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Factors Influencing EPA+DHA Levels in Red Blood Cells in Japan

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Abstract. *The blood eicosapentaenoic and docosahexaenoic acid (EPA+DHA) concentration is an important inverse risk factor for sudden cardiac death. However, it is not known what kinds of factors influence the EPA+DHA levels in the total phospholipid fraction in red blood cells (RBC EPA+DHA) in Japan, who regularly eat more fish with increasing age. Four hundred and fifty-six healthy individuals (320 men and 136 women, 18 to 70 years old) were recruited between 2002 and 2005. RBC EPA+DHA were measured by gas chromatography and questionnaires were administered. Multivariate analysis indicated that there were significant correlations between RBC EPA+DHA and (i) dietary EPA+DHA (β=0.31), (ii) age (β=0.33), (iii) gender (β=–0.15) and (iv) physical activity (β=–0.11) but not with body mass index or smoking.*

The percentage of eicosapentaenoic and docosahexaenoic acids (EPA+DHA) in the blood is a useful biomarker of long-chain n-3 fatty acid intake. It was termed the Omega-3 Index, and might be an important negative risk factor for coronary heart disease (CHD) deaths (1). This index was actually shown to be significantly correlated with human myocardial n-3 fatty acid content (2), and strongly associated with reduced risk of sudden cardiac deaths (3).

Recently Sands *et al.* (4) determined the Omega-3 Index in red blood cells (RBCs) and other basic characteristics from 163 adults, and found that four factors significantly and independently influenced the Omega-3 Index in RBCs: fish servings, age, body mass index (BMI) and diabetes. In the present study the relationship between the EPA+DHA levels in the total phospholipid fraction in RBCs (RBC EPA+DHA) and several factors including physical activity was investigated in 456 Japanese subjects. Considering that Japanese people regularly eat fish and that the rate of CHD death is only one fourteenth of all-cause mortality in Japan (5), it might also be interesting to compare RBC EPA+DHA in Japan and the Omega-3 Index of the United States.

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Key Words: Age, fish intake, gender, Omega-3 Index, physical activity.

Materials and Methods

We performed four intervention studies in the Tokyo, Shizuoka and Toyama areas between 2002 and 2005. Intervention included changes in lipid nutrition. The fatty acid composition of the total phospholipid fraction in RBCs was measured at both start and end of each intervention study. In the present study, the data obtained at the start of studies (while study participants were still under no effects of intervention) were compiled for statistical calculation. Each intervention trial was approved by the ethics committee of Toyama Medical and Pharmaceutical University (the former name before its union with University of Toyama) and written informed consent was obtained from each participant. Those who were under treatment of diabetes mellitus, hypertension and hyperlipidemia were not eligible for any of the four studies described above. Those who were taking supplements were also excluded.

Food intake including alcoholic beverages was calculated with a semi-quantitative food-frequency questionnaire and the food calculation software, Eiyokun 3.0 (Kenpakusha Co. Ltd., Tokyo). To assess nutrients, study participants were asked how often on average they had eaten a portion size of each food during the previous 4 weeks. The daily physical activity and smoking status (smoker, non-smoker, ex-smoker) were also determined with a simple questionnaire. With regard to the daily physical activity, participants were asked to choose one of four activity levels, namely low, relatively low, moderate or high. For each activity level, there was a simple explanation with approximate hours spent for standing, walking, rapid walking and strong muscle activity.

Fasting EDTA-anticoagulated blood samples were collected early in the morning and RBCs were separated from the samples. They were washed twice with saline and frozen at –80˚C until analysis. The fatty acid composition of the total phospholipid fraction of washed RBCs was determined as described elsewhere

Table I. *Relation between RBC EPA+DHA and participants' characteristics.*

	Univariate analysis				Multivariate analysis			
		95% CI				95% CI		
Independent variable	r	Lower	Upper	*p*-value	standardized β	Lower	Upper	*p*-value
Dietary EPA+DHA %	0.40	0.32	0.47	<0.0001	0.31	0.22	0.41	<0.0001
Age	0.40	0.32	0.48	<0.0001	0.33	0.25	0.44	<0.001
Gender (Female)	–0.19	–0.28	–0.10	<0.0001	–0.15	–0.24	–0.05	<0.0001
BMI	0.15	0.06	0.24	0.0015	0.03	–0.06	0.12	0.45
Smoking	0.12	0.03	0.21	0.01	0.05	–0.04	0.14	0.25
Physical activity	–0.07	–0.17	0.02	0.11	–0.11	–0.21	–0.02	0.01

Results from 456 healthy participants. RBC EPA+DHA=the area percentage of EPA and DHA in the total phospholipid fraction in RBCs. Dietary EPA+DHA =the weight percentage of the total fatty acid intake.

and expressed as area % (6). Briefly, total lipids were extracted with chloroform/methanol; the total phospholipid fraction was separated by thin-layer chromatography; after transmethylation with HCl-methanol, the fatty acid composition was analyzed by gas chromatography (GC14A Shimadzu Corporation, Kyoto) with a capillary column DB-225 (0.25 mm i.d., 30 m length, 0.25 μm; J&W Scientific, Folsom, CA, USA).

Data are expressed as means±SD. Univariate regression and multivariate analyses were performed to assess the associations between RBC EPA+DHA and characteristics of the participants. In the case of multivariate analysis, the following items were included into the calculation as independent predictors: age, gender, BMI, smoking status, physical activity and dietary EPA+DHA. The mean dietary EPA+DHA levels in age groups in both sexes were compared with two-way ANOVA. SPSS, version13.0 (SPSS 13.0J Base System, 2005, SPSS Japan Inc., Tokyo) was used for calculation. $P<0.05$ was considered as significant.

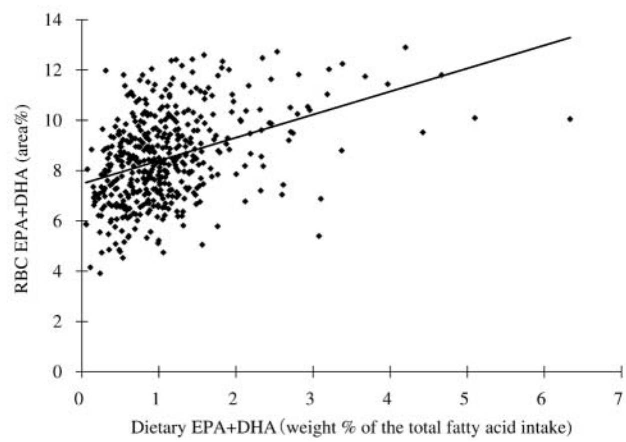

Figure 1. *The relationship between RBC EPA+DHA and the dietary ratios of EPA+DHA to the total fatty acid intake. r=0.40, p<0.0001, n=456.*

Results

From the four intervention studies above, 456 complete data sets were collected. The study participants constituted 320 Japanese men and 136 women. The age distribution was as follows: age 18-29, 47; age 30-39, 142; age 40-49, 126; age 50-59, 126 and age 60-70, 15 with an average age of 42.5±10.6 years. Other baseline characteristics of the study participants were as follows: BMI, 22.5±3.3 (range: 16-38), smokers (men: 51%, women: 24%), and physical activity (low: 27%, relatively low: 41%, moderate: 29%, and high: 3%). The average values of RBC EPA, DHA and EPA+DHA were 1.6±0.7% (range: 0.4-4.4%), 6.8±1.3% (range: 3.5-10.4%) and 8.5±1.8% (range 3.9-12.9%), respectively. There was only one participant whose RBC EPA+DHA was below 4%.

Univariate and multivariate analyses of RBC EPA+DHA and independent variables are shown in Table I. Dietary EPA+DHA expressed as a weight % of the total fatty acid intake (dietary EPA+DHA %), age, gender, BMI and smoking status were significantly correlated with RBC EPA+DHA on univariate analysis, but physical activity was not correlated with RBC EPA+DHA. However, on multivariate analysis, BMI and smoking ceased to be significant, while physical activity became significant.

Figure 1 shows the relationship between RBC EPA+DHA and dietary EPA+DHA %. Figure 2 shows the relationship between RBC EPA+DHA and age in both sexes. Figure 3 shows the relationship between dietary EPA+DHA % and age in both sexes.

Discussion

The Omega-3 Index is strongly associated with reduced risk of sudden cardiac death (3). Sands *et al.* (4) proposed that an Omega-3 Index in RBCs of 8% or above was the target

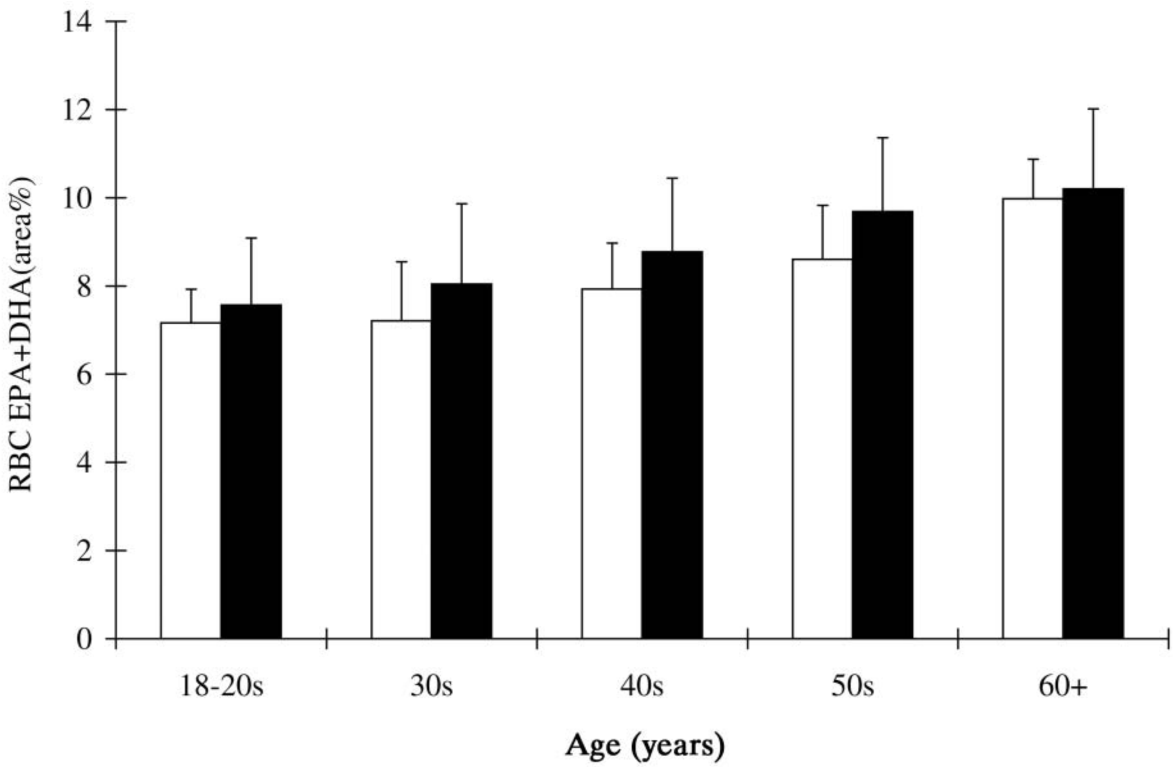

Figure 2. *The relationship between RBC EPA+DHA and age in both genders on a univariate analysis. Men, closed columns; women, open columns. Bars indicate one SD.*

cardioprotective level. The average RBC EPA+DHA in the present study participants were above that value, actually 8.5%. In contrast, the average value in Sands *et al.*'s study was only 4.9% (4). They also proposed that the high risk horizon of the Omega-3 Index in RBCs was 4%. Interestingly, there was only one participant with a value below 4% in the present study. This low incidence is probably one of the major reasons why the Japanese do not frequently die from acute myocardial infarction.

In the present study, we analyzed the total phospholipid fraction of RBCs. This fraction was different from that most of the other workers used for analysis. Taking into account that about 95% of all fatty acids in RBCs are located in the total phospholipid fraction (not published data), it might be reasonably safe to compare the differences in EPA+DHA levels between the total lipid (not total phospholipid) fraction in RBCs measured by others and our RBC EPA+DHA, especially where there was a sizable difference in the fatty acid composition between two study populations. Nevertheless, we should realize that there are a number of different methods to measure fatty acids and to describe the fatty acid composition, and that all these differences make a direct comparison difficult, especially if the difference in the fatty acid composition was not great.

It has generally been thought that fish consumption increases with age in Japan and some data support this belief (7). As shown in Figure 3, our data concur with such a belief. Interestingly, as a factor, age survived as a significant predictor of RBC EPA+DHA even after adjustment for n-3 fatty acid intake. This finding was similar to what Sands *et al.* found in their study (4) and also to an animal experiment of Gudbyarnason (8) who showed that DHA contents in the myocardium of old rats were higher than those of young rats, although both groups of rats ate the same diet.

BMI did not remain a significant predictor of RBC EPA+DHA on multivariate analysis (Table I). This was different from the results of Sands *et al.* (4). There was a big difference in average BMIs between the two study populations (22.5±3.3 in the present study *vs.* 26.2±4.8 (4)). The relationship between BMI and EPA+DHA might be different in a country where the BMI is rather high such as in the United States. Another reason may be the difference in the unit of EPA+DHA intake. In their study (4), n-3 fatty acid intake was registered as the frequency of fish servings, which was not adjusted to participants' body weights or their total fat (or energy) intake, whereas the EPA+DHA intake in the present study was adjusted for the

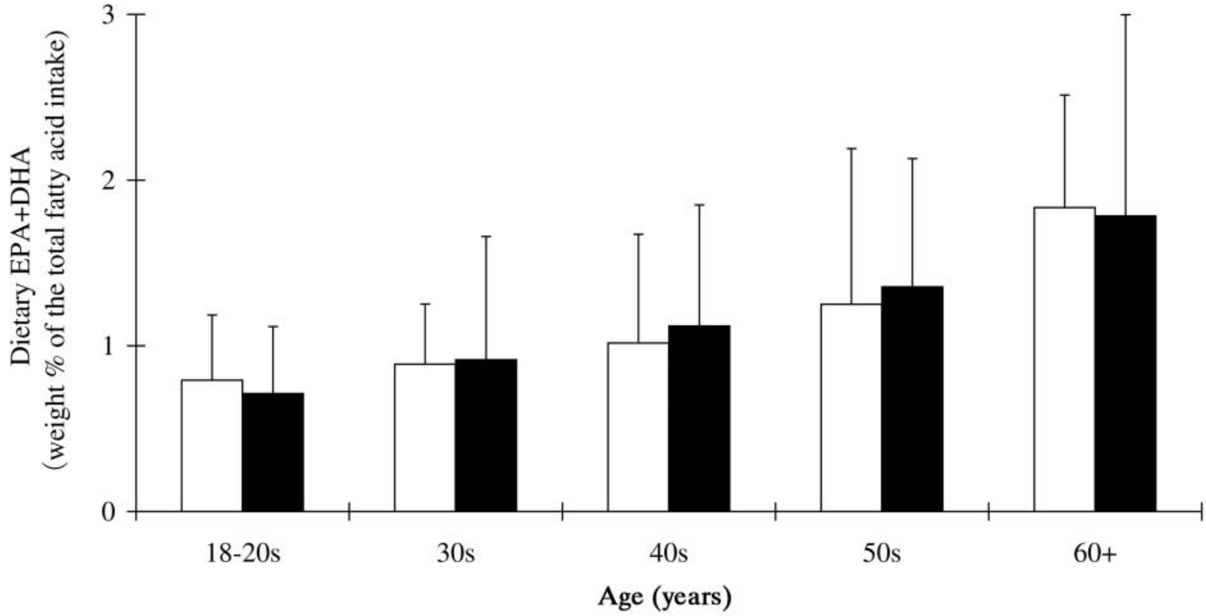

Figure 3. *The relationship between the mean dietary EPA+DHA and age in both genders. Men, closed columns; women, open columns. There was a significant difference in the mean dietary EPA+DHA % among age groups (p<0.0001, two-way ANOVA).*

total fatty acid intake. Consequently, our data of EPA+DHA intake were less influenced by obesity.

In the present study, physical activity was inversely related to RBC EPA+DHA (Table I). The mechanism of this relationship is not immediately clear, but there is a possibility that EPA and DHA might be preferentially catabolized for energy production when physical activity is increased. EPA and DHA are two of the least viscous fatty acids (9) and are probably not suitable for storage in the adipose tissue. In fact, the percentages of EPA and DHA in the human adipose tissue did not exceed 1% even after more than one year of daily administration of 10 g fish oil (10).

In previous studies on a smaller scale (11-13), n-3 fatty acid levels tended to be rather higher in women than in men. The same trend was seen in the report of Sands *et al.* before adjustment for some confounding factors, but this was reversed after adjustment (4). Our study showed that RBC EPA+DHA was higher in men than in women both before and after adjustment for confounding factors (see Table I). For women who eat enough EPA and DHA, such as those women in our study, a greater capacity to produce EPA and DHA from α-linolenic acid in women (14) does not seem to influence RBC EPA+DHA.

Hibbeln *et al.* reported that smoking status predicted lower levels of RBC n-3 fatty acids in schizophrenic patients (13). However, the effect of smoking was not confirmed by Sands *et al.* (4). In our study with essentially normal participants, a positive association of smoking with RBC EPA+DHA was found before adjustment, but this association disappeared after adjustment (Table I).

In conclusion, multivariate analysis indicated that there were significant correlations between RBC EPA+DHA and (i) dietary EPA+DHA, (ii) age, (iii) gender and (iv) physical activity, but not with BMI or smoking in Japan.

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