Original article

High concentrations of omega-3-fatty acids are associated with the development of atrial fibrillation in the Japanese population

Short title: Impact of EPA and DHA on the Development of AF

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Total word count: 4753

Abstract

Background: The favorable effect of fish oils rich in n-3 polyunsaturated fatty acids (PUFAs) on the development of atrial fibrillation (AF) is controversial. The relationship between the serum concentrations of n-3 PUFAs and the incidence of AF is unclear; therefore, in the present study, we aimed to elucidate this relationship. **Methods:** We evaluated the serum concentrations of n-3 PUFAs in 110 patients with AF, 46 patients with ischemic heart disease (IHD) and no AF, and 36 healthy volunteers. Thirty-six patients had a history of IHD (IHD-AF group) and 74 did not (L-AF group). **Results:** The eicosapentaenoic acid (EPA) levels in the L-AF group were higher than those in the IHD-AF and control groups (117 ± 64 μg/ml, 76 ± 30 μg/ml, and 68 ± 23 μg/ml, respectively); the docosahexaenoic acid (DHA) levels showed the same pattern (170 ± 50 μg/ml, 127 ± 27 μg/ml, and 126 ± 35 μg/ml, respectively). In both the L-AF and IHD-AF groups, the EPA levels in patients with persistent and permanent AF were higher than those in patients with paroxysmal AF (L-AF: 131 ± 74 μg/ml vs 105 ± 51 μg/ml; IHD-AF: 82 ± 28 μg/ml vs 70 ± 33 μg/ml). Multivariate analysis showed that cases of AF were associated with higher levels of EPA but not DHA. **Conclusion:** In this Japanese population study, the EPA and DHA levels in patients with L-AF were higher than those in normal subjects. In particular, the EPA level was associated with the incidence of AF. These findings suggest that an excess of EPA might be a precipitating factor of AF.

Keywords: Atrial fibrillation; Fatty acids; Omega-3 fatty acids; Risk factors

Introduction

Two n-3 polyunsaturated fatty acids (n-3 PUFAs) found in fish oil, i.e., eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have recently become commercially available as dietary supplements. The favorable effects of n-3 PUFA on cardiac disorders have been demonstrated. Frequent intake of fish oils decreases the incidence of ischemic cardiovascular disease [1]. The balance of EPA/arachidonic acid (n-6 PUFA) is related to the events of ischemic cardiovascular disease [2]. Furthermore, regular daily ingestion of fish oil fatty acids reduces the incidence of potentially fatal ventricular arrhythmias [3]. The blood levels of n-3 PUFA have also been associated with a reduced risk of sudden death in cases where there is no evidence of prior cardiovascular disease [4]. Conversely, other investigators have shown that compared to a control diet, fish oil and EPA/DHA supplementation results in proarrhythmia not only in animal models [5] but also in patients with implantable defibrillators [6]. Thus, the favorable effects of n-3 PUFA on arrhythmic events are still being debated.

The relationship between n-3 PUFA and the development of atrial fibrillation (AF) is also unclear. Several reports have suggested that n-3 PUFA has a preventive effect on AF. In a prospective population-based cohort study, tuna and boiled fish intake was associated with a low incidence of AF in elderly adults [7]. In a randomized controlled trial, EPA/DHA supplementation resulted in a lower incidence of AF in patients who underwent coronary

artery bypass surgery [8]. Conversely, several reports have described a lack of this preventive effect on AF. In cohort studies, fish intake did not reduce the risk of AF [9, 10]. Previous randomized placebo-controlled trials determining the preventive effects of n-3 PUFA on recurrent AF showed that prescribed n-3 PUFA supplements did not reduce the risk of recurrent AF to a level lower than that seen in placebos [11,12]. The percentage of n-3 PUFA in the erythrocyte membranes of patients with atrial flutter/fibrillation was higher than that in the erythrocyte membranes of control subjects [13]. Thus, the preventive effect of n-3 PUFA on AF is controversial. The aims of the present study were as follows: (1) to determine the concentrations of n-3 PUFA and n-6 PUFA in patients with and without AF, and (2) to define the differences in n-3 PUFA and n-6 PUFA levels in patients with each type of AF (paroxysmal and persistent) in the presence or absence of IHD.

Methods

Subjects

We designed a case-control study and reviewed 12-lead electrocardiograms of outpatients at Shinshu University Hospital between April 2007 and July 2009. We consecutively enrolled patients with a history of AF. All patients underwent clinical screening that included medical history, atherosclerotic risk factors, routine blood chemistry and hematologic analyses, physical examination, resting electrocardiography, echocardiography, chest radiography, an

exercise tolerance test, and, if needed, coronary angiography. AF patients without any abnormal findings were included in the lone AF (L-AF) group. Patients with ischemic heart disease (IHD) diagnosed by coronary angiography were recruited and divided into 2 groups depending to the presence of AF (IHD and IHD-AF). Atherosclerotic risk factors were defined as follows: Hypertension was defined as systolic blood pressure greater than 140 mm Hg and/or diastolic blood pressure greater than 90 mm Hg. Dyslipidemia was defined as an LDL cholesterol level greater than 140 mg/dl, an HDL cholesterol level lesser than 40 mg/dl, or triglyceride levels greater than 150 mg/dl. Diabetes mellitus was defined as fasting plasma glucose levels greater than 126 mg/ml or an HbA1c level greater than 6.1% in patients already receiving medications. Patients were excluded from the study if they took fish oil supplements or had one or a combination of the following: valvular disease, cardiomyopathy, or hormonal disease. Furthermore, each AF group was divided into 2 subgroups based on whether the patients were experiencing (1) paroxysmal AF (PAF) or (2) persistent and permanent AF (PeAF). PAF was defined as the occurrence of several AF episodes that continued for at least 1 hour and terminated spontaneously within 7 days. Persistent AF was defined as AF that continued for longer than 7 days. Permanent AF was defined as AF that could not be terminated or that continued for more than 1 year. Thirty-six age-matched healthy volunteers without AF and cardiac disease were recruited as the control group for comparison of clinical features such as n-3/n-6 PUFA levels and echocardiographic findings

(control group: mean age 65 ± 12 years, 17 men).

Fatty acid analysis

Fasting blood samples were collected from all subjects by using vacuum blood collection tubes containing heparin sodium in order to evaluate the serum concentrations of the free fatty acids n-3 PUFA and n-6 PUFA. Plasma was prepared by centrifugation and was then frozen (-80 °C). Plasma phospholipids were extracted with chloroform, methanol, and distilled water. Following centrifugation, the samples were centrifuged, the supernatant was removed, and the organic layer was treated with sulfuric anhydride sodium to obtain phospholipids. A 5% solution of potassium hydroxide in ethanol (w/v) was added to saponify the phospholipids. Free fatty acids were isolated using hexane after removing the desaponified fraction. Fatty acids were converted to methyl esters by using 14% BF_3-methanol:methanol:benzene (35:35:30) and were analyzed by gas chromatography with a DEGS capillary column. The major peaks of long-chain n-3 fatty acids, including DHA (C22:6n-3) and EPA (C20:5n-3), were analyzed. Arachidonic acid (C20:4n-6) levels were also evaluated.

Statistical analysis

Continuous variables have been expressed in terms of mean ± standard deviation, and categorical variables have been expressed in terms of numbers and frequencies. Echocardiographic parameters and blood sampling data were analyzed using one-way

ANOVA for comparisons between groups. Statistically significant differences were determined using Tukey's post-hoc test for comparing individual group data. Non-normally distributed variables were analyzed using the nonparametric Kruskal–Wallis test. We tested the influence of patients' clinical characteristics and n-3 and n-6 PUFA levels on the incidence of AF by using univariate and multivariate logistic regression analyses. $P < 0.05$ was considered significant.

Results

Patient characteristics

We evaluated 110 cases of AF (mean age, 67 ± 11 years; 76 men) of which 36 had IHD and AF (IHD-AF group: mean age, 74 ± 10 years; 26 men) and the remaining 74 had no history of IHD or structural heart disease, as determined by health screening and echocardiography (L-AF group: mean age, 66 ± 9 years; 50 men) (Table 1). Forty-six patients with IHD had no history of AF (IHD group: mean age, 65 ± 12 years; 18 men).

The mean age did not differ among the 4 groups. The incidence of hypertension, dyslipidemia, and diabetes in the IHD-AF and IHD groups was significantly higher than those in the control and L-AF groups. The left atrial size in the IHD-AF group was larger than those in the L-AF, IHD, and control groups (50 ± 10 mm, 47 ± 7 mm, 46 ± 9 mm, 46 ± 9 mm and 32 ± 10 mm, respectively). The left ventricular systolic and diastolic dimensions in

the IHD-AF group were also larger than those in the other 3 groups.

From the patients who experienced AF episodes, 49 experienced paroxysmal AF (PAF group: mean age, 68 ± 10 years; 29 men) and 55 patients experienced persistent/permanent AF (PeAF group: mean age, 68 ± 11 years; 50 men). The left atrial and left ventricular sizes in the PeAF group were larger than those in the control group. The mean age, left atrial size, and left ventricular size did not differ significantly between the PAF and PeAF groups (Table 2).

Intergroup differences in n-3/n-6 PUFA levels

The EPA levels in the L-AF group were higher than those in the IHD-AF, IHD, and control groups (L-AF: 117.2 ± 63.9 μg/ml; IHD-AF: 76.4 ± 32.1 μg/ml; IHD: 52.7 ± 27.1 μg/ml; control: 62.2 ± 22.7 μg/ml). The EPA levels differed between the IHD-AF and IHD groups (Fig. 1). The DHA levels in the L-AF group were higher than those in the other groups (L-AF: 170.4 ± 55.2 μg/ml; IHD: 122.9 ± 40.7 μg/ml; IHD-AF: 126.4 ± 30.2 μg/ml; control: 125.9 ± 35.2 μg/ml). The DHA levels in the IHD group did not differ from those in the control group. The arachidonic acid levels in the IHD group were higher than those in the control group. The other groups showed similar levels of arachidonic acid (L-AF: 139.4 ± 29.6 μg/ml; IHD: 148.9 ± 45.0 μg/ml; IHD-AF: 131.9 ± 34.4 μg/ml; control: 123.9 ± 37.4 μg/ml).

The mean EPA level in the PeAF group was higher than those in the PAF and control

groups (PeAF: 114.4 ± 66.4 μg/ml; PAF: 93.1 ± 46.7 μg/ml; control: 62.2 ± 22.7 μg/ml) (Fig. 2). The EPA levels differed between the PAF and control groups. The DHA level in the PeAF group was higher than that in the control group; however, it did not differ from that in the PAF group (PeAF: 162.5 ± 57.3 μg/ml; PAF: 149.8 ± 45.2 μg/ml; control: 125.9 ± 35.2 μg/ml). The arachidonic acid levels were similar in all the groups (PeAF: 137.4 ± 31.9 μg/ml; PAF: 137.2 ± 30.9 μg/ml; control: 123.9 ± 37.4 μg/ml).

The AF patients were divided into 4 subgroups according to AF etiological factors and AF type. The EPA level in patients with PeAF without IHD was the highest among the 4 groups (PeAF without IHD: 130.9 ± 73.8 μg/ml; PAF without IHD: 105.0 ± 51.1 μg/ml; PeAF with IHD: 80.2 ± 27.4 μg/ml; PAF with IHD: 72.1 ± 36.9 μg/ml) (Fig. 3). In patients with PAF, the EPA levels were higher when IHD was absent than when it was present. The same pattern was seen in patients with PeAF. The DHA levels in patients with PAF or PeAF but no IHD were also higher than those in both AF types with IHD (PeAF without IHD: 183.2 ± 60.8 μg/ml; PAF without IHD: 158.5 ± 47.2 μg/ml; PeAF with IHD: 125.2 ± 24.7 μg/ml; PAF with IHD: 120.7 ± 36.4 μg/ml).

Influence of n-3/n-6 PUFA levels on the development of AF

We performed univariate and multivariate logistic regression analyses to evaluate the odds ratios of all clinical characteristics and n-3 and n-6 PUFAs levels in order to determine the incidence of AF (Table 3). Univariate analysis showed that IHD (odds ratio [OR]), 2.18;

95% confidence interval (CI), 1.22–3.99; *P* = 0.008), EPA (OR, 1.03; CI, 1.02–1.04; *P* < 0.0001), DHA (OR, 1.02; CI, 1.01–1.03; *P* < 0.0001), and arachidonic acid (OR, 1.00; CI, 0.99–1.01; *P* = 0.004) were associated with the incidence of AF. In contrast, age (OR, 1.01; CI, 0.99–1.04; *P* < 0.26), gender (OR, 1.36; CI, 0.75–2.50; *P* < 0.32), hypertension (OR, 1.14; CI, 0.61–2.13; *P* < 0.68), dyslipidemia (OR, 0.75; CI, 0.40–1.42; *P* < 0.38), diabetes (OR, 0.52; CI, 0.24–1.14; *P* < 0.10), and all echocardiography parameters were not associated with the incidence of AF.

We subsequently performed multivariate logistic regression analysis to determine the adjusted odds ratios of clinical characteristics and n-3 and n-6 PUFAs levels that might influence the incidence of AF. In multivariate logistic regression analysis, the EPA levels (OR, 1.02; CI, 1.00–1.04; *P* = 0.03) were associated with the incidence of AF. Age (OR, 1.02; CI, 0.99–1.02; *P* = 0.21), gender (OR, 2.19; CI, 0.93–5.51; *P* = 0.07), a history of IHD (OR, 1.24; CI,0.39–3.97; *P* = 0.72), hypertension (OR, 2.01; CI, 0.78–5.17; *P* = 0.15), dyslipidemia (OR, 0.63; CI, 0.24–1.64; *P* = 0.34), diabetes (OR, 0.63; CI, 0.19–1.70; *P* = 0.31), DHA (OR, 1.01; CI, 0.99–1.02; *P* = 0.55), and AA (OR, 1.00; CI, 0.99–1.01; *P* = 0.64) were not associated with the incidence of AF.

Discussion

Previous studies have shown varying effects of fish oil and DHA/EPA supplementation on

the incidence of AF, and whether EPA and DHA exert favorable effects on health and cardiac function in particular remains controversial. In our case-control study involving the Japanese population, the EPA and DHA levels in L-AF patients were higher than those of normal subjects. The EPA levels in patients with persistent and permanent AF were higher than those in patients with paroxysmal AF and either L-AF or IHD-AF. Further, multivariate analysis showed that the incidence of AF was associated with higher levels of EPA but not DHA. To our knowledge, the present study is the first to describe differences in EPA and DHA concentrations among patients who exhibited different types and etiological factors of AF.

The present study shows that the EPA/DHA levels in patients with L-AF were higher than those of healthy volunteers. In contrast, the EPA/DHA levels in IHD patients did not differ from those of normal subjects, indicating that high levels of EPA/DHA might be associated with the development of AF in the normal population. Virtanen et al. showed that increased concentrations of DHA might protect against AF, while EPA is not associated with reduction of risk for AF [14]. In the current study, multivariate analysis showed that EPA was associated with the prevalence of AF but DHA did not significantly. The results of 2 studies suggested that EPA does not protect against AF. A study on a healthy Danish population that evaluated consumption of n-3 fatty acids from fish showed that n-3 fatty acids were not associated with a reduction in the risk of atrial fibrillation or flutter. Inversely, frequent consumption of n-3 PUFA from fish increased the adjusted hazard ratio for AF or atrial

flutter [10]. These results are comparable with those from the present study. Furthermore, the n-3 PUFA levels of PeAF patients were higher than those of PAF patients in both the L-AF and IHD-AF groups (Fig. 3). In particular, the EPA levels in the PeAF group were higher than those in the PAF group. A recent report by Sakabe et al. that addresses the development of PeAF showed that interatrial dyssynchrony on tissue Doppler imaging is associated with the progression to chronic AF in patients with nonvalvular paroxysmal AF [15]. EPA might also play an important role in the development of PeAF.

Influence of high EPA/DHA concentrations on L-AF

The origins of AF are multifactorial in nature, and the incidence of this condition increases with age even in the absence of valvular and coronary heart disease. We found that excess concentrations of EPA/DHA might present the risk of AF at least in normal subjects (Fig. 1). Coumel classified AF into vagotonic and adrenergic types according to its time of onset and relation with autonomic tone [16] and showed that patients with vagally mediated AF were younger, always exhibited idiopathic AF, and experienced AF episodes at night; the adrenergically mediated PAF differed in all respects.

Fluctuation in autonomic balance is involved in the initiation and termination of PAF [17]. Vagal and sympathetic nerve stimulation results in shortened atrial effective refractory periods and increased dispersion of refractoriness [18, 19]. In particular, activation of the vagus nerve is an established determinant of vulnerability for L-AF. Holguin et al. reported

that fish oil supplementation was associated with a significant increase in heart rate variability, indicating vagal activity [20]. Therefore, enhancement of vagal nerve tone by chronically high levels of EPA/DHA could increase the risk of PAF in normal individuals.

Influence of EPA/DHA on AF in IHD

In patients with IHD, n-3 PUFA was not significantly associated with the incidence of AF. We think that the favorable effects of n-3 PUFA on the vasculature (protection against atherosclerosis) predominate over the adverse effects on AF. Virtanen et al. showed that a high level of DHA was associated with a low incidence of AF. Approximately 15% of the subjects in their study were diagnosed with IHD [14]. IHD is an important risk factor of AF. Atrial ischemia induced by occlusion of the atrial artery has been shown to cause local conduction slowing, with re-entry contributing to increased AF [21]. The cardiovascular effects of myocardial ischemia in ventricular infarction and dysfunction, increased atrial stretch, pericarditis, and neurohormonal and autonomic nervous system changes are potentially important contributors to a heterogeneous electrical and structural milieu, thereby increasing the risk of AF [22-27]. Therefore, cardiac ischemia itself can cause AF.

The influence of n-3 PUFA on the development of AF could be relatively small. In the present study, the n-3 PUFA levels did not differ between the IHD-AF and control groups. However, the EPA levels in the IHD-AF group were higher than those in the IHD group (Fig. 1). In a study performed in Iceland, the results showed that high levels of n-3 PUFA did not

prevent AF and might be harmful, even in ischemic coronary bypass patients [28]. In the present study, IHD was associated with the incidence of AF as shown by univariate analysis. However, multivariate analysis showed that AF was associated with a higher level of EPA but not IHD. These results suggest that EPA itself is a precipitating factor for AF in patients with IHD, as found for normal subjects.

The beneficial effects of n-3 PUFA on the prevention of AF suggest that these fatty acids are associated with improvement of ischemic states [8, 29-32]. Even in a population with normal left ventricular function, a slightly elevated level of N-terminal pro-brain natriuretic peptide can predict coronary artery disease [33]. EPA and DHA supplementation might, therefore, prevent AF by inhibiting atherosclerosis in IHD patients. Moreover, the activation of vagal tone by EPA could also inhibit adrenergically mediated AF in patients with heart failure. The preventive effect of n-3 PUFA supplementation on AF has also been shown in patients with other types of structural heart disease [34, 35]. However, our results could not show that n-3 PUFA was associated with a reduced risk of AF, irrespective of etiological factors. Because our present study included a relatively limited number of subjects, further worldwide studies will be required to determine whether n-3 PUFA influences the development of AF in healthy individuals and heart disease patients.

Conclusion

In the present study involving the Japanese population, EPA and DHA levels in patients with L-AF were higher than those in normal subjects. In particular, the EPA level was associated with the incidence of AF. These findings suggest that an excess of EPA might be a precipitating factor for AF. Further investigation is required to determine the effect of n-3 PUFAs on AF.

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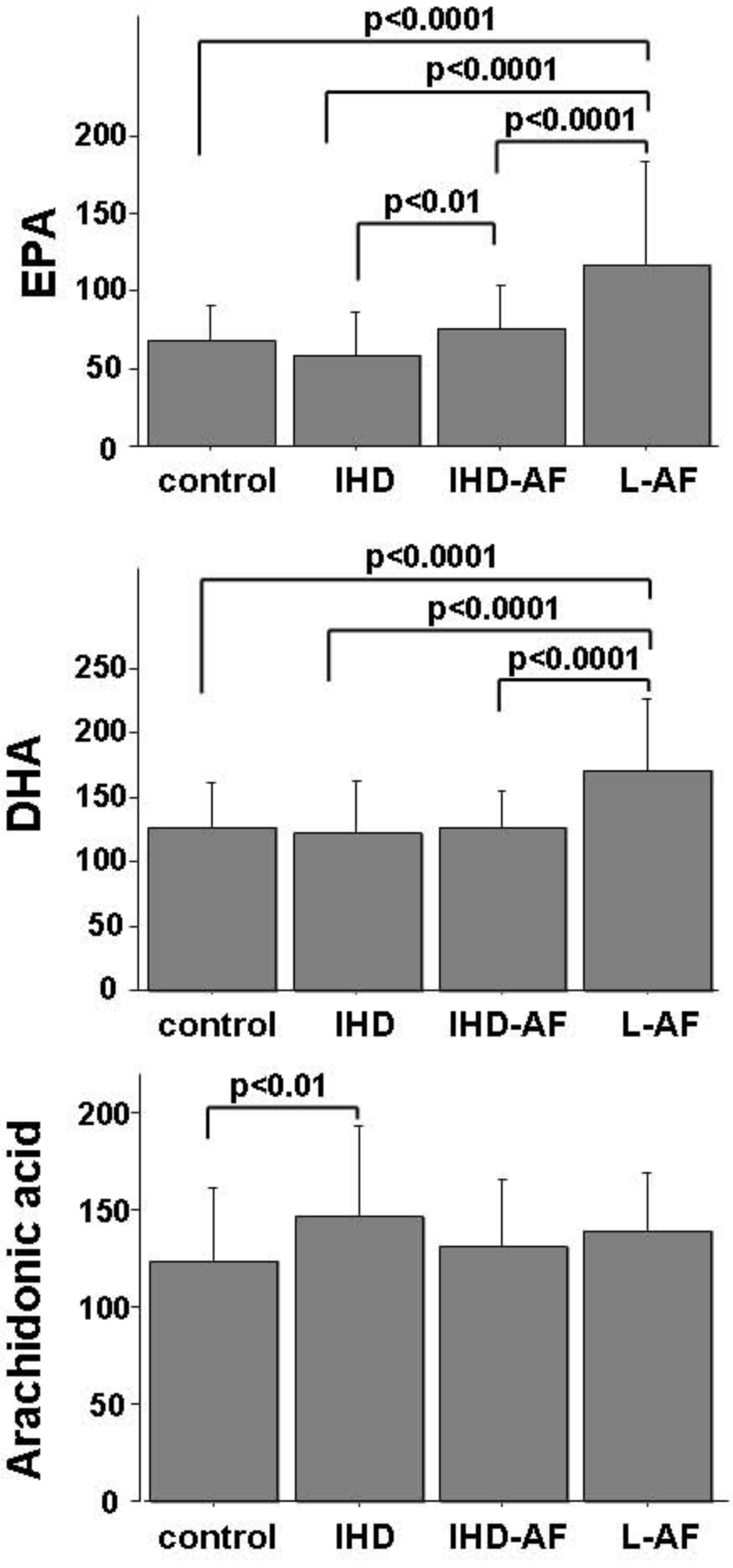
Figure legends

Fig. 1 Serum concentrations of EPA, DHA, and arachidonic acid in subjects grouped according to AF etiological factors. The *P* value is indicated for cases wherein there was a statistically significant difference between the groups. IHD, ischemic heart disease; L-AF, lone atrial fibrillation; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; AA, arachidonic acid

Fig. 2 Serum concentrations of EPA, DHA, and arachidonic acid in groups according to AF types. PAF, paroxysmal atrial fibrillation; PeAF, persistent or permanent atrial fibrillation (additional abbreviations are the same as in Fig. 1).

Fig 3 Serum concentrations of EPA and DHA in subgroups classified according to AF etiological factors and types. EPA: PeAF without IHD, 130.9 ± 73.8 μg/ml; PAF without IHD, 105.0 ± 51.1 μg/ml; PeAF with IHD, 80.2 ± 27.4 μg/ml; PAF with IHD, 72.1 ± 36.9 μg/ml. DHA: PeAF without IHD, 183.2 ± 60.8 μg/ml; PAF without IHD, 158.5 ± 47.2 μg/ml; PeAF with IHD, 125.2 ± 24.7 μg/ml; PAF with IHD, 120.7 ± 36.4 μg/ml.

Fig.1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p<0.0001
p<0.0001
p<0.01
EPA
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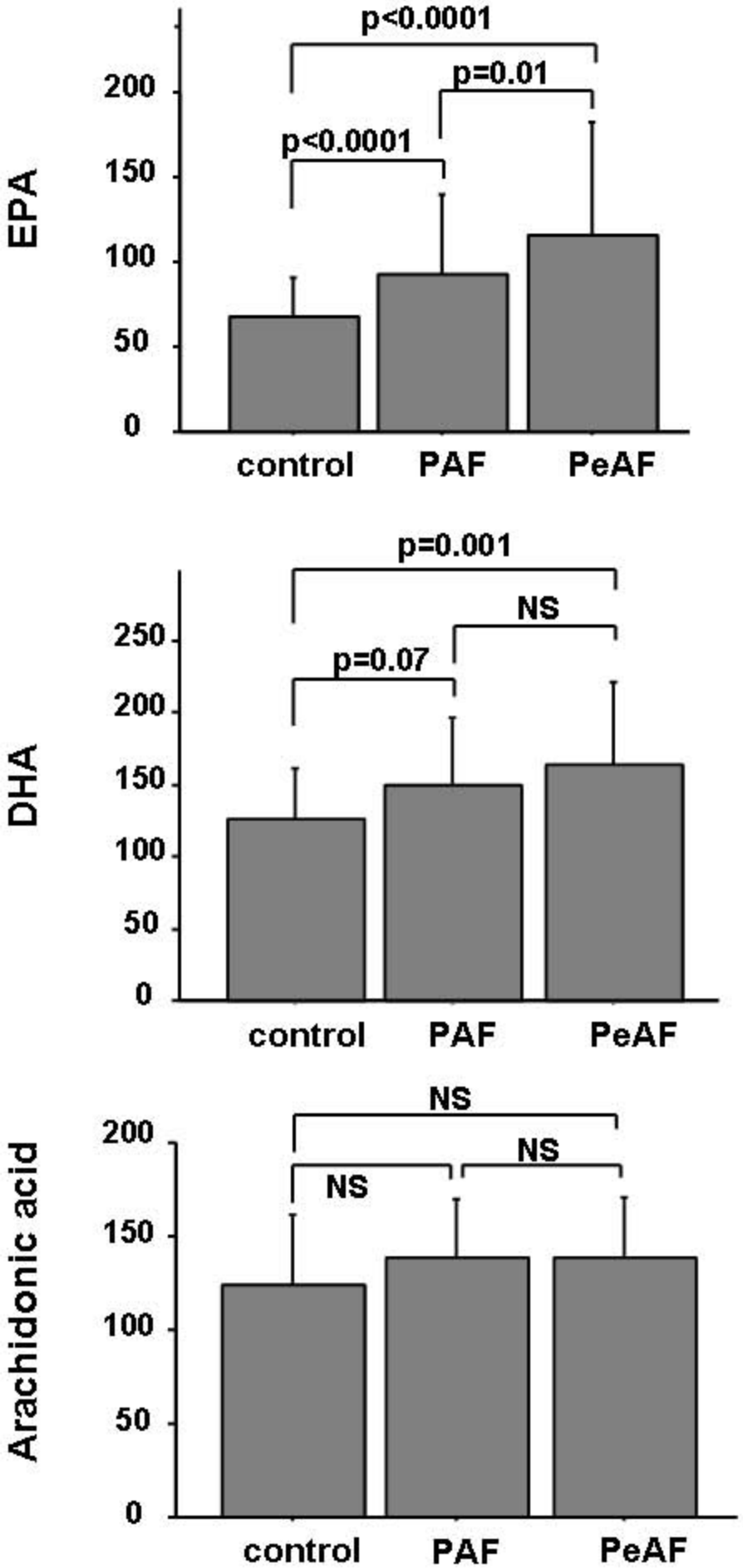
150
100
50
0
control
IHD
IHD-AF
L-AF
p<0.0001
p<0.0001
p<0.0001
DHA
250
200
150
100
50
0
control
IHD
IHD-AF
L-AF
p<0.01
Arachidonic acid
200
150
100
50
0
control
IHD
IHD-AF
L-AF

Fig.2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50
0
control
PAF
PeAF
DHA
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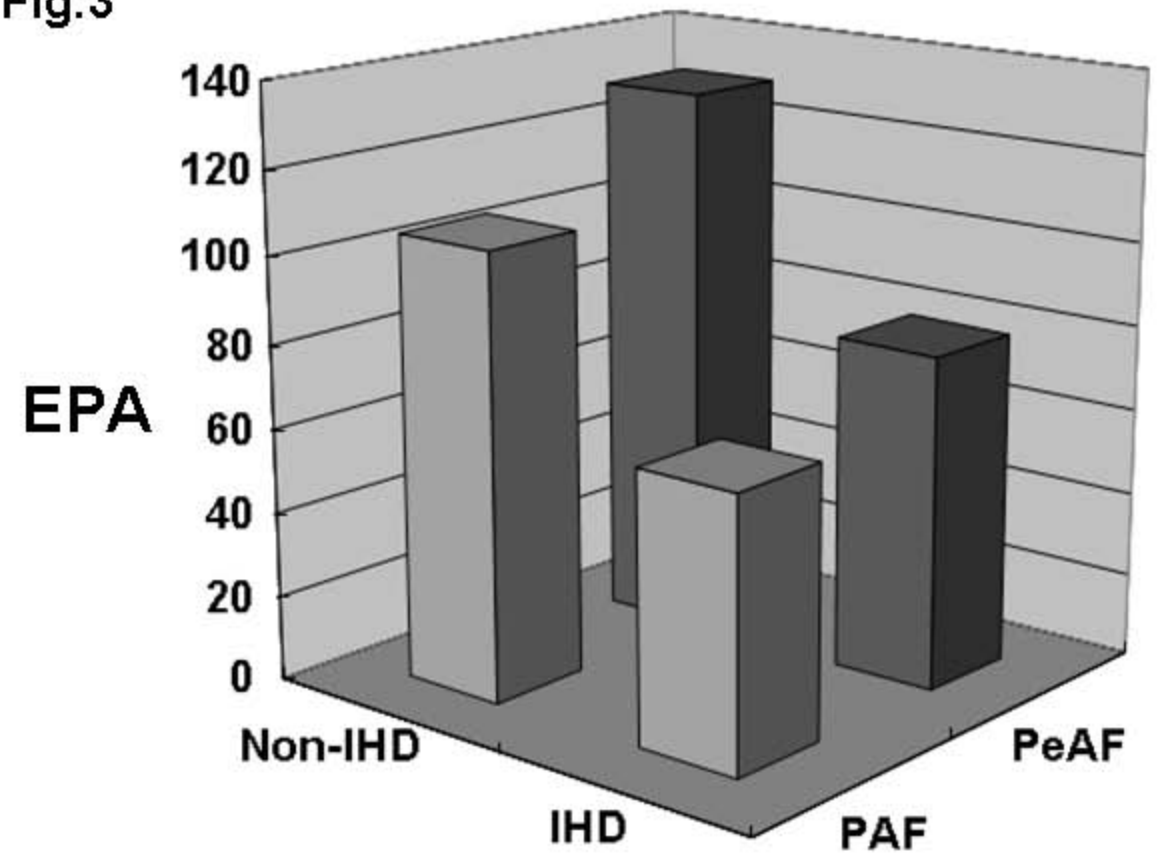
NS
p=0.07
250
200
150
100
50
0
control
PAF
PeAF
Arachidonic acid
NS
NS
NS
200
150
100
50
0
control
PAF
PeAF

Fig.3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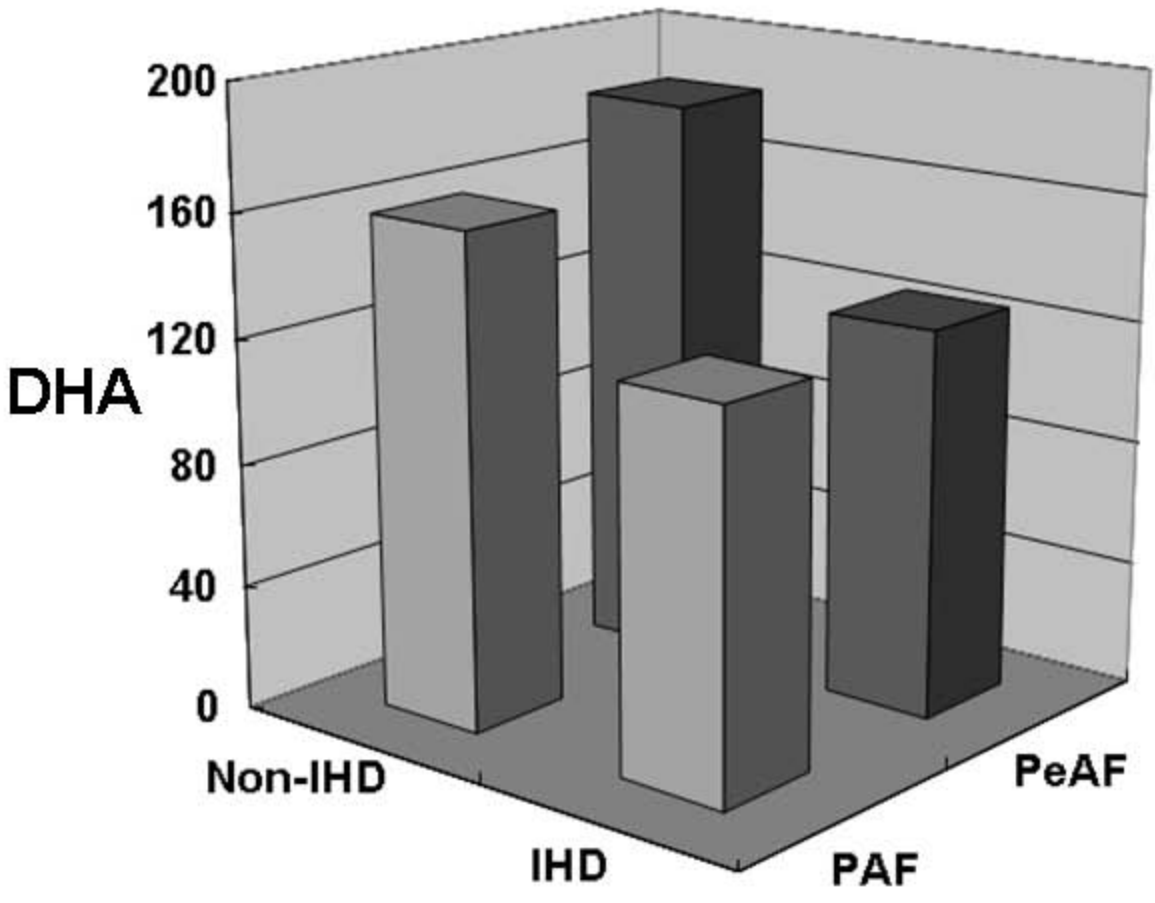
120
100
80
60
40
20
0
Non-IHD
IHD
PAF
PeAF


DHA
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160
120
80
40
0
Non-IHD
IHD
PAF
PeAF