

Relationship Between Plasma, Erythrocyte Membrane, and Dietary Intake Levels of ω -3 Fatty Acids in Young Korean Females : Effect of Diet Survey for Two Months*

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ABSTRACT

This study was conducted to assess the ω -3 fatty acid intake levels and to evaluate the relationship between the levels of ω -3 fatty acids in plasma and erythrocyte membrane and the dietary intake of these fatty acids over the period of two months in 56 young Korean females. Dietary survey was conducted to obtain 7-day weighed records and six 24-hour recalls. Fasting blood sample was collected from each subject after the dietary survey period. Mean daily intakes of energy, fat, and cholesterol were 1,569kcal, 41.8g, and 217mg, respectively. Fat supplied 24% of total energy intake. Mean daily intake levels of eicosapentaenoic acid(EPA), docosahexaenoic acid(DHA), and total ω -3 fatty acids were 0.04g, 0.06g, and 0.48g, respectively. Plasma cholesterol levels of most of the subjects were within normal range, and there was no significant correlation between plasma cholesterol levels and intake levels of any specific fatty acid. Levels of EPA, DHA, EPA+DHA in plasma and erythrocyte membrane, but not the level of α -linolenic acid(LNA), were significantly correlated with dietary intake of respective fatty acids. Such a correlation, however, was not observed in a previous study where dietary intake was assessed for 3 days. The results of this study show that dietary intakes of ω -3 fatty acids are low in the subject and that about two-month period is required to assess dietary intake levels of ω -3 fatty acids with a reasonable accuracy.

KEY WORDS : ω -3 fatty acids · blood fatty acid composition · EPA · DHA.

Introduction

Effects of dietary fat on cardiovascular diseases

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(CVD) depend not only on the total amount of fat but also on the kinds of fatty acids composing dietary fat¹⁾²⁾. Omega-3 fatty acids, which are generally low in diets of most of the population in the world, are reported to reduce blood lipids³⁾, thrombosis⁴⁾, and blood pressure⁵⁾⁶⁾ and thus considered as an important factor for prevention of CVD⁷⁾⁸⁾. Since the effects of

dietary fat on the development of CVD are long-term, perhaps a life-long process, any study investigating the relationship between ω -3 fatty acid intake and health/disease of an individual requires an estimation of intake levels of these fatty acids over an extended period of time. This is difficult to achieve by usual dietary survey methods⁹⁾. Therefore, many studies have been conducted to identify biomarkers of ω -6 and ω -3 fatty acid intakes¹⁰⁾¹¹⁾. Fatty acid composition in plasma total lipid or subfractions of lipids, such as phospholipid and cholesterol ester, reflects the pattern of recent fatty acid intake. On the other hand, fatty acid composition of erythrocyte membrane reflects the pattern of fatty acid intake during the previous several months while that of adipose tissue reflects the intake of fatty acid over many years. These biomarkers are very useful in estimating ω -3 fatty acid intake of individual, over the appropriate time span¹⁰⁻¹⁹⁾.

Fat intake among Koreans is generally low. According to the National Nutrition Survey of 1992, about 16% of energy comes from dietary fat²⁰⁾ although the figure goes beyond 30% in several studies conducted with young population in urban areas²¹⁾²²⁾. Among the food items commonly consumed by Koreans, perilla oil, rapeseed oil, and walnuts are high in α -linolenic acid(LNA), while sardines, mackerel, mackerel pikes are high in eicosapentaenoic acid(EPA) and docosahexaenoic acid(DHA)²³⁾. However, these foods are not generally consumed in large amounts, so the intake level of ω -3 fatty acids of Koreans may not be high. In studies with young female subjects, mean daily intake of ω -3 fatty acids was 1.3g by 24-hour recalls²²⁾, and 0.99g by 3-day diet records²¹⁾. Jung et al²⁴⁾ estimated the mean daily intake of ω -3 fatty acids per household to be 3-4g with the data from National Nutrition Survey of 1992, and 0.62g per adult living in rural area with data obtained by 24-hour recall method. However, no report is available about the intake levels of ω -3 fatty acids on a period longer than three days in Koreans, either by dietary survey or with biomarkers. Kim and Paik²¹⁾ looked into the relationship between blood fatty acid composition and dietary intake levels but did not find significant re-

lationships. The reason for this failure might be that dietary intake obtained by 3-day records in that study was not long enough.

The present study was conducted to estimate a more typical intake level of ω -3 fatty acids, and to evaluate plasma and erythrocyte membrane ω -3 fatty acid levels as biomarkers to reflect dietary intake levels of ω -3 fatty acids in 56 young Korean adult females. Dietary intake levels were obtained by 7-day dietary record and six 24-hour recalls obtained over the period of two months while they maintained normal diet and activity pattern.

Materials and Methods

Subjects

This study was conducted with 56 young healthy females of 19-24 years of age. The average values of age, height, weight, and BMI were 21.0 ± 1.4 years, 159.0 ± 4.2 cm, 51.8 ± 6.2 kg, and 20.5 ± 2.2 , respectively. No one was considered obese by any criteria.

Diet Survey

Diet survey was conducted to collect dietary intake of 13 days from each subject during the 2 months of study period. During the study period, each subject selected the most convenient time to conduct weighed dietary records for 7 consecutive days. Six 24-hour recalls, evenly spread over the rest of the study period, were also conducted. Every effort was made to include different weekdays including one Sunday for each subject. Intake levels of energy, fat, and different fatty acids were calculated using several resources on nutrient contents of Korean and Asian foods²³⁾²⁵⁻²⁷⁾. For each subject, an average value of 13 days for particular nutrient was used as the mean daily intake for that nutrient.

Blood Sampling and Analysis

Immediately after dietary assessment, fasting blood samples were drawn from each subject in vacuum tubes with 3% EDTA solution. Blood samples were centrifuged at 4°C, 3000rpm for 15 minutes and the

plasma samples were kept frozen at -20°C until fatty acid compositions were analyzed. Buffy coats were removed and the erythrocytes were washed three times with phosphate-buffered saline (PBS, pH 7.2). After washing, an equal volume of PBS was added to erythrocytes and was also kept frozen at -20°C until fatty acid analysis. Sample tubes were filled with N_2 gas before freezing and analyzed within one month to minimize oxidation.

Plasma total cholesterol was analyzed with commercial kit based on enzymatic method (Young-dong Pharmaceutical Co., Korea) and triglyceride was analyzed by commercial kit utilizing glycerol-3-phosphate oxidase-p-chlorophenol coloring method (GPO-PAP method, Wako Co., Japan). HDL-cholesterol was determined with the same analytical method as total cholesterol, after the precipitation of LDL and VLDL with dextran sulfate- Mg^{2+} . The level of LDL-cholesterol was derived by Friedewald formula²⁸⁾.

For the analysis of fatty acid composition, plasma lipid was extracted by the method of Folch et al²⁹⁾, and fatty acid methyl esters were prepared by the method of Lepage and Roy³⁰⁾. For the erythrocyte membrane analysis, an aliquote of erythrocyte sample was saponified and nonsaponifiable lipids were extracted and removed by the method of Tilvis et al³¹⁾. The saponified lipids were then extracted and fatty acids were methylated using the same method as plasma lipid. Fatty acid methyl esters were analyzed by gas chromatograph (Schimadzu GC-9A, Japan) equipped with capillary column (30m \times 0.25mm, fused silica)²³⁾.

Data Analysis

Means and standard deviations of dietary intake levels or blood analysis data were calculated for the subjects. Relationship between dietary intakes of ω -3 fatty acids and the composition of fatty acids in plasma and erythrocyte membrane were analyzed by Pearson's correlation coefficients as well as Duncan's multiple range test using SAS-PC version 6.04³²⁾.

Results

Mean daily intakes of energy, total fat, and cho-

lesterol of the subjects were 1,569kcal, 41.8g, and 216.9mg respectively. Korean RDA³³⁾ recommends daily intake of 2,000kcal for young adult females but the study subjects consumed about 20% less energy than the recommended level. Dietary fat composed about 24% of total energy intake. The mean daily intake levels of different fatty acids are shown in Table 1. Oleic acid was consumed in the largest quantity followed by palmitic, linoleic, and stearic acids. The mean daily intakes of total ω -3 fatty acids and EPA+DHA were 0.48g(0.3% of energy intake) and 0.13g(0.

Table 1. Dietary intake of fat, cholesterol and fatty acids (N=56)

	Mean	S.D. ¹⁾		
Energy(kcal)	1,569.1	262.5		
Total Fat(g)	41.78	9.48		
Cholesterol(mg)	216.9	78.9		
Fatty acid	grams per day		% energy	
	Mean	S.D.	Mean	S.D.
4 : 0	0.07	0.06	0.04	0.03
6 : 0	0.04	0.04	0.02	0.02
8 : 0	0.02	0.02	0.01	0.01
10 : 0	0.25	0.12	0.14	0.06
12 : 0	0.31	0.13	0.17	0.07
14 : 0	1.68	0.51	0.96	0.24
16 : 0	10.32	2.94	5.91	1.33
16 : 1	1.28	0.51	0.74	0.27
18 : 0	3.87	1.14	2.22	0.56
18 : 1(ω -9)	17.57	5.85	10.07	2.81
18 : 2(ω -6)	7.88	2.48	4.51	1.18
18 : 3(ω -3)	0.36	0.24	0.21	0.13
20 : 1(ω -9)	0.07	0.07	0.04	0.04
20 : 4(ω -6)	0.07	0.03	0.04	0.02
20 : 5(ω -3)	0.04	0.05	0.02	0.07
22 : 6(ω -3)	0.09	0.11	0.05	0.07
Total SFA	16.59	4.61	9.50	2.06
Total MUFA	18.96	6.35	10.87	3.06
Total PUFA	8.44	2.65	4.84	1.25
Total ω -6	7.95	2.48	4.55	1.18
Total ω -3	0.48	0.31	0.28	0.15
EPA+DHA	0.13	0.16	0.07	0.10
ω -6/ ω -3 ratio	30.57	20.39		
P/M/S ratio	0.69/1.09/1			

¹⁾Standard Deviation

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07% of energy intake), respectively.

Levels of plasma total, LDL-, and HDL-cholesterol in the study subjects were 168.4 ± 32.3 mg/dl, 102.7 ± 31.2 mg/dl, and 54.5 ± 8.3 mg/dl, respectively. Mean plasma triglyceride level was 55.7 ± 22.4 mg/dl. Ratios of LDL-/HDL-cholesterol and total-/HDL-cholesterol were 1.92 ± 0.63 and 3.13 ± 0.65 . Reported levels of plasma lipid of Korean young females are 170-193mg/dl⁽²¹⁾⁽²²⁾, similar to the present study. Six subjects fell in the moderate risk group(> 200mg/dl) for total cholesterol as proposed by Kim et al.⁽³⁶⁾ for Koreans. Plasma cholesterol levels of the subjects were not significantly related to dietary fatty acid intake(data not shown).

Fatty acid compositions of plasma and erythrocyte membrane show some differences(Table 2). The highest concentration of fatty acid in plasma was linoleic acid followed by palmitic and oleic acids. However, palmitic acid was present in the highest concentration in erythrocyte membrane followed by stearic, arachidonic, and oleic acids. Erythrocyte membrane contained significantly higher concentrations of stearic acid, arachidonic acid, and DHA and lower concentrations of linoleic and palmitoleic acid. Overall, erythrocyte membrane contained considerably higher concentration of saturated fatty acids with less monounsaturated fatty acids. While the percentage of ω -3 fatty acids was only 4% in plasma, their con-

tribution in erythrocyte membrane was more than 10%, solely due to the increase of DHA. On the other hand, ω -6 fatty acids was lower in erythrocyte membrane compared to plasma lipid. Also the major type

Table 2. Fatty acid composition of plasma and erythrocyte membranes in the subjects (unit=% ; N=56)

Fatty acid	Plasma		RBC Membrane	
	Mean	S.D. ¹⁾	Mean	S.D.
14 : 0	0.84	0.26	0.33	0.09
16 : 0	23.16	1.48	22.07	1.74
16 : 1	2.35	0.74	0.18	0.15
17 : 0	0.27	0.06	0.30	0.06
18 : 0	7.04	0.57	19.10	1.32
18 : 1(ω -9)	19.78	2.27	15.08	1.00
18 : 2(ω -6)	35.06	3.03	11.63	1.42
18 : 3(ω -3)	0.52	0.17	0.15	0.29
20 : 3(ω -6)	1.06	0.32	1.38	0.26
20 : 4(ω -6)	5.81	1.03	16.00	1.40
20 : 5(ω -3)	0.65	0.37	0.84	0.42
22 : 6(ω -3)	2.69	0.75	7.96	1.27
Total SFA	31.53	1.86	42.03	1.88
Total MUFA	22.25	2.54	15.52	1.04
Total PUFA	46.22	2.75	42.45	2.24
Total ω -6	42.21	2.78	31.45	2.31
Total ω -3	3.86	1.08	10.74	1.74
ω -6 / ω -3 ratio	11.89	3.77	3.01	0.54
P / M / S ratio	1.47/0.71/1		1.01/0.37/1	

1) Standard Deviation

Table 3. Pearson correlation coefficients between the concentrations of ω -3 fatty acids in plasma and erythrocyte membrane dietary intake levels in the study subjects (N=56)

Blood Fatty Acids		Dietary Fatty Acids				
		18 : 3	EPA	DHA	Total ω -3	EPA+DHA
plasma	18 : 3(ω -3)	0.11	0.17	0.20	0.21	0.19
	EPA	0.02	0.54***	0.56***	0.35**	0.56***
	DHA	0.09	0.36**	0.37**	0.30*	0.37**
	Total ω -3	0.09	0.47***	0.48***	0.36**	0.48***
	EPA+DHA	0.08	0.46***	0.47***	0.34**	0.47***
erythrocyte membrane	18 : 3(ω -3)	-0.07	0.01	0.00	-0.06	0.00
	EPA	-0.01	0.43***	0.47***	0.26*	0.46***
	DHA	-0.07	0.45***	0.48***	0.22	0.47***
	Total ω -3	-0.04	0.47***	0.50***	0.25	0.49***
	EPA+DHA	-0.05	0.47***	0.49***	0.24	0.49***

*p < 0.05 ** p < 0.01 *** p < 0.001

of ω -6 fatty acid changed from linoleic acid in plasma to arachidonic acid in erythrocyte membrane.

Relationship among the dietary intake of ω -3 fatty acids, the levels of these fatty acids in plasma, and those of erythrocyte membranes are shown in Tables 3 and 4. Except LNA, ω -3 fatty acid intakes and blood levels were significantly correlated. Correlation coefficients between erythrocyte membrane ω -3 fatty acids and the dietary intake of total ω -3 fatty acid were much lower than the correlation coefficients with individual ω -3 fatty acid intake. When subjects are divided into 4 groups by the intake levels of EPA and DHA, subjects in the highest group had significantly higher contents of EPA and DHA, both in plasma and in erythrocyte membrane (Table 4).

Discussions

Fat intake of the subjects is certainly much lower than the levels reported in studies conducted in the U. S. or Europe. However, it is considerably higher than the levels found in recent reports of the National Nutrition Survey²⁰. Cholesterol intake was within the range of safe intake (below 300mg) proposed by

dietary guidelines of the U.S.A.³⁴. Dietary intake levels of ω -3 fatty acids of the study subjects are lower when they are compared with previous reports in similar subjects, ranging from 0.99g to 1.3g^{21,22}. This difference may partly come from the differences in dietary survey methodology. Previous reports used 24-hour recalls or 3-day records, and it may not have been long enough to estimate the intake of nutrients which are consumed with great variability. Coefficients of variation (CV) of intakes of fatty acids range from 28% to 125% in the present study and they are considerably lower than CV's obtained by 3-day records by Kim and Paik²¹. The highest CV's were observed in EPA (125%) and DHA (124%) in this study, compared to 217% and 212% respectively, in the study of Kim and Paik²¹. Whether this variability would further decrease with longer dietary survey period remains to be determined. The intake level of ω -3 fatty acids of the study subjects are lower when it is compared to the results in other countries, including Japan³⁵. In this international comparison, dietary intake levels of Japan were calculated from dietary records of 7 days and 4 days obtained in 1964 and 1971. Considering the variability of ω -3 fatty acid intake discussed above,

Table 4. Comparison of ω -3 fatty acids in plasma and erythrocyte membrane fatty acids by quartiles of dietary EPA plus DHA intakes (n=56)

	Quartile of Dietary Intake of EPA+DHA			
	1st(n=14)	2nd(n=14)	3rd(n=14)	4th(n=14)
Intake(g/d)	0.021 \pm 0.004 ¹⁾	0.041 \pm 0.008	0.098 \pm 0.034	0.348 \pm 0.195
plasma FA(%)				
18 : 3(ω -3)	0.53 \pm 0.16	0.49 \pm 0.16	0.52 \pm 0.15	0.54 \pm 0.22
EPA	0.57 \pm 0.28 ^a	0.56 \pm 0.22 ^a	0.55 \pm 0.32 ^a	0.92 \pm 0.50 ^b
DHA*	2.25 \pm 0.69 ^a	2.73 \pm 0.62 ^{ab}	2.78 \pm 0.66 ^{ab}	3.00 \pm 0.86 ^b
Total ω -3*	3.35 \pm 0.90 ^a	3.78 \pm 0.73 ^{ab}	3.84 \pm 0.97 ^{ab}	4.47 \pm 1.40 ^b
EPA+DHA	2.81 \pm 0.89 ^a	3.29 \pm 0.69 ^{ab}	3.33 \pm 0.92 ^{ab}	3.93 \pm 1.30 ^b
erythrocyte membrane FA(%)				
18 : 3(ω -3)	0.11 \pm 0.11	0.13 \pm 0.20	0.13 \pm 0.31	0.22 \pm 0.44
EPA*	0.63 \pm 0.22 ^a	0.84 \pm 0.31 ^{ab}	0.82 \pm 0.34 ^{ab}	1.04 \pm 0.64 ^b
DHA*	7.20 \pm 0.91 ^a	8.11 \pm 0.91 ^{ab}	7.80 \pm 1.13 ^{ab}	8.73 \pm 1.61 ^b
Total ω -3	9.60 \pm 1.14 ^a	10.95 \pm 1.24 ^b	10.60 \pm 1.44 ^{ab}	11.82 \pm 2.28 ^b
EPA+DHA*	7.83 \pm 1.05 ^a	8.95 \pm 1.10 ^{ab}	8.63 \pm 1.43 ^{ab}	9.78 \pm 2.19 ^b

1) MEAN \pm SD

*Differences among the groups and significantly different at $p < 0.05$ by Duncan's multiple range test. Values within the same row with different superscripts are significantly different.

long-term extensive survey is required for accurate estimation of dietary intake of these fatty acids. The differences in ω -3 fatty acid intake levels between the present study and that reported in Japan may or may not reflect true differences in intake levels. Confirmation of such differences requires a comparable extensive dietary surveys in the two countries.

Lack of the relationship between the concentration of blood cholesterol and dietary fatty acid intake is not consistent with many other studies³⁵⁾. Hayes and Khosla reported that the major determinants of serum cholesterol in humans consuming dietary cholesterol below 400mg/d are myristic and linoleic acids³⁶⁾. Calculation of predicted serum cholesterol level of the subjects based on regression equation proposed by Hayes {serum cholesterol=229+8(% energy from myristic acid)-36log(% energy from linoleic acid)} comes out to be 213mg/dl, a value significantly higher than the actual mean value of 168.9mg/dl. The discrepancy may partly come from the much lower intake of energy, total fat, and cholesterol in the subjects of the present study than those of western population, whom the regression equation was derived from.

The concentrations of polyunsaturated fatty acids, both ω -6 and ω -3 families, in RBC membrane of the subjects in the present study are much higher than those of rural Chinese population in the report of Wenxun et.al.¹⁵⁾. Compared to English and Canadian populations, Chinese population was reportedly higher in concentrations of monounsaturated fatty acids, but lower in polyunsaturated fatty acids. There was no analysis of the relationship between blood fatty acid composition and dietary intake levels of Chinese population in the above mentioned report.

EPA and DHA levels in plasma lipid and erythrocyte membrane seem to reflect intakes of these fatty acids, but such a relationship was not found in LNA. When subjects were divided into quartiles according to intake levels of EPA+DHA, mean daily intake level increased from 0.021mg in the lowest quartile to 0.348mg in the highest quartile. Levels of ω -3 fatty acids in plasma and erythrocyte membranes generally

increased with increasing dietary intake. Such a tendency, however, was not observed for LNA either in plasma or in erythrocyte membrane. These results indicate that plasma and erythrocyte levels of EPA and DHA, but not the levels of LNA, reflect dietary intake levels of these fatty acids during the previous 2 months. In the previous study²¹⁾, no significant correlation was observed between blood concentrations and dietary intakes of ω -3 fatty acids in female Korean college students when dietary intake levels were obtained by 3-day records. Therefore, accurate assessment of dietary intake of ω -3 fatty acids seems to require diet survey for longer than 3 days. A period of two months seems to be adequate to assess intake of ω -3 fatty acids accurate enough to find significant correlation with blood concentration of these fatty acids.

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= 국 문 초 록 =

한국 성인 여성의 혈장 및 적혈구막의 ω -3 지방산함량과 2개월간의 식이섭취와의 관계

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혈장 및 적혈구막의 지방산 조성은 식이 지방산 섭취패턴의 영향을 받는 것으로 알려져 있다. 본 연구는 한국 성인 여성 56명에서 ω -3 지방산 섭취와 혈장 및 적혈구막의 ω -3 지방산 섭취의 관계를 파악하기 위하여 실시되었다. 식이섭취조사는 약 2개월간의 기간에 걸쳐 연속 7일간의 식이기록과 6 회에 걸친 24시간 회상을 실시하여 각 대상자별 1일 평균 지방산 섭취량을 계산하였다. 식이섭취조사가 끝난 직후 공복시 혈액을 채취하여 혈장과 적혈구를 분리하여 혈장 지질과 적혈구막을 추출하여 지방산조성을 분석하였다. 대상자들의 1일 평균 영양소 섭취량은 열량 1,569kcal, 지방 41.8g, 콜레스테롤 217mg 이었으며, 총 열량중 지방열량의 비율은 24% 였다. 1일 평균 eicosapentaenoic acid(EPA) 섭취량은 0.04g, docosahexaenoic acid(DHA) 섭취량은 0.06g, 총 ω -3 지방산 섭취량은 0.48g 이었다. 대상자들의 혈장 및 적혈구막의 EPA 와 DHA 함량은 각각의 지방산 섭취량과 유의적인 상관관계가 있었으나, α -linolenic acid 에서는 그러한 상관관계가 나타나지 않았다. 이전의 연구에서 3일간의 식이섭취와 혈액 지방산 조성에서는 유의적인 상관관계가 없었으므로 혈액에 영향을 미칠정도의 식이섭취상태를 파악하기 위해서는 3일간의 조사는 충분하지 못한 것으로 생각된다. 이상의 결과에서 우리나라 젊은 성인 여성의 ω -3 지방산 섭취량은 낮은 편이며 그 패턴을 파악하기 위해서는 2개월정도의 식이섭취조사가 필요한 것으로 생각된다.