,407.4歩以上）を基準として，各分位における前頭葉萎縮進行のリスクを検討したところ，歩行量が最も少ない群（5,736.0歩未満）では前頭葉萎縮進行のリスクが約3.7倍高いことが示された．このことから，男性では前頭葉萎縮の進行を予防する日常の歩行量の最少閾値が，約5,800歩付近に存在している可能性が考えられた．一般に歩行量は加齢に伴い減少することが知られている．日本人男性の一日平均歩数は，50歳代が7,772歩，60歳代が6,949歩，70歳以上が4,707歩と報告されており[17]，70歳以降の男性では前頭葉萎縮の進行リスクが他の年代と比較して特に高いことが推察される．従って日本人の中高年男性では，一日の歩数を概ね5,800歩以上に保つこと，また特に70歳以降において歩行量を増やすことが，加齢による前頭葉の萎縮進行の予防において重要である可能性が示唆された．

対照的に，女性では加齢による前頭葉萎縮進行と日常の歩行量の間に関連を認めなかった（表4）．一般的に，男性は女性と比較して脳萎縮の頻度は高い[25]．そして実際に本研究においても，男性では前頭葉萎縮進行の頻度が女性と比較して高く（表2），身体活動の効果が男性でより明確化したことが考えられる．また，テストステロンやエストロゲンなどの性ホルモンについても，脳量に影響を及ぼす因子であることが報告されており[8,15]，身体活動に対する脳の可塑性には性差が存在する可能性も考えられる．

本研究はヒトを対象とし，脳萎縮の進行についてMRI画像を基に評価した非侵襲的研究であることから，身体活動が前頭葉萎縮進行を抑制したメカニズムを明らかにすることはできない．マウスの脳ではアミロイドβの蓄積量と活動量との間に関連が認められることが報告されており[14]，身体活動が高いことでアミロイドβの蓄積が抑制された可能性が考えられる．また，身体活動により神経細胞の増殖や生存に不可欠とされる成長因子の発現量が変動した可能性もある[26]．

運動が脳量に与える効果は，前頭葉に限らず，側頭葉や頭頂葉，海馬など多くの脳領域に及ぶことが報告されている[3,5,11]．興味深いことに，本研究では前頭葉と側頭葉を脳萎縮進行の評価の対象としたが，高い身体活動量との関連は前頭葉に限られている．脳における神経新生を促す要因として，脳血流量の増加が指摘されている[19]．そして身体運動は脳血流量を変動させるが，その変動様式は運動の種類や強度により異なるとされる[12,18]．本研究は加速度計を用いて得た歩数を身体活動レベルの指標としており，身体活動の種類や強度などは考慮されていない．今後は，身体活動の種類や強度などの影響を考慮した上で，さらなる検討を行う必要があると思われる．

4．まとめ

本研究は地域から無作為に抽出された50歳から79歳までの男女774名を対象に，日常歩行量と加齢による脳萎縮進行の関連について，縦断的に検討した．その結果，男性において日常の

歩行量を高く保つことが，前頭葉萎縮進行を抑制することが示された．また5,800歩が，前頭葉萎縮の進行を抑制する一日当たりの歩行量閾値として示され，認知機能低下の予防に繋がる身体活動量の目標値の一つとなる可能性が示唆された．

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資　料

地域在住中高年男女における性・年齢群別の血清脂肪酸構成比率

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要旨：地域から無作為抽出した一般住民における年齢群別の血清脂肪酸構成比率を明らかにすることを目的とした。対象者は「国立長寿医療研究センター・老化に関する長期縦断疫学研究（NILS-LSA）」の第5次調査（2006-2008年）に参加し，空腹時採血による血清中脂肪酸24分画を測定した40-88歳の男性1,070名，女性1,098名である。血清脂肪酸構成比率は各脂肪酸について脂肪酸総量（μg/mL）に占める各脂肪酸の濃度（μg/mL）を重量比（wt%）で示した。性別の各年齢群（40，50，60，70，80-歳）の脂肪酸構成比率は，男女ともに高齢群ほどα-リノレン酸，EPA，DHAを含むn-3系多価不飽和脂肪酸は増加し，リノール酸やアラキドン酸を含むn-6系多価不飽和脂肪酸は減少した。女性では高齢群ほど飽和脂肪酸と一価不飽和脂肪酸が増加した。年齢階級の上昇に伴い血清脂肪酸構成比率が変化する可能性が示唆された。

キーワード：血清脂肪酸，日本人，中高年男女，年齢群

認知症は日常生活や社会生活に支障を来す深刻な老年病のひとつであり，高齢化に伴い今後患者数が増大することが懸念されている[1]。n-3系多価不飽和脂肪酸のドコサヘキサエン酸（docosahexaenoic acid：DHA）やエイコサペンタエン酸（eicosapentaenoic acid：EPA），n-6系多価不飽和脂肪酸のアラキドン酸は哺乳類の脳や神経細胞の膜を構成する主要な脂肪酸であり，脳機能の維持に重要と考えられている[2]。最近，米国在住の非認知症患者において赤血球のDHA濃度が低いことがその後の脳容量の低下と関連したこと[3]，仏国の地域在住高齢者において血漿中のEPAが4年間の認知症病態に伴う脳萎縮と逆相関したことが報告されており[4]，血中脂肪酸が認知機能と関連する可能性が示唆されている[5]。

我々は認知機能低下のリスクが高まる中高年者を対象とし，血中脂肪酸と認知機能との関連を疫学的に明らかにする予定であるが，海馬のリン脂質中アラキドン酸量やDHA量は高齢者ほど低かったことや[6]，日本人において高齢群は若年群に比し血中EPAおよびDHAが高く，アラキドン酸が低かったことが報告されており[7]，血中脂肪酸と認知機能との関連性を検討する上で，第一に年齢群による血中脂肪酸構成を明らかにする必要があると考える。

日本人は世界の中でも海産物摂取量が多いため，DHAやEPAなどのn-3系多価不飽和脂肪酸濃度が他民族に比し高く[8]，日本人の中高年者は，他民族とは異なる脂肪酸構成を有している可能性がある。これまでに日本人の女性栄養士において高齢群（51-66歳）は中年群に比し血漿中のEPA，DHA構成比が高かったことや[7]，赤血球あるいは血漿リン脂質中のアラキドン酸構成比が高齢群（56-75歳）の男女では20歳代に比し低かったことなどが報告されているが[9]，80歳代も含めた地域在住の一般住民における性・年齢群別の脂肪酸構成は明らかでない。そこで本研究は，地域から無作為に抽出された一般住民における年齢群別の血清脂肪酸構成比率を明らかにすることを目的とした。

方　法

1. 対　象

対象者は「国立長寿医療研究センター・老化に関する長期縦断疫学研究（National Institute for Longevity Sciences - Longitudinal Study of Aging：NILS-LSA）」の第5次調査（2006-2008年）に参加した地域在住中高年者で

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ある。NILS-LSAは，愛知県大府市および知多郡東浦町在住の住民（2005年10月1日国勢調査公表の人口[10]：大府市80,262人，東浦町48,046人）から，年齢および性別で層化無作為抽出した一般地域住民約2,400名（初回調査参加時40-79歳）を対象に1997年の第1次調査以降，追跡中のドロップアウトを性別・年齢層ごとに新たに補充しながら，約2年に一度繰り返し追跡調査を実施している[11]。無作為抽出にあたっては，研究対象集団約2,400名を構成する性・年齢群（40，50，60，70歳代）別の人数が約250-300名程度を維持できるよう見積もった上で，研究参加を呼び掛ける対象者を住民台帳に基づき無作為抽出している。第5次調査では，第4次調査までの参加者に加え，追跡中のドロップアウトを補充するために新たな対象者を住民台帳から性・年齢群別に層化無作為抽出し，郵送により調査説明会への参加を呼びかけ（郵送配布者数に対する説明会参加率は約3割），説明会参加者のほぼ全数から調査参加への同意を得た。

本研究は，NILS-LSA第5次調査に参加した2,419人（男性1,200人，女性1,219人）のうち，下記に示す空腹時採血を行い（2,366人），解析に必要な項目に欠損のない40歳から88歳の男性1,070名，女性1,098名の計2,168人を解析対象者とした。

なおNILS-LSAは，国立長寿医療研究センター倫理委員会で承認を得ており（承認番号No. 369-2），前述のように参加対象者に事前に説明会を行い，参加者全員の文書による同意を得て実施している。

2. 血清コレステロール・トリグリセリドおよび血清脂肪酸24分画の測定

12時間以上の空腹時採血による血液は，30分室温静置後，3,000 rpmにて15分間遠心分離し，血清を分離した。その後速やかに，血清を冷蔵状態と−80℃の凍結状態に分けた（2006年7月-2008年7月）。

冷蔵状態の血清は，採血後速やかに株式会社SRL分析センターにて，トリグリセリドは酵素法（GK-GPO・遊離グリセロール消去），総コレステロールは脱水素酵素（UV）法，HDLコレステロールは直接法により測定を行った。

−80℃で凍結保存した血清は，2010年の7月から10月にかけて，凍結状態で株式会社SRLに血清脂肪酸24分画の測定を依頼した。全脂質中の脂肪酸24分画は，血清からFolch法により脂質を抽出後，加水分解，メチル化処理を行った後，ガスクロマトグラフィにより検量線法を用いて測定した。血清脂肪酸構成比率は，各脂肪酸について，脂肪酸総量（μg/mL）に占める各脂肪酸の濃度（μg/mL）を重量比（wt%）で示した。

3. その他の調査項目

第5次調査参加日（2006年7月-2008年7月）に，調査センターにて身長，体重を測定し，Body Mass Index（BMI; kg/m^2）を算出した。また，調査参加日に全対象者に，栄養素等摂取量を把握するための3日間の食事秤量記録調査（3DR）への協力を依頼した。具体的には，使い捨てインスタントカメラとはかり，記録用紙を配布し，記録方法の説明を行い，調査参加日以降1カ月以内の特別食（行事食）を含まない休日1日と連続する平日2日，計3日間の食事秤量記録調査および記録用紙の返却を依頼した。返却された記録用紙と写真をもとに，専属の管理栄養士が全食品のコーディング作業を行った後，五訂増補食品成分表に基づき栄養素等摂取量を算出した[12]。

自記式質問票により喫煙の有無，既往歴（高血圧，高脂血症，糖尿病）と，食物摂取頻度調査票により過去1年間の平均的な飲酒量を把握した。

4. 統計解析

すべての解析は性別毎に行った。年齢群は5群（40，50，60，70歳代と80歳以上）に分類した。年齢群によるBMI，栄養素等摂取量，血清脂質，および血清脂肪酸構成比率の差は，一般線形モデルを用い群間差の有無と傾向性の検定により評価した。傾向性の有無は，年齢群における結果変数が年齢群の上昇とともに有意に増減しているかを検討するために，GLMプロシジャ（分散分析）を用い，線形パラメーターとして年齢群5群に“−2，−1，0，1，2”をあてはめ，対比による結果変数の平方和および自由度(1)に基づくF値の有意差検定をもって評価した。

年齢群における既往歴，喫煙状況の割合の差はカイ二乗検定または，人数が5以下のカテゴリがある場合はコクランマンテルヘンツェル検定により評価した。カテゴリ変数の年齢群における比率の傾向性の検定は，コクランアーミテージ検定を用いた。

解析にはSAS 9. 3（SAS Institute, Cary, NC, USA）を用い，$p < 0.05$を統計的有意とみなした。

結　　果

性・年齢群別の対象者の特性を表1に示した。BMIの平均値は男性の50歳代の23.8 kg/m^2，女性の70歳代の22.9 kg/m^{2}が頂値を示した。栄養素等摂取量は，男女ともに年齢群が上昇するに従い，エネルギー摂取量，たんぱく質摂取量，脂質摂取量が低下し，脂肪酸摂取量では男女ともに飽和，一価不飽和，n-6系多価不飽和脂肪酸摂取量が減少した。n-3系多価不飽和脂肪酸摂取量は男女ともに年齢群の上昇に伴う有意な増減は認められなかった。

表2に性・年齢群別の血清中脂肪酸総量，コレステロール濃度，トリグリセリド濃度を示した。男性では脂肪酸総量，総コレステロール，トリグリセリドともに50歳代で頂値を示した。女性では脂肪酸総量，トリグリセリドが70歳代で頂値を示し，HDLコレステロールは高齢群ほど低下した。

表3に性・年齢群別の血清脂肪酸構成比率を示した。測定対象の脂肪酸24分画のうち，一価不飽和脂肪酸の