Relationship Between Aging, Menopause, and Eicosapentaenoic Acid/Arachidonic Acid Ratio in Women With Dyslipidemia in Tokyo

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Original

Relationship Between Aging, Menopause, and Eicosapentaenoic Acid/Arachidonic Acid Ratio in Women With Dyslipidemia in Tokyo

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Background: The serum eicosapentaenoic acid (EPA)/arachidonic acid (AA) ratio (sEAR), which is affected by diet, is negatively correlated with the risk of coronary artery disease. The effects of aging and menopause on sEAR in women with dyslipidemia in an urban environment were investigated.

Methods: To compare sEAR and serum fatty acids in 24 fractions, 89 patients were categorized into age tertiles as follows: group A, < 52 years (n = 28); group B, 52-58 years (n = 30); and group C, > 58 years (n = 31). Altogether, 82 participants with menstrual data were investigated to compare the sEAR between 66 postmenopausal patients (group M) and 16 premenopausal patients (group P).

Results: The sEAR, serum EPA, and docosahexaenoic acid (DHA) levels were significantly higher in group C than in the other groups. The weight ratios of linoleic acid and docosatetraenoic acid were significantly lower in group C than in group A. However, sEAR was not significantly different between groups M and P.

Conclusions: Among women in an urban area, the sEAR was significantly higher in the oldest group than in the other groups, with no effects on menopause. These results may be caused by the difference in the intake of EPA and DHA by age, rather than by menopause.

Keywords: serum eicosapentaenoic acid/arachidonic acid ratio (serum EPA/AA ratio), serum fatty acids in 24 fructions, women, menopause, dietary habits

Introduction

Several reports have revealed an association between the serum eicosapentaenoic acid (EPA)/arachidonic acid (AA) ratio (sEAR) and coronary artery disease.[1-7] A lower sEAR is reportedly associated with a greater risk of coronary heart disease.[1,2] EPA is an oceanic ω-3 polyunsaturated fatty acid (PUFA), and arachidonic acid is one of the ω-6 PUFAs. Since EPA and arachidonic acid (AA) are essential fatty acids in a broad sense, their contents in

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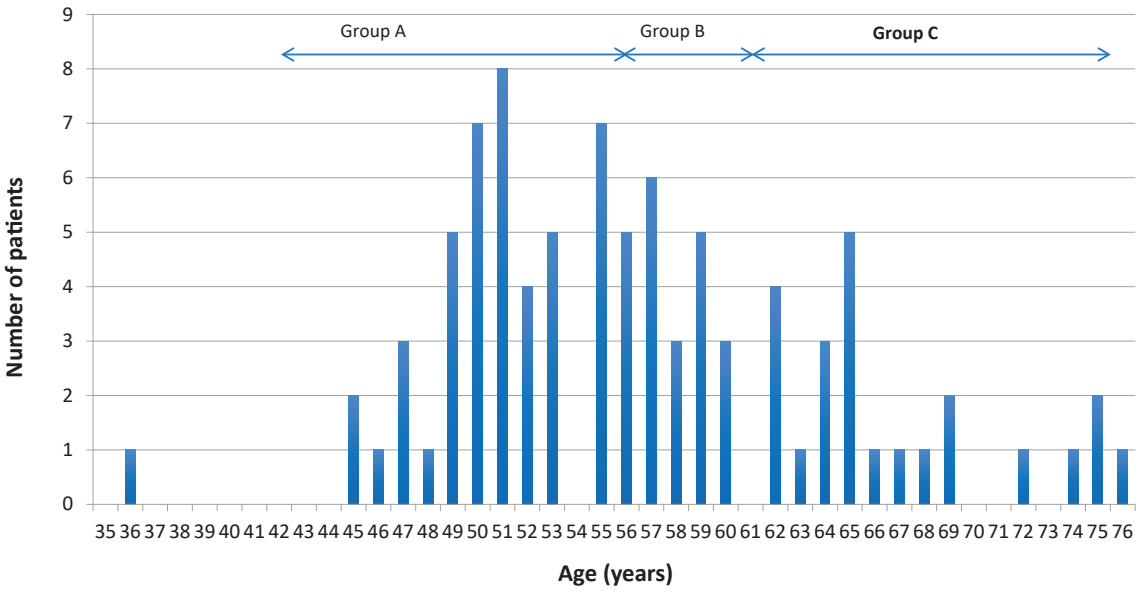

Figure 1. Age distribution of all patients. The participants were 89 female patients aged between 35 and 76 years (average 56.5 years) with hyperlipidemia whose serum free fatty acid concentrations were measured.
Group A, lower age tertile; Group B, middle age tertile; Group C, upper age tertile.

the body depend on oral intake. Recently, the dietary habits of Japanese people have changed, which may be accompanied by changes in urban lifestyles and an increase in the number of women working in society. However, studies on sEAR in women around the menopausal period living in metropolitan areas are scarce, although several cases involving women in the countryside have been recorded.[2-4] In the present study, we examined the effects of aging and menopause on sEAR in female patients with dyslipidemia living in a metropolitan area. Therefore, this study aimed to investigate the following research points: the sEAR in female patients with dyslipidemia in the metropolitan area by age; the serum fatty acids in 24 fractions (sFA24F, which are free fatty acids contained in foods ingested daily and are involved in health) according to age; and any effects of menopause on sEAR.

Materials and Methods

The participants were 89 female patients with dyslipidemia who visited our clinic between January 2012 and 2015, and whose weight ratio (%) of sFA24F was measured. The age distribution ranged from 36 to 76 years [mean 56.6 years (standard deviation, SD 7.6)]. Women treated for dyslipidemia other than EPA were included. Meanwhile, the exclusion criteria were taking EPA; history of ischemic heart disease, cerebrovascular events, arteriosclerosis obliterans, surgery for gynecological diseases, hormone replacement therapy, or intake of oral contraceptive/low-estrogen progestin; and received treatment for malignant diseases.

This study has been approved by the Ethics Committee of Tokyo Women's Medical University (approval number 3884-R).

The clinical records of the participants, including age, body mass index (BMI), low-density lipoprotein cholesterol (LDL-C) level, and menopausal status, were examined retrospectively and evaluated as patient attributes. The weight ratio (%) of sFA24F was compared.

First, the 89 participants were divided into three groups by age: group A [n = 28, 36-51 years, mean 48.7 years (SD 3.1)]; group B [n = 30, 52-58 years, mean 55.1 years (SD 2)]; and group C [n = 31, 59-76 years, mean 65 years (SD 5.1)] (**Figure 1**). The sEAR and weight ratios (%) of sFA24F of EPA and AA (%) (SRL Inc.'s gas-chromatograph method) were compared among the three groups, and significant differences were determined by a one-way analysis of variance (ANOVA) and the Tukey's test. Statistical significance was set at $p < 0.05$. The

Table 1. Background characteristics of the three groups.

	Group A n = 28	Group B n = 30	Group C n = 31
Mean (SD) of age (years)	48.7 (3.1)	55.1 (2.0)	65.0 (5.1)
Age range (years)	36-51	52-58	59-76
Mean (SD) of BMI (kg/m^2)	23.3 (5.7)	21.8 (4.2)	22.7 (2.5)
Mean (SD) of LDL-C (mg/dL)	135.3 (26.8)	142.5 (30.3)	125.4 (26.9)
Mean (SD) of TG (mg/dL) [a]	106.4 (58.3)	122.0 (61.4)	134.3 (75.3)
Mean (SD) of HDL-C (mg/dL) [b]	69.5 (18.2)	64.5 (12.4)	59.6 (12.0)
DM (person)	4	5	6
HT (person)	5	7	10

BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; DM, diabetes mellitus; HT, hypertension; SD, standard deviation; Group A, bottom third of age; Group B, middle third of age; Group C, upper third of age.

[a]n = 74 (Group A, 25; Group B, 27; Group C, 22).

[b]n = 76 (Group A, 28; Group B, 26; Group C, 22).

weight ratio was used instead of the weight as the former allows easier comparison owing to the variation of the total amount of free fatty acids in the blood between individuals depending on nutritional status and physical constitution.

Subsequently, the weight ratio (%) of each fatty acid in the entire sFA24F was measured in all the 89 patients. The measurement results were compared among the three groups (A, B, and C) using the one-way ANOVA and Tukey's test. Differences were considered significant at $p < 0.05$.

After excluding seven women without information on menstrual status, 82 of the 89 patients were divided into the post-menopausal group and pre-menopausal group and compared. The postmenopausal group (group M) was defined as follows: patients who had amenorrhea for at least one year, and had follicle-stimulating hormone levels > 40 mIU/mL and E2 < 20 pg/mL. The remaining participants were included in the pre-menopausal group (group P). Patients with secondary amenorrhea were excluded from this study.

The sEAR and the respective weight ratios (%) of EPA and AA were compared using the Student's *t*-test (*t*-test) between the two unpaired groups. A p value ≤ 0.05 was considered significant.

Results

1. Background characteristics of the three age groups

The average age, age distribution, BMI, and LDL-C levels of the three groups are presented in **Table 1**.

No significant differences in BMI and LDL-C levels were observed among the three groups in the one-way ANOVA ($p = 0.402$ and $p = 0.064$, respectively).

2. Comparison of sEAR among the three groups by age

The mean sEAR for was 0.35 ± 0.20 for all patients (89 patients), 0.30 ± 0.19 for group A, 0.29 ± 0.14 for group B, and 0.44 ± 0.22 for group C (**Figure 2**). No significant difference in the mean sEAR was observed between groups A and B ($p = 0.866$). Group C had a significantly higher mean sEAR than group A ($p = 0.010$). Furthermore, group C had a significantly higher mean sEAR than group B ($p = 0.005$).

3. Comparison of the weight ratio (%) of serum EPA in sFA24F among the three groups by age

The weight ratio (%) of serum EPA in the sFA24F was 1.82% ± 1.01% in group A, 1.85% ± 0.78% in group B, and 2.62% ± 1.14% in group C (**Figure 3**). No significant difference in the weight ratio (%) of serum EPA in sFA24F was observed between groups A and B ($p = 0.988$). The weight ratio (%) in sFA24F of serum EPA

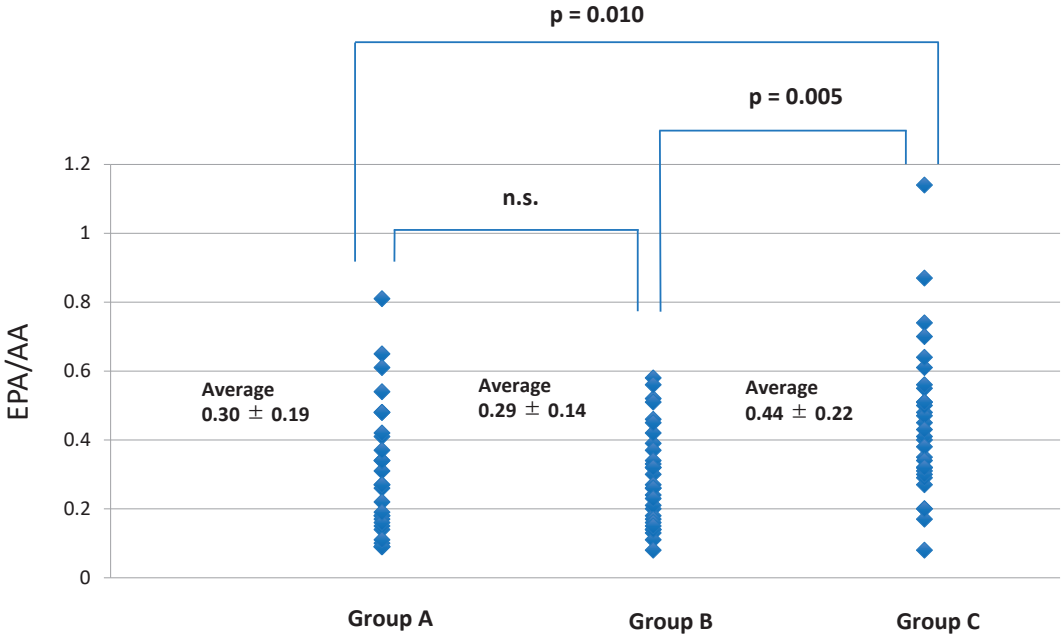

Figure 2. Serum EPA/AA ratios of the three groups.
n.s., not significant; EPA/AA, serum eicosapentaenoic acid/arachidonic acid ratio; p, p-value on one-way analysis of variance.

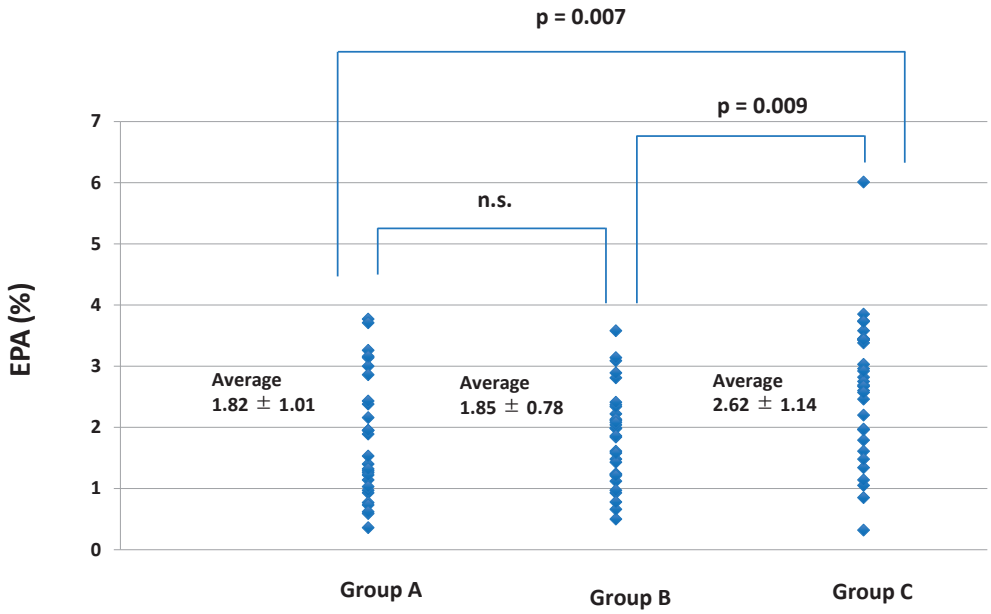

Figure 3. Ratio (%) of EPA in all serum fatty acids in the three groups.
n.s., not significant; EPA (%), ratio of eicosapentaenoic acid to all serum fatty acids; p, p-value on one-way analysis of variance.

was significantly higher in group C than in groups A or B ($p = 0.007$ and $p = 0.009$, respectively).

4. Weight ratios of each fatty acid in whole sFA24F (%)

The mean weight ratio (%) of each sFA24F was compared among the three groups (A, B, and C), and the results for fatty acids that demonstrated significant differences among the three groups are presented in **Table 2**. The weight ratios (%) of linoleic acid, docosatetraenoic acid, and lignoceric acid in sFA24F were significantly lower in group C than in group A (**Table 3**). The weight ratios (%) of EPA and docosahexaenoic acid (DHA) in sFA24F were significantly higher in group C than in

Table 2. The percentage of all fatty acids of EPA and fatty acids was significantly different between age groups.

FFA	Group	Mean (SD) %	p value in one-way analysis of variance	Significant difference in Tukey HSD
Linoleic acid (ω6)	A	30.55 (4.68)	p = 0.025	A > C (p = 0.018)
	B	29.10 (3.04)	NEV	A vs B (p = 0.323)
	C	27.77 (3.68)		B vs C (p = 0.372)
EPA (ω3)	A	1.82 (1.01)	p = 0.003	C > A (p = 0.007)
	B	1.85 (0.78)	NEV	C > B (p = 0.009)
	C	2.62 (1.14)		A vs B (p = 0.989)
Behenic acid	A	0.74 (0.13)	p = 0.001	A > C (p = 0.001)
	B	0.71 (0.14)	NEV	B > C (p = 0.012)
	C	0.62 (0.11)		A vs B (p = 0.737)
DTA (ω6)	A	0.16 (0.04)	p = 0.037	A > C (p = 0.045)
	B	0.16 (0.04)	NEV	A vs B (p = 0.901)
	C	0.14 (0.04)		B vs C (p = 0.112)
Lignoceric acid	A	0.67 (0.15)	p = 0.038	A > C (p = 0.030)
	B	0.63 (0.13)	NEV	A vs B (p = 0.476)
	C	0.58 (0.13)		B vs C (p = 0.323)
DHA (ω3)	A	4.29 (1.51)	p = 0.002	C > A (p = 0.014)
	B	4.09 (1.32)	NEV	C > B (p = 0.002)
	C	5.34 (1.38)		A vs B (p = 0.856)

FFA, free fatty acids; HSD, honest significant difference; EPA, eicosapentaenoic acid; DTA, docosatetraenoic acid; DHA, docosahexaenoic acid; NEV, not equal variances; SD, standard deviation.

Table 3. Percentage of fatty acids with no significant differences in each age group.

	Group A mean (SD) (%)	Group B mean (SD) (%)	Group C mean (SD) (%)	p value
Lauric acid	0.09 (0.07)	0.14 (0.12)	0.11 (0.10)	0.248
Myristic acid	0.80 (0.28)	0.87 (0.25)	0.92 (0.32)	0.287
Palmitic acid	21.71 (1.85)	21.71 (1.54)	21.88 (1.79)	0.904
Stearic acid	7.16 (0.66)	7.29 (0.52)	7.14 (0.59)	0.528
Arachidic acid	0.29 (0.05)	0.30 (0.04)	0.28 (0.04)	0.447
Myristoleic acid	0.03 (0.03)	0.04 (0.03)	0.04 (0.03)	0.252
Palmitoleic acid	21.71 (1.85)	21.71 (1.54)	21.88 (1.79)	0.763
Oleic acid	18.77 (3.19)	19.93 (2.35)	19.79 (3.29)	0.287
Eicosenoic acid	0.14 (0.03)	0.15 (0.03)	0.16 (0.04)	0.195
Erucic acid	0.03 (0.02)	0.03 (0.02)	0.03 (0.02)	0.926
Nervonic acid	1.24 (0.27)	1.17 (0.30)	1.23 (0.32)	0.566
Alpha-linolenic acid	0.82 (0.52)	0.80 (0.29)	0.96 (0.40)	0.284
Docosapentaenoic acid	0.60 (0.14)	0.58 (0.13)	0.66 (0.17)	0.063
γ-Linolenic acid	0.33 (0.16)	0.40 (0.18)	0.32 (0.13)	0.132
Eicosadienoic acid	0.21 (0.04)	0.20 (0.04)	0.21 (0.03)	0.402
Dihomo-γ-linolenic acid	1.27 (0.42)	1.32 (0.30)	1.19 (0.32)	0.334
Arachidonic acid	6.31 (1.21)	6.62 (1.47)	6.02 (1.51)	0.255
Eicosatrienoic acid	0.07 (0.03)	0.08 (0.04)	0.07 (0.04)	0.197

SD, standard deviation.

groups A and B. The weight ratio (%) of behenic acid in sFA 24 F was significantly lower in group C than in groups A and B.

5. Serum AA weight fraction in sFA24F (%)

The average weight ratio (%) of serum AA in sFA24F was 6.31% ± 1.21% in group A, 6.62% ± 1.47% in group B, and 6.02% ± 1.51% in group C (**Table 3**), with no significant difference among the three groups (p = 0.255 by one-way ANOVA).

Table 4. Background characteristics of the two groups.

	Group M n = 66	Group P n = 16	
Mean (SD) of age (years)	58.6 (7.2)	48.6 (4.1)	
Age range (years)	45-76	36-53	
Mean (SD) of BMI (kg/m^2)	22.2 (3.0)	24.3 (5.4)	(p = 0.471)
Mean (SD) of LDL-C (mg/dL)	132.8 (26.9)	137.4 (34.1)	(p = 0.560)
Mean (SD) of TG (mg/dL) [a]	119.8 (63.8)	125.9 (73.3)	(p = 0.396)
Mean (SD) of HDL-C (mg/dL) [b]	62.9 (12.6)	67.9 (20.6)	(p = 0.754)
DM (person)	14	1	
HT (person)	16	3	

BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; DM, diabetes mellitus; HT, hypertension; SD, standard deviation; Group M, postmenopausal patients; Group P, non-postmenopausal patients; p, p-value of the unpaired *t*-test.

[a]n = 71 (Group M, 56; Group P, 15).

[b]n = 72 (Group M, 57; Group P, 15).

6. Background characteristics of groups M and P

Group M was the postmenopausal group (n = 66), with ages ranging from 45 to 76 years (mean age: 58.6 ± 7.2 years). Group P was the pre-menopausal group (n = 16), with ages ranging from 36 to 53 years (mean age: 48.6 ± 4.1 years) (**Table 4**). No significant differences in BMI, LDL-C, triglycerides, or high-density lipoprotein cholesterol were observed between the two groups.

7. sEAR and weight ratios of serum EPA and AA in sFA24F (%) in groups M and P

No significant differences in sEAR and in the EPA and AA weight ratios (%) in sFA24F were noted between groups M and P (p = 0.561, p = 0.267, and p = 0.134, respectively) (data not shown).

Discussion

1. Value of sEAR in female patients with dyslipidemia in metropolitan areas by age

The mean sEAR for all patients in this study was 0.35 ± 0.20, which is lower than the mean reported by Kitagawa et al. (0.42 ± 0.34) for women.[8] In the present study, the mean sEAR ratio was significantly higher in the upper age tertile group than in the other two age groups. Thus, no significant difference in the weight ratio (%) of AA in sFA24F was identified among the three groups. Group C demonstrated a significantly higher weight ratio (%) of serum EPA in the sFA24F group than the other two groups. Therefore, the weight ratio (%) of EPA in sFA24F may have affected the sEAR in this study. Here, the sEAR was significantly higher in the upper age tertile group than in the other groups, with no significant difference between the lower and middle age tertile groups. EPA is derived from α-linolenic acid, an essential fatty acid of the n-3 series, and is synthesized in the body. However, EPA is an essential fatty acid in a broad sense because the required amount cannot be provided by conversion alone. It is mainly found in marine products, and its concentration in the blood depends on oral intake. Of the three groups divided by age, the upper tertile group had a higher oral intake of EPA than the other groups, or the lower and middle age tertile groups (mainly in their 40s and 50s) may have had a lower intake of EPA.

For serum AA levels, the present data revealed no significant differences among the age groups. By contrast, according to the report by Kitagawa et al.,[8] serum AA levels were significantly higher in patients in the 50s group, and then demonstrated a downward trend thereafter. AA is an essential fatty acid in a broad sense because linoleic acid, which is an n-6 essential fatty acid, is converted in the body and cannot be provided in the necessary amount by conversion alone. It is abundant in eggs and meat, and its concentration in the blood depends on oral intake. In the results of the abovementioned two studies[8,9] and this study, sEAR tended to generally increase with age, although slight differences were ob-

served in the relationship between age and sEAR in each study. Thus, both EPA and AA levels in the blood may be affected by oral intake.

In a study examining the relationship between fish intake frequency and n-3 fatty acid levels in the blood, a relationship with fish intake frequency was observed not only in Japanese but also in Caucasian, African American, Hispanic American, and Chinese American populations. It has been reported that the concentration of n-3 fatty acids in the blood increases as intake increases.[10, 11] According to a report by the Ministry of Health, Labor and Welfare, the intake of seafoods by women all over Japan (per person per day) is approximately 53 g between the ages of 20 and 49 years, 72.5 g for those aged 50-59 years, 86.0 g for those aged 60-69 years, and 82.1 g for those aged ≥70 years; thus, the intake of seafoods increases as age increases.[12] Chung et al. have reported that a higher fish intake was associated with a higher concentration of n-3 fatty acids in the blood.[10] Similar to a previous study,[13-15] the present study revealed that group C (aged between 59 and 72 years, mean age 65 years) had higher EPA/AA ratios than the other groups, because the participants in this group are suspected to have consumed more seafood and to have had less opportunities of eating out than the other groups. As lifestyle habits are changing, the number of people consuming seafood may also increase.

2. sFA24F by age

The weight ratio (%) of the sFA24F of EPA (ω3 series) and DHA (ω3 series) was significantly higher in the older group (group C) than in the other two groups. The weight ratios (%) of linoleic acid (ω6 series), docosatetraenoic acid (ω6 series), and lignoceric acid in the sFA24F were significantly higher in the younger group (group A) than in the older group (group C). The weight ratio (%) in the sFA24F to behenic acid was significantly higher in the younger and middle-aged groups than in the older group. No significant difference in arachidonic acid levels (ω6 series) was observed among the three groups.

EPA is an essential fatty acid in a broad sense, synthesized from α-linolenic acid as a raw material. Therefore, most of the EPA concentrations in the blood reflect oral intake. DHA is also an essential fatty acid in a broad sense synthesized from EPA, using α-linolenic acid as a raw material. Similar to EPA, most DHA concentrations in the blood reflects its ingestion. Older age groups may have higher oral EPA and DHA intakes than other age groups.

Oral intake affects the concentrations of linoleic acid, an essential n-6 fatty acid and docosatetraenoic acid, which is synthesized from linoleic acid, in the blood. Decreases in the weight ratios (%) of serum linoleic and docosatetraenoic acids in sFA24F in the older adult group may indicate that their oral intake was low. The decrease in the weight ratio of linoleic acid to docosatetraenoic acid in the older adult group may be related to the increase in the weight ratio of EPA to DHA.

Lignoceric acid is a saturated fatty acid with 24 carbon atoms (C24), with one synthesized from palmitic acid having 16 carbon atoms (C16) via behenic acid (C22) by the elongation of fatty acids and one ingested from the diet.

Behenic acid is a saturated fatty acid with 22 carbon atoms (C22), and is synthesized by extending palmitic acid and those taken from the diet.

Higher levels of lignoceric and behenic acid concentrations in the blood have been demonstrated to reduce the risk of heart failure. Studies in older people have suggested that groups with higher lignoceric acid and behenic acid levels in the blood have a better prognosis than those with lower levels.[15-17] In the present study, no significant difference in the weight ratio (%) of palmitic acid in sFA24F was observed among the three groups classified by age. However, behenic and lignoceric acid levels, which are synthesized after elongation, tended to be higher in the younger group, indicating that the oral intake of behenic and lignoceric acids was higher in the younger group. Moreover, the synthesis of behenic or lignoceric acids from palmitic acid may be promoted in younger age groups. Lignoceric acid is found in small amounts in most fats, especially in peanut oil. Behenic acid is often found in cosmetics, such as seeds, shampoos, and facial cleansers. More detailed dietary surveys are needed to elucidate the differences of saturated fatty acids, such as lignoceric and behenic acids, in different age groups.

3. Effects of menopause on sEAR

n-3PUFAs are reportedly involved in estrogen metabo-

lism, especially in human breast cancer cells, where n-3PUFAs affect the promotion of intracellular estrogen-induced apoptosis and suppress cancer cell growth. Moreover, n-3PUFAs may be involved in estrogen degradation (metabolism or excretion).[18, 19] In rats, circulating estrogens have been reported to enhance plasma and tissue levels of n-3PUFAs, and dietary fish oil intake of n-3PUFAs promoted cytochrome p450, which is involved in estrogen metabolism. Estrogen may be reduced by n-3PUFAs.[18-20]

Kitagawa et al. have reported that the EPA/AA ratio in women was significantly higher than that in men between the ages of 30 and 39 years and similar to that in men between the ages of 40 and 49 years with no significant difference. Moreover, it was significantly lower in women than men between the ages of 50 and 70 years.

Here, the essential fatty acids EPA and DHA in the n-3 series were higher in the older adult group than in the other groups, and in this group, the intake of seaweeds containing the n-3 system was higher than in the other groups. Thus, the group may have eaten more fish. Even among urban women, older women may be able to consume fish relatively easily compared with other age groups. Considering the Ministry of Internal Affairs and Communications' report that people in larger cities eat out more often than those in smaller municipalities because women in the Tokyo metropolitan area spend more time working in the morning and returning to work; eating out at a restaurant or convenience store may be associated with a lower chance of consuming fish and seaweeds, although long commutes and other reasons are possible. However, the content of meals was not evaluated in this study, so further research is necessary in the future to confirm this hypothesis along with analyzing the impact of employment, working hours, and meal content.

4. Limitations

This study had some limitations. First, the number of patients in the pre-menopausal group was small, which may have caused the lack of difference between the two groups. Second, this retrospective study used data from a single institute, which may have resulted in potential bias. Third, as the effect of menopause was insignificant, the effect may not be detectable. Lastly, the issue on multiplicity analysis may have not been completely eliminated despite ranking the second research point below the first research point based on clinical importance.

Conclusion

Among menopausal women in a metropolitan area, the sEAR was higher in the older adult group than in the other groups. Serum EPA and DHA levels, which are essential fatty acids of the n-3 family, were higher in the older adult group than in the other groups. The sEAR between the menopausal and non-menopausal groups had no significant differences. Thus, menopausal women in younger age groups in the Tokyo metropolitan area may consume less fish and shellfish containing n-3 fatty acids than the older adult population.

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Conflicts of Interest: The authors declare that there are no conflicts of interest.

Author Contributions: MS contributed to data acquisition, data analysis, statistical analysis, data interpretation, and partial drafting of the manuscript in English; takes responsibility for the accuracy of the data analysis and the literature search; has full access to all the data in the study; and takes responsibility for its integrity. MK (the second author) contributed to the study design, data acquisition, data analysis, statistical analysis, data interpretation, and writing in English; the second author is the guarantor of this work and, as such, had full access to all the data, analyses, and interpretation. NK contributed to the data acquisition and literature search. MK (the fourth author) and KS contributed to the data interpretation. All authors contributed to the final approval of the version to be published. Parts of this study were previously presented at the International Gender Medicine Congress, Sendai, Japan, held on September 14-16, 2017.

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Ethical Approval: This study was approved by the Ethics Committee of Tokyo Women's Medical University (approval

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