Sex Differences in the Association between the Eicosapentaenoic Acid/Arachidonic Acid Ratio and the Visceral Fat Area among Patients with Type 2 Diabetes

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Abstract

Objective To examine the serum levels of eicosapentaenoic acid (EPA) and the ratios of docosahexaenoic acid (DHA), and the EPA/arachidonic acid (AA) and DHA/AA and to clarify their association with the areas of subcutaneous and visceral fat separately by sex among patients with type 2 diabetes.

Methods The study participants included 118 men and 96 women who were hospitalized to receive treatment for type 2 diabetes. We examined the serum levels of EPA and DHA and the ratios of EPA/AA and DHA/AA, and analyzed their association with the total fat area (TFA), subcutaneous fat area (SFA), and visceral fat area (VFA), as measured by computed tomography.

Results The mean age of the study participants was 62.6 ± 13.6 years. The mean HbA1c level was $9.37 \pm 2.27\%$. Among men, a multivariate regression analysis adjusted for age and BMI, revealed a significant negative association between VFA and the EPA/AA ratio. When the multivariate regression analysis was adjusted for age, BMI, and HbA1c level, VFA was still found to be significantly negatively associated with the EPA/AA ratio. Although a crude analysis revealed a significant negative association between SFA and the EPA/AA ratio in women, no association was observed in multivariate regression analyses.

Conclusion These results suggest the possibility that EPA inhibits the accumulation of visceral fat in men. Furthermore, there appear to be marked differences in the relationships between EPA and DHA and visceral fat accumulation.

Key words: type 2 diabetes, eicosapentaenoic acid, arachidonic acid, docosahexaenoic acid, visceral fat area

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Introduction

Obesity is known to induce low-grade inflammation and insulin resistance (1, 2), conditions which lessen the efficacy of type 2 diabetes treatment. The accumulation of visceral fat plays a central role in diseases related to obesity, such as atherosclerosis (3). In addition, waist circumference (as a representation of abdominal obesity) is one of several important clinical criteria for the diagnosis of metabolic syndrome. However, the cut-off value for abdominal obesity differs between men and women, because the distribution of subcutaneous and visceral fat differs between the

sexes (4-6).

In epidemiological studies and clinical trials, fish oils rich in n-3 polyunsaturated fatty acids (PUFAs) have been demonstrated to reduce the incidence of coronary heart disease (7, 8). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are two of the most well-known n-3 PUFAs. In contrast, arachidonic acid (AA), one of the chief sources of n-6 PUFAs, has been reported to be a source of mediators that cause inflammation through the synthesis of vasoactive eicosanoids in humans (9). Of note, the Japan EPA Lipid Intervention Study (JELIS) reported that treatment with highly purified EPA taken in conjunction with a statin significantly reduced the incidence of major coronary

events in comparison to treatment with a statin alone (10). A report from the Hisayama Study (11) showed that a lower serum EPA/AA ratio is associated with a greater risk of cardiovascular disease among subjects with high levels of high-sensitivity C-reactive protein (hs-CRP).

One important reason for the protective effects of n-3 PUFAs against atherosclerotic disease is the anti-inflammatory effects that these fatty acids are reported to exert (12). A separate study reported that the n-3/n-6 ratio was negatively correlated with the insulin-resistance status of subjects with metabolic syndrome (13). Accordingly, n-3 PUFAs might have a preventive effect against cardiovascular or metabolic diseases by canceling the inflammatory effects of visceral fat obesity. However, the relationships between EPA and DHA and the visceral fat area (VFA) are not well understood, because it is difficult to measure VFA and SFA separately.

The aim of this retrospective study was to examine the serum levels of EPA and DHA, and the ratios of EPA/AA and DHA/AA, and investigate their association with body composition (including the areas of subcutaneous and visceral fat) separately by sex using the data of patients who were hospitalized to receive treatment for type 2 diabetes.

Materials and Methods

Study population

The study participants included 118 men and 96 women who had been hospitalized to receive treatment for type 2 diabetes at Hiroshima Red Cross Hospital & Atomic-bomb Survivors Hospital at some point between April 2012 and December 2014. Subjects with a serum creatinine level of >3 mg/dL, a history of cardiovascular disease, who were currently taking EPA or DHA medications, or who were being treated for type 2 diabetes using pioglitazone, sodium-glucose cotransporter 2 (SGLT-2) inhibitors, or glucagon-like peptide-1 (GLP-1) analogues were excluded from the study. A medical interview was conducted to ensure that all of the subjects were free of infectious symptoms, autoimmune diseases, and any other acute conditions. This study was approved by the ethical committee of the Hiroshima Red Cross Hospital & Atomic-bomb Survivors Hospital.

Measurements

At the beginning of hospitalization, we measured the patients' height, weight, and waist circumference in the standing position. We calculated the body mass index (BMI) for each patient as body weight (kg) divided by the square of standing height (m).

CT (SYMBIA T6; Siemens, Erlangen, Germany) was performed with subjects in a supine position (130 kV, 100 mA, section thickness: 5 mm, scanning time: 3 s, field of view: 250 mm, gantry rotation time: 600 ms) at the beginning of hospitalization. The total fat area (TFA), SFA, and VFA were measured using a high-quality two-dimensional computed

tomography image analysis system (Synapse Vincent, Fuji Film, Tokyo, Japan) (14).

Blood and urine samples were taken from all participants after overnight fasting. The measurement of the participants' EPA, DHA, and AA levels was outsourced to SRL (Tokyo, Japan). In brief, plasma lipids were extracted by Folch's procedure, and fatty acids (tricosanoic acid, C23:0, as the internal standard) were methylated with boron trifluoride and methanol. The methylated fatty acids were then determined and analyzed using a capillary gas chromatograph (Shimadzu GC-2010, Kyoto, Japan).

Statistical analysis

The results were expressed as the mean $\pm$ standard error. Since the triglyceride, BMI, EPA, DHA, AA, TFA, SFA, and VFA data were not normally distributed, these data were analyzed after logarithmic transformation. Continuous variables were compared using an age-adjusted analysis of variance (ANOVA) for comparisons between men and women. The TFA, SFA, and VFA values were each compared as dependent variables by regression analyses in order to assess their association with the serum EPA/AA or DHA/AA ratio and the serum EPA, DHA, or AA level as an independent variable in an unadjusted model. Multivariate analyses were performed, adjusting for age, BMI, and HbA1c as potential confounders. These indexes were adopted because BMI is an important anthropometric marker reflecting body fat mass, and because HbA1c is an important marker of glucose status in patients with type 2 diabetes. The data of the male and female patients were analyzed separately. The associations between the categorical data were evaluated using the χ^2 test. The SAS software program (version 8.2, SAS Institute, Cary, USA) was used for all of the statistical analyses.

Results

The clinical characteristics of the study subjects

The mean duration of type 2 diabetes (since diagnosis) was 13.3 ± 12.4 years. The mean age of the participants was 62.6 ± 13.6 years. The mean HbA1c was $9.37 \pm 2.27\%$. The mean serum EPA, DHA, AA levels, and EPA/AA and DHA/AA ratios were 63.3 ± 32.5 $\mu\text{g/mL}$, 143.1 ± 44.2 $\mu\text{g/mL}$, 202.9 ± 65.1 $\mu\text{g/mL}$, and 0.33 ± 0.17 and 0.73 ± 0.22 , respectively. The mean TFA, SFA, and VFA values were 268.1 ± 136.0 cm^2 , 155.9 ± 100.0 cm^2 , and 111.6 ± 56.3 cm^2 , respectively. The clinical characteristics of the study participants, separated by sex, are shown in Table 1.

The associations between the areas of total, visceral and subcutaneous fat and serum levels of EPA, DHA, and AA (separated by sex)

We first performed simple and multivariate regression analyses by gender. In the crude analyses of the male participants, TFA and VFA, but not SFA, were found to be significantly negatively associated with the serum EPA level

Table 1. The Baseline Clinical Characteristics.

	Men	Women	p value
N	118	96	0.133
Age (years)	61.7±14.1	63.6±13.0	0.314
Duration of type 2 diabetes	13.1±13.9	13.6±10.3	0.855
BMI (kg/m ²)	24.2±3.9	25.1±5.0	0.115
Body weight (kg)	68.0±12.8	59.6±14.4	<0.0001
Systolic blood pressure (mmHg)	130.2±16.2	131.8±18.5	0.650
Diastolic blood pressure (mmHg)	74.1±12.8	70.6±11.9	0.059
HbA1c (%)	9.55±2.34	9.16±2.18	0.360
Total cholesterol (mg/dL)	198.2±46.1	204.8±55.4	0.346
Triglycerides (mg/dL)	169.6±104.3	131.9±73.3	0.006
HDL cholesterol (mg/dL)	52.3±18.0	61.9±18.6	0.0003
AST (U/L)	31.1±28.4	29.1±24.2	0.668
ALT (U/L)	33.4±27.6	30.4±28.3	0.596
BUN (mg/dL)	15.2±6.0	14.7±4.4	0.342
Creatinine (mg/dL)	0.91±0.30	0.71±0.17	<0.0001
Uric acid (mg/dL)	5.44±1.67	5.05±1.44	0.235
EPA (μg/mL)	61.3±31.6	65.7±33.5	0.390
DHA (μg/mL)	141.4±44.3	145.2±44.3	0.626
AA (μg/mL)	199.9±66.9	206.7±62.9	0.307
EPA/AA ratio	0.33±0.18	0.32±0.15	0.952
DHA/AA ratio	0.74±0.23	0.72±0.20	0.422
TFA (cm ²)	241.9±128.2	300.3±144.4	0.0005
VFA (cm ²)	115.0±59.4	107.6±52.2	0.353
SFA (cm ²)	126.1±77.3	192.5±112.3	<0.0001

Data are expressed as the mean and standard error or number.

The data were analyzed using the Chi-square test or by an analysis of variance adjusted for age.

TFA: total fat area, VFA: visceral fat area, SFA: subcutaneous fat area, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid, AA: arachidonic acid

($\beta=-0.273$, $p=0.031$, $\beta=-0.287$, $p=0.034$, and $\beta=-0.283$, $p=0.198$, respectively), but not with the serum levels DHA or AA. No significant associations were found in multivariate regression analyses adjusted for age and BMI, or adjusted for age, BMI, and HbA1c.

Among women, no associations were found to exist with the three fat areas.

The associations between the areas of total, visceral and subcutaneous fat and the EPA/AA and DHA/AA ratios (separated by sex)

Next, to clarify the associations between TFA, VFA and SFA with the EPA/AA and DHA/AA ratios, we performed simple and multivariate regression analyses by gender (Table 2). In the crude analyses, TFA and VFA were found to be significantly negatively associated with the EPA/AA ratio ($\beta=-0.314$, $p=0.008$, $\beta=-0.324$, $p=0.010$, and $\beta=-0.336$, $p=0.102$, respectively) in men, but not with the DHA/AA ratio ($\beta=-0.365$, $p=0.076$, $\beta=-0.332$, $p=0.132$, and $\beta=-0.428$, $p=0.231$, respectively).

In multivariate regression analyses adjusted for age and BMI, only VFA was significantly negatively associated with EPA/AA ($\beta=-0.224$, $p=0.043$, $\beta=-0.159$, $p=0.068$, and $\beta=-0.086$, $p=0.637$, respectively). This significant negative association remained after the adjustment for age, BMI, and HbA1c ($\beta=-0.228$, $p=0.041$).

Among women, in the crude analyses, TFA and SFA were found to be significantly negatively associated with the EPA/AA ratio ($\beta=-0.385$, $p=0.006$, $\beta=-0.461$, $p=0.004$, and $\beta=-0.272$, $p=0.242$, respectively), but not with the DHA/AA ratio. However, no associations were observed in the multi-

variate analyses adjusted for age, and BMI, or for age, BMI, and HbA1c.

Discussion

In the present study, the EPA/AA ratio (but not the serum levels of EPA or DHA or the DHA/AA ratio), was significantly negatively associated with VFA (but not TFA or SFA), as assessed by CT measurement, in the male participants. To the best of our knowledge, this is the first study to find any sex differences in the association between the n-3 fatty acids and subcutaneous or visceral fat.

BMI is widely used as an immediate indicator in the diagnosis of obesity (15). That the EPA/AA ratio was negatively associated with VFA, even after adjustment for age and BMI, suggests that the anti-inflammatory potential of EPA (16, 17) directly affects visceral fat through the inhibition of the adipose tissue inflammation that is associated with obesity (18) - independent of anthropometric factors. Indeed, in rodent models of obesity, EPA was found to increase the production of adiponectin, possibly through the attenuation of the inflammatory changes and the reduction of adipocyte cell size (19). Thus, the negative association between the EPA/AA ratio and VFA that was observed in men might have been (at least partially) because the high serum level of EPA (relative to the level of AA), leads to a reduction of adipose tissue weight through the anti-inflammatory effects of EPA. In addition, visceral and subcutaneous adipocytes are known to have different biological characteristics, which are represented by the expression of lipid metabolism-related genes, such as hormone sensitive li-

Table 2. The Associations between the EPA/AA and DHA/AA Ratios and the Areas of Total, Visceral and Subcutaneous Fat.

	Crude		Age and BMI adjusted		Age, BMI and HbA1c adjusted	
	β	p	β	p	β	p
Men						
TFA						
EPA/AA	-0.314	0.008	-0.159	0.068	-0.165	0.058
DHA/AA	-0.365	0.076	-0.051	0.731	-0.069	0.644
VFA						
EPA/AA	-0.324	0.010	-0.224	0.043	-0.228	0.041
DHA/AA	-0.332	0.132	-0.097	0.610	-0.112	0.557
SFA						
EPA/AA	-0.336	0.102	-0.086	0.637	-0.102	0.570
DHA/AA	-0.428	0.231	0.040	0.897	-0.008	0.979
Women						
TFA						
EPA/AA	-0.385	0.006	-0.000	0.999	0.007	0.945
DHA/AA	-0.272	0.242	0.165	0.277	0.170	0.260
VFA						
EPA/AA	-0.242	0.089	-0.049	0.707	-0.033	0.799
DHA/AA	-0.022	0.925	0.238	0.226	0.251	0.188
SFA						
EPA/AA	-0.461	0.004	0.014	0.901	0.016	0.893
DHA/AA	-0.400	0.132	0.122	0.481	0.123	0.479

β : Standardized regression coefficient. TFA: total fat area, VFA: visceral fat area, SFA: subcutaneous fat area, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid, AA: arachidonic acid

Figure. A schematic illustration of the assumed mechanism of the sex differences in the association between EPA/AA or DHA/AA ratio and the areas of subcutaneous and visceral fat. The gender-based differences in the anti-inflammatory effects of EPA or DHA on adipose tissue may be a key factor.

pase (HSL), and peroxisome proliferator-activated receptor (PPAR)-gamma (20). The characteristics of EPA include the stimulation of HSL phosphorylation (21) and the promotion of the binding of PPAR-gamma to PPAR-response element (22). Accordingly, the effect of EPA on adipocytes might be demonstrated more strongly in VFA than in SFA.

In contrast with EPA, no associations between DHA and SFA or VFA were found. The reason for the difference in the results of these n-3 fatty acids is unclear. The serum

EPA level increases and decreases rapidly in response to the consumption of fish oils, a rich source of n-3 fatty acids, whereas DHA responses are more subdued (23). Accordingly, a high serum EPA level, rather than DHA level, might be an effective surrogate marker of high fish or fish oil consumption, which may lead to body weight loss (24), or the consumption of other unmeasured nutrition components derived from fish oils might affect adipose tissue. Consequently, a high EPA/AA ratio might be correlated with

smaller fat areas. In addition, the anti-atherosclerotic effect of DHA is controversial (11, 25). This study may indicate that serum EPA and DHA play different roles in adipose tissue.

The reason why the negative correlation between the EPA/AA ratio and VFA was found in men but not in women is also unclear. One report demonstrated an association between EPA/AA level and VFA (as measured by the impedance method) in men (26). However, that particular report did not examine women. In the Framingham Heart Study, abdominal adiposity, especially VFA, was observed to be more strongly associated with CRP in women than in men (2). Accordingly, the anti-inflammatory effects of EPA may be comparatively weak or insufficient relative to the serum levels in women. In addition, physiological sexual differences regarding body composition may have affected our results. Estrogen is known to affect adipose tissue hypertrophy and body composition (27-29). Consequently, this may lead to increased visceral adipose tissue in postmenopausal women (30). Accordingly, there is a possibility that the effect of EPA on visceral fat in women may be weakened by hormonal changes. Several mechanisms could be proposed (Figure), however, further study is needed to clarify the reasons for the differences between the sexes.

This study is associated with several limitations. First, the data were derived from hospital-based, cross-sectional patient information, which makes it difficult to evaluate cause and effect. Second, although it may have had an impact on our results, we did not investigate n-3 PUFA consumption through dietary intake was not investigated in our study. In addition, certain medication might have modified our data because all of the subjects had been diagnosed with type 2 diabetes and were already taking medication. However, the subjects taking thiazolidine, SGLT-2 inhibitors, GLP-1 analogues, or n-3 fatty acid supplements and those who had a history of cardiovascular disease or who were experiencing severe renal failure were excluded from the study. Third, the testing of the n-3 fatty acid levels and CT examinations were conducted on only one occasion for each participant.

In summary, we accurately assessed SFA and VFA using CT, and found a negative association between n-3 fatty acids and these areas. EPA appears to inhibit the accumulation of visceral fat, especially in men. Moreover, we found evidence that the effects of EPA and DHA on visceral fat might be significantly different.

The authors state that they have no Conflict of Interest (COI).

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