

Japan Diet Intake Changes Serum Phospholipid Fatty Acid Compositions in Middle-Aged Men: A Pilot Study

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Aim: The Japan Diet nutritional education program effects on serum fatty acid compositions for prevention of atherosclerotic cardiovascular disease in middle-aged men brought up in the westernized dietary environment of modern Japan were examined.

Methods: Thirty-three men, 30–49 years of age, attended a nutrition education class and were recommended to consume Japan Diet volumes (more fish, soybeans and soy products, vegetables, seaweed, konjak, mushrooms, and unrefined cereals and less animal fat, meat and poultry with fat, sweets, desserts and snacks, and alcoholic drinks) for 6 weeks. Three-day weighted dietary records were kept, and fatty acid intakes were calculated. Serum phospholipid fatty acid compositions were examined.

Results: During the 6 weeks, fish, soy, and seaweed and/or mushrooms and/or konjak were consumed 1.0, 1.1, and 1.0 times daily on average, whereas daily fatty meat and poultry and sweet consumptions were 0.3 and 0.3, respectively. These changes were attributed to increased intake of n-3 polyunsaturated fatty acids (PUFAs) such as C20:5(n-3), C22:6(n-3), C18:4(n-3), and C20:4(n-3) and decreased intakes of all saturated fatty acids and unsaturated fatty acids such as C18:1 and C18:2(n-6). As to the phospholipid fatty acid composition, C18:0 decreased, whereas C15:0, C17:0, and C20:0 increased. Marked increases in C20:5(n-3) and C22:6(n-3) raised total n-3 PUFA from 10.30% to 13.20% along with n-6 PUFA decreasing from 33.92% to 31.16%. Despite decreases in C20:4(n-6) and C20:3(n-6), the C20:4(n-6)/C20:3(n-6) ratio used as an estimate of delta-5 desaturase activities increased and correlated positively with fish intake at completion of the intervention.

Conclusions: The Japan Diet is effective for changing the fatty acids to an anti-atherosclerotic profile. The clinical trial registration number: UMIN000020639.

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Key words: Japan Diet, Fish, Delta-5 desaturase activities, Icosapentaenoic acid, Docosahexaenoic acid

Introduction

There has been major research and clinical interest in elucidating the effects of dietary fatty acids on cardiovascular disease risk reduction. Over the years, numerous studies have corroborated the blood cholesterol predictive equations showing saturated fatty acids (SFAs) to be hypercholesterolemic and that polyunsaturated fatty acids (PUFAs) reduce cholesterol⁽¹⁾.

In the Seven Countries Study, a high serum cholesterol level was found to be a risk factor for coronary artery disease, and high SFA intake was recognized as leading to a high serum cholesterol level⁽²⁾. From the end of World War II, problems of malnutrition remained in Japan up to 1960. The traditional Japanese diet in the 1960s to mid-1970s, which was rich in fish, with relatively low consumptions of meat, poultry, and animal fats, was described as a cardioprotective dietary

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style. From the 1960s, the Japanese dietary style has changed markedly, with Westernization and diversification, and the percentage of energy derived from lipid increasing from 10.6% in 1960 to 18.9% in 1970 and finally to 22.3% in 1975. From 1975 to 1990, the changes were moderate, and the percentage of energy derived from lipid was rather stable. Ever since, lipid intake has been remarkably high, especially in the younger generation. In 2016, people with energy intake derived from lipid of over 30% were 40% in the all three age groups (20s, 30s, and 40s). Lack of fish intake and excessive oils and meat and poultry intakes have been recognized as major problems in younger generations³). Along with these trends, fatty acid intakes have also changed, and this is contributing to increased obesity, dyslipidemia, and type 2 diabetes. There is now a need to verify the effects of the traditional Japanese diet on dietary fatty acid intakes and circulating fatty acid levels.

We previously conducted a pilot study showing the anti-atherosclerotic effects of the Japan Diet (higher consumptions of fish, soybeans and soy products, vegetables, seaweed/mushrooms/konjak, and unrefined cereals with reduced consumptions of animal fat, meat and poultry with fat, sweets including desserts and snacks, and alcoholic drinks). At baseline, their food consumption was rich in meat and poultry, but poor in fish and soybeans. The percentage of energy derived from lipid was 31%, twice as high as that of the 1960–1970s. After the 6 week education program, fish; soy; and the sum of seaweed, mushroom, and konjak intakes doubled, whereas intakes of meat and poultry, sweets including desserts and snacks, and margarine and shortening all decreased. These changes contributed to significant decreases in serum low-density lipoprotein (LDL) cholesterol, malondialdehyde-modified LDL, and triglyceride concentrations and to the maintenance of high levels of high-density lipoprotein (HDL) cholesterol⁴).

The Japan Diet has a characteristic fatty acid content derived from seafood and plant foods. Serum fatty acid compositions would be expected to evolve together with the anti-atherogenic pattern in response to Japan diet consumption. The effects of purified fatty acid supplementation as part of a prescribed dietary intervention that increased circulating n-3 PUFA have been reported⁵). However, there have been no studies examining changes in serum fatty acid profiles achieved by the educational approach of recommending intake of the Japan Diet.

Aim

This study aimed to examine how the Japan Diet affects fatty acid intakes and serum fatty acid compo-

sitions.

Methods

Study Design and Subjects

The study design, methods, and subjects were described in detail in our previous report⁴). The 34 participants were male, between 30 and 49 years of age, with a body mass index (BMI) over 22 kg/m², and all were non-smokers. Users of dietary supplements or health food products were excluded. After being given a detailed explanation of the program, all 34 subjects consented to participate. The present study was conducted according to the guidelines of the Declaration of Helsinki, and all procedures were approved by the ethics committee of Japan Women's University (No. 209). Written informed consent was obtained from all subjects prior to participation. The clinical trial registration number is UMIN000020639.

Intervention Program

The 6 week intervention program was explained in our previous report⁴). Briefly, a lecture and group work were conducted focusing on easy ways to consume the foods classified as constituting the Japan Diet (consumption of more fish, soybeans and soy products, vegetables, seaweed, mushrooms, konjak, and unrefined cereals while reducing animal fat, meat and poultry with fat, sweets including desserts and snacks, and alcoholic drink intakes). Each participant was required to keep a daily performance record in terms of the intakes of foods comprising the Japan Diet for 6 weeks. The subjects were instructed to maintain their habitual physical activity levels during the study period.

Food Intake

Subjects were instructed to record their daily food intake, counting the number of dishes (fish, soybeans and soy products, vegetables, seaweed/mushrooms/konjak and unrefined cereals, fatty meat and poultry, and sweets/desserts/snacks) during the intervention period.

Dietary Fatty Acid Intake

Three-day weighted dietary records were kept at baseline and at the end of the intervention as previously reported⁴). Fatty acid intakes were calculated employing Excel-Eiyokun Ver.6, (Kenpaku-sha Co., Ltd., Tokyo, Japan) software.

Measurement of Metabolic Parameters and Serum Phospholipid Fatty Acid Compositions

Fasting blood collections were conducted on day 1 and after 6 weeks, in the morning following a 12 h overnight fast. Plasma and serum samples were obtained

Table 1. Number of dishes which provide each of target foods in a day during the 6 week intervention

	number in a day	(minimum, maximum)
Fish	1.0 ± 0.3	(0.4, 2.1)
Soybeans and other soy products	1.1 ± 0.5	(0.1, 2.4)
Vegetables	2.3 ± 0.9	(0.7, 5.0)
Seaweed, mushrooms and konjak	1.0 ± 0.5	(0.2, 2.0)
Fatty meat and poultry	0.3 ± 0.2	(0.0, 0.9)
Sweets, desserts and snacks	0.3 ± 0.2	(0.0, 0.9)

$n = 33$, Values are expressed as means ± SD.

and stored at -80°C until analysis. Serum biochemical parameters were measured as described in our previous report⁴. For fatty acid measurement, serum lipids were extracted by the method of Folch *et al.*⁶. The phospholipid fraction was obtained with thin-layer chromatography and methylated as described in our previous report with minor modifications⁷. The fatty acid methyl esters were separated by gas-liquid chromatography (Shimadzu GC14B, Tokyo, Japan) using a 0.25 mm × 30 m capillary column containing DB-FFAP (J&W Scientific, CA, USA). The injector temperature was 230°C , and the column temperature rose at $2^{\circ}\text{C}/\text{min}$ from 160°C to 200°C . Nitrogen was employed as the carrier gas, and the split ratio was 22.0.

Statistical Analysis

The data of the 33 Japanese men who completed this program were analyzed. One participant was hospitalized for an acute illness and had to drop out.

Statistical analyses were carried out using SPSS for Windows (version 16.0J; SPSS Japan, Inc.). All values are presented as means with standard deviations. The statistical significance of differences in values obtained at baseline and at the end of the intervention was assessed using the non-parametric Wilcoxon matched pair's signed-rank test. Spearman correlation analyses were performed to identify correlations between metabolic parameters and the fatty acid ratio in the serum phospholipids. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Subjects

The mean age of the subjects was 39 ± 5 years. Other clinical parameters were shown in our previous report⁴. In short, after the intervention, body weight, BMI, and umbilical circumference decreased with a coincident decrease in the serum leptin concentration. Serum LDL-cholesterol, malondialdehyde-LDL, and triglyceride concentrations decreased significantly, whereas HDL cholesterol was unchanged⁴.

Food Intake

Food intake volumes per day at baseline and at the end of this intervention were reported previously; that is, mean fish and soy intakes increased from 49.0 and 39.8 g to 98.0 and 86.5 g, respectively, and seaweed intake from 9.6 to 33.4 g. The sum volume of meat and poultry intake, which totaled 134.3 g at baseline, had decreased to 95.4 g at the end of the intervention⁴. The number of dishes consumed daily, which provided each type of target food during the 6 weeks, is shown in **Table 1**. Fish, soy, and seaweed and/or mushrooms and/or konjak were consumed 1.0, 1.1, and 1.0 times per day on average, respectively, whereas fatty meat and poultry, and sweets were consumed 0.3 and 0.3 times daily, respectively.

Dietary Fatty Acid Intakes

Energy intakes derived from lipids and SFAs were 31.4 (23.7, 42.9) (mean (minimum, maximum)) % and 8.8 (5.61, 15.0) % at baseline, and had decreased to 30.6 (18.4, 44.9) % and to 7.8 (3.6, 14.2) %, respectively, at the end of the intervention⁴.

Almost all SFAs intake including C12:0, C16:0, and C18:0 significantly decreased ($p = 0.002$, 0.002 , 0.005), and C14:0 tended to decrease ($p = 0.053$). The sum of C12:0, C14:0, and C16:0, which are known to increase the LDL-C concentration, was decreased by 3882 mg corresponding to 1.4% of total energy intake.

Monounsaturated fatty acid (MUFA) intake decreased ($p = 0.008$), principally that of C18:1, which is the major MUFA ($p = 0.003$).

C18:2 intake, which accounts for the majority of n-6 PUFA, tended to decrease ($p = 0.055$), though C20:3 (n-6) and C20:4(n-6) did not change ($p = 0.122$, 0.922).

Mean n-3 PUFA intake was 3103 mg at baseline and had increased to 3723 mg ($p = 0.036$), mainly due to C20:5(n-3) and C22:6(n-3), by the end of the intervention ($p = 0.005$, 0.016).

The SFA/MUFA/PUFA ratio changed from 1/1.3/0.7 to 1/1.4/0.9, and the n-6/n-3 ratio decreased from 4.9 to 3.7 (**Table 2**).

At the end of the intervention, a positive correla-

Table 2. Fatty acid intakes at baseline and the end of the 6 week intervention

	Baseline (mg)	6 weeks (mg)	Change (mg)	<i>p</i>
C4:0	328 ± 241	223 ± 284	-105 ± 301	0.003
C6:0	214 ± 156	144 ± 177	-70 ± 187	0.002
C7:0	1 ± 2	1 ± 2	-0 ± 2	0.129
C8:0	198 ± 171	119 ± 134	-79 ± 172	0.002
C10:0	328 ± 232	215 ± 233	-113 ± 267	0.004
C12:0	820 ± 840	454 ± 573	-366 ± 895	0.002
C13:0	4 ± 4	3 ± 5	-1 ± 6	0.127
C14:0	1733 ± 867	1426 ± 969	-307 ± 1072	0.053
C15:0	165 ± 85	135 ± 104	-30 ± 112	0.024
C15:0ant	47 ± 34	32 ± 40	-15 ± 46	0.002
C16:0	14504 ± 3626	11296 ± 5105	-3209 ± 5222	0.002
C16:0iso	23 ± 17	16 ± 20	-7 ± 23	0.004
C17:0	202 ± 76	173 ± 103	-30 ± 115	0.075
C17:0ant	44 ± 32	30 ± 38	-14 ± 43	0.003
C18:0	5533 ± 1616	4236 ± 1970	-1297 ± 2361	0.005
C20:0	259 ± 59	218 ± 58	-42 ± 82	0.009
C22:0	129 ± 56	110 ± 37	-19 ± 68	0.140
C24:0	62 ± 28	47 ± 17	-16 ± 33	0.014
SFA subtotal	24593 ± 7168	18870 ± 8946	-5723 ± 9572	0.003
C10:1	26 ± 19	18 ± 22	-8 ± 26	0.004
C14:1	146 ± 80	114 ± 159	-32 ± 160	0.006
C15:1	0 ± 0	0 ± 0	-0 ± 0	0.144
C16:1	1240 ± 424	1242 ± 758	2 ± 836	0.448
C17:1	134 ± 52	121 ± 95	-13 ± 106	0.161
C18:1	29991 ± 6651	24137 ± 8618	-5855 ± 10664	0.003
C20:1	628 ± 321	802 ± 486	174 ± 582	0.106
C22:1	329 ± 472	511 ± 608	182 ± 781	0.102
C24:1	49 ± 43	74 ± 56	25 ± 74	0.031
MUFA subtotal	32583 ± 6931	27013 ± 9518	-5570 ± 11440	0.008
C18:2(n-6)	14937 ± 3197	13384 ± 3468	-1552 ± 4710	0.055
C18:3(n-6)	4 ± 5	8 ± 7	4 ± 8	0.021
C20:2(n-6)	79 ± 28	76 ± 44	-4 ± 47	0.280
C20:3(n-6) DGLA	43 ± 15	38 ± 24	-5 ± 22	0.122
C20:4(n-6) AA	207 ± 67	220 ± 128	13 ± 115	0.922
C22:2(n-6)	0 ± 0	0 ± 0	0 ± 0	0.213
C22:4(n-6)	17 ± 7	16 ± 11	-2 ± 10	0.249
C22:5(n-6)	9 ± 8	19 ± 17	10 ± 16	0.001
n-6 PUFA subtotal	15143 ± 3150	13760 ± 3594	-1534 ± 4786	0.067
C18:3(n-3)	2195 ± 627	2141 ± 701	-54 ± 912	0.741
C18:4(n-3)	71 ± 105	125 ± 148	54 ± 185	0.039
C20:4(n-3)	28 ± 30	54 ± 43	26 ± 56	0.020
C20:5(n-3) EPA	237 ± 235	454 ± 313	217 ± 398	0.005
C21:5(n-3)	6 ± 10	14 ± 15	8 ± 16	0.010
C22:5(n-3)	84 ± 55	144 ± 93	61 ± 111	0.007
C22:6(n-3) DHA	490 ± 440	797 ± 547	306 ± 730	0.016
n-3 PUFA subtotal	3103 ± 1022	3723 ± 1476	620 ± 1637	0.036
PUFA subtotal	18427 ± 3768	17541 ± 4639	-886 ± 5589	0.249

n = 33, Values are expressed as means ± SD.

SFA: saturated fatty acid, MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid

DGLA: di-homo gamma linolenic acid, AA: arachidonic acid, EPA: icosapentaenoic acid, DHA: docosahexaenoic acid

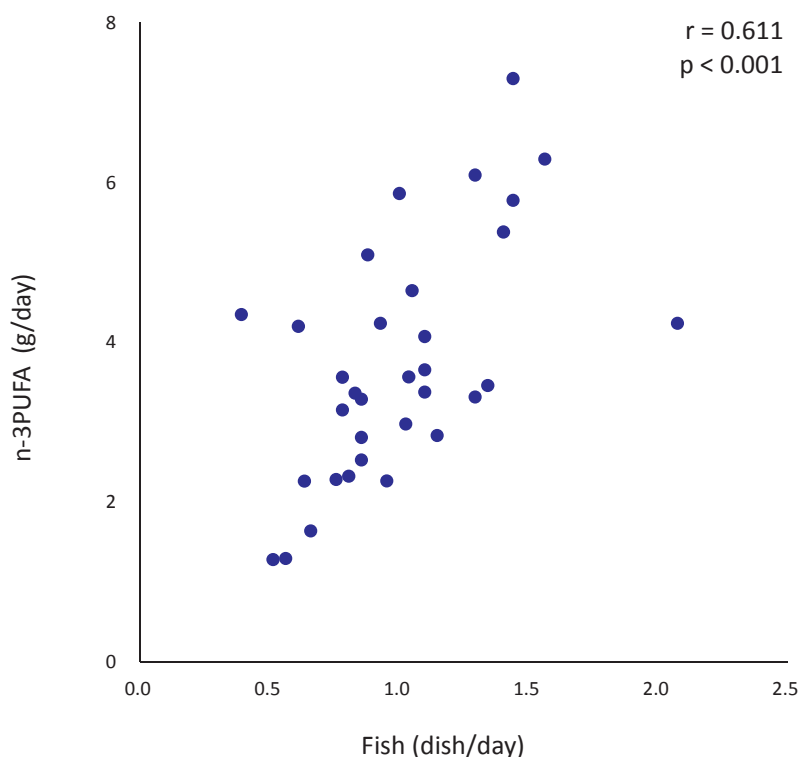


Fig. 1. Simple correlation between fish dish intake during the 6 week intervention and n-3 PUFA intake at the end of the 6 week intervention
 $n = 33$, PUFA: polyunsaturated fatty acid

tion was observed between n-3 PUFA intake and the number of fish dishes consumed ($p < 0.001$) (**Fig. 1**).

Serum Phospholipid Fatty Acid Compositions

Changes in the fatty acid composition in serum phospholipids are presented in **Table 3**.

As to the SFAs, the C18:0 percentage had decreased by the end of the intervention as compared with baseline ($p = 0.046$), whereas C15:0 ($p = 0.044$), C17:0 ($p = 0.020$), and C20:0 ($p = 0.003$) were increased.

The total MUFA percentage did not change significantly, though cisC18:1(n-9) decreased ($p = 0.009$), whereas C20:1, C22:1, and C24:1 increased ($p < 0.001$, $p = 0.002$, $p < 0.001$).

As to n-3 PUFA, marked increases in C20:5(n-3) and C22:6(n-3) ($p < 0.001$, both) resulted in a significant increase in total n-3 PUFA from 10.30% to 13.20% ($p < 0.001$) along with a total n-6 PUFA decrease from 33.92% to 31.16% ($p < 0.001$). The n-3 PUFA percentage increased as much as the n-6 PUFA percentage decreased, suggesting n-6 PUFAs to have been replaced by n-3 PUFAs. Almost all n-6 PUFAs, including C18:2(n-6), 20:4(n-6), C20:3(n-6), and C20:2(n-6) decreased ($p = 0.004$, $p = 0.003$, $p < 0.001$, $p = 0.010$).

Fatty acid ratios such as C20:5(n-3)/C20:4(n-6), C22:6(n-3)/C20:4(n-6), and (C20:5(n-3) + C22:6(n-3))/C20:4(n-6) increased ($p < 0.001$). Furthermore, the C20:4(n-6)/C20:3(n-6) ratio, which serves as an estimate of delta-5 desaturase (D5D) activities also increased ($p = 0.002$) (**Table 4**).

Correlations between Changes in Metabolic Parameters and Fatty Acid Ratios in Serum Phospholipids

Changes in the C20:4(n-6)/C20:3(n-6) ratio correlated negatively with changes in body weight ($p < 0.001$), BMI ($p < 0.001$), total cholesterol ($p = 0.013$), LDL cholesterol ($p = 0.012$), non-HDL cholesterol ($p = 0.002$), triglycerides ($p = 0.040$), and the insulin concentration ($p = 0.036$). However, other fatty acid ratios showed no significant correlations with metabolic parameters (**Table 5**).

Correlations between Food Intake Volume and Fatty Acid Ratios in Serum Phospholipids

There was a tendency toward a positive association between the number of fish dishes consumed and the C20:4(n-6)/C20:3(n-6) ratio. Fish intake volume showed a positive association with the C20:4(n-6)/C20:3(n-6) ratio in serum phospholipids at the end of

Table 3. Fatty acid compositions in serum phospholipids at baseline and the end of the 6 week intervention

	Baseline	6 weeks	Change	<i>p</i>
C14:0	0.14 ± 0.04	0.13 ± 0.04	-0.01 ± 0.05	0.406
C15:0	0.09 ± 0.02	0.10 ± 0.05	0.01 ± 0.05	0.044
C16:0	23.04 ± 1.32	22.96 ± 1.12	-0.08 ± 1.13	0.809
C17:0	0.32 ± 0.05	0.34 ± 0.04	0.02 ± 0.04	0.020
C18:0	14.64 ± 0.93	14.24 ± 1.19	-0.39 ± 1.12	0.046
C20:0	0.62 ± 0.11	0.66 ± 0.12	0.04 ± 0.08	0.003
C22:0	1.84 ± 0.38	1.85 ± 0.34	0.02 ± 0.30	0.586
C24:0	1.60 ± 0.29	1.56 ± 0.26	-0.04 ± 0.28	0.623
SFA subtotal	42.28 ± 1.58	41.85 ± 1.55	-0.43 ± 1.82	0.140
C14:1	0.04 ± 0.02	0.03 ± 0.03	-0.01 ± 0.02	0.092
C16:1	0.29 ± 0.07	0.27 ± 0.07	-0.02 ± 0.08	0.118
C17:1	0.14 ± 0.08	0.15 ± 0.09	0.01 ± 0.09	0.561
C18:1(n-9)	8.10 ± 0.94	7.47 ± 0.88	-0.63 ± 1.18	0.009
C20:1	0.21 ± 0.04	0.29 ± 0.15	0.08 ± 0.15	<0.001
C22:1	0.01 ± 0.02	0.09 ± 0.18	0.09 ± 0.18	0.002
C24:1	3.19 ± 0.66	3.88 ± 0.95	0.69 ± 0.81	<0.001
MUFA subtotal	11.98 ± 0.93	12.18 ± 1.01	0.21 ± 0.99	0.257
C18:2(n-6)	19.83 ± 2.34	18.26 ± 3.08	-1.57 ± 2.81	0.004
C20:2(n-6)	0.32 ± 0.04	0.29 ± 0.07	-0.03 ± 0.07	0.010
C20:3(n-6) DGLA	2.33 ± 0.81	1.79 ± 0.69	-0.54 ± 0.57	<0.001
C20:4(n-6) AA	10.74 ± 1.71	10.14 ± 1.87	-0.60 ± 1.26	0.003
C22:2(n-6)	0.69 ± 0.14	0.68 ± 0.17	-0.01 ± 0.19	0.823
n-6 PUFA subtotal	33.92 ± 2.64	31.16 ± 2.74	-2.75 ± 2.99	<0.001
C18:3(n-3)	0.28 ± 0.10	0.28 ± 0.13	0.00 ± 0.11	0.741
C18:4(n-3)	0.17 ± 0.03	0.17 ± 0.03	0.00 ± 0.03	0.908
C20:4(n-3)	0.12 ± 0.06	0.13 ± 0.07	0.01 ± 0.09	0.782
C20:5(n-3) EPA	1.91 ± 1.40	3.21 ± 1.83	1.30 ± 2.01	<0.001
C22:5(n-3)	0.98 ± 0.17	1.03 ± 0.14	0.05 ± 0.15	0.201
C22:6(n-3) DHA	6.84 ± 1.61	8.37 ± 1.67	1.53 ± 1.83	<0.001
n-3 PUFA subtotal	10.30 ± 2.95	13.20 ± 3.33	2.89 ± 3.58	<0.001
PUFA subtotal	44.22 ± 1.85	44.36 ± 1.92	0.14 ± 1.54	0.574

n = 33, Values are expressed as means ± SD. Results are presented as the percentage of total fatty acids.

SFA: saturated fatty acid, MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid

DGLA: di-homo gamma linolenic acid, AA: arachidonic acid, EPA: icosapentaenoic acid, DHA: docosahexaenoic acid

the intervention (*p* < 0.001) (Fig. 2). No associations were observed between intake volumes of other foods and the C20:4(n-6)/C20:3(n-6) ratio.

Discussion

Our educational intervention recommending the Japan Diet resulted in daily consumption of one dish each on average of fish, soy, and seaweed, as well as increased vegetable intake, and decreased consumptions of meat and poultry, sweets including desserts and snacks, and margarine and shortening⁴. These changes were attributed to increased intakes of n-3 PUFAs such

as C20:5(n-3), C22:6(n-3), C18:4(n-3), and C20:4(n-3) and decreased intakes of all SFAs, as well as unsaturated fatty acids such as C18:1 and C18:2(n-6).

For dietary treatment of dyslipidemia, the fatty acid intake ratio of SFAs (S)/MUFAs (M)/PUFAs (P) was to be 1/1.3/1 according to dietary guidelines⁸. The Japanese S/M/P ratio in the 1970s was equivalent to this recommended ratio on average⁹. In this study, the baseline average was 1/1.3/0.7, clearly moving in a worsening direction, approaching the ratio in the UK, which is 1/1/0.5¹⁰ while showing an improvement to 1/1.4/0.9 with SFA decreasing from 8.8% to 7.8% after the educational intervention. Most notably, the sum

Table 4. Fatty acid ratios of serum phospholipids at baseline and at the end of the 6 week intervention

	Baseline	6 weeks	<i>p</i>
C20:5(n-3)/C20:4(n-6)	0.188 ± 0.174	0.329 ± 0.210	< 0.001
C22:6(n-3)/C20:4(n-6)	0.656 ± 0.211	0.854 ± 0.242	< 0.001
(C20:5(n-3) + C22:6(n-3))/C20:4(n-6)	0.844 ± 0.372	1.183 ± 0.418	< 0.001
C20:4(n-6)/C20:3(n-6)	5.149 ± 1.928	6.365 ± 2.252	0.002

n = 33, Values are expressed as means ± SD.

Table 5. Correlations between changes in metabolic parameters and fatty acid ratios in serum phospholipids

	ΔC20:5(n-3)/ C20:4(n-6)		ΔC22:6(n-3)/ C20:4(n-6)		Δ(C20:5(n-3) + C22:6(n-3))/ C20:4(n-6)		ΔC20:4(n-6)/ C20:3(n-6)	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
ΔBody weight (kg)	-0.147	0.415	-0.293	0.099	-0.179	0.320	-0.618	< 0.001
ΔBody Mass Index (kg/m ²)	-0.146	0.418	-0.296	0.094	-0.179	0.318	-0.600	< 0.001
ΔSBP (mmHg)	-0.086	0.636	-0.061	0.736	-0.062	0.732	-0.305	0.084
ΔDBP (mmHg)	-0.055	0.762	0.005	0.978	-0.025	0.890	-0.264	0.138
ΔTC (mg/dL)	0.111	0.537	0.108	0.551	0.154	0.392	-0.426	0.013
ΔLDL-C (mg/dL)	-0.020	0.914	0.060	0.742	0.065	0.721	-0.434	0.012
Δnon-HDL-C (mg/dL)	0.181	0.314	0.202	0.259	0.250	0.160	-0.517	0.002
ΔHDL-C (mg/dL)	0.025	0.888	-0.120	0.504	-0.068	0.706	0.058	0.749
ΔTriglyceride (mg/dL)	-0.055	0.761	0.119	0.510	0.028	0.876	-0.360	0.040
ΔGlucose (mg/dL)	0.003	0.989	-0.068	0.707	-0.022	0.904	-0.098	0.589
ΔInsulin (μU/mL)	0.037	0.836	0.037	0.836	0.044	0.807	-0.367	0.036

n = 33

r: Spearman correlation coefficients, *p*: *P* value with two-sided tests

SBP: systolic blood pressure, DBP: diastolic blood pressure

TC: total cholesterol, LDL-C: LDL-cholesterol, non-HDL-C: non-HDL-cholesterol, HDL-C: HDL-cholesterol

of dietary C12:0, C14:0, and C16:0 fatty acids, which are known as hyper-LDL-cholesterolemic fatty acid concentrations^{1, 11, 12}, were reduced by 3882 mg, corresponding to 1.4% of total energy intake, and the LDL cholesterol concentration was significantly decreased as mentioned in our previous report⁴. Reduced consumptions of meat and poultry, sweets including deserts and snacks, as well as margarine and shortening, were confirmed in our study to effectively limit SFA consumption.

Dietary n-3 PUFA has attracted attention from the perspective of maintaining the physiological balance of active substances¹³. The dietary n-6/n-3 ratio was 4.9, thus being much higher than the recommended level¹⁴ at baseline. The subjects achieved improvement to 3.7 by increasing seafood consumption, as one dish each of fish and seaweed per day. Consuming one fish dish daily could increase n-3 PUFA intake by at least 2.1 g per day, according to the results of this study (Fig. 1), being consistent with the results of a Japanese

cohort study, in which subjects who ate fish eight times per week consumed 2.1 g of n-3 PUFA per day. Such high n-3 PUFA consumers were reported to have significantly lower coronary heart disease risk than those with lower fish intakes¹⁵.

Dramatic changes in the fatty acid compositions of serum phospholipids were observed after the 6 week intervention. Although C20:5(n-3) and C22:6(n-3) compositions were much higher than those of subjects in other countries at baseline¹⁶, these n-3 PUFA compositions were additionally increased. The ratios of C20:5(n-3)/C20:4(n-6) and C22:6(n-3)/C20:4(n-6) were increased as C20:4(n-6) decreased.

Decreased serum C20:5(n-3)/C20:4(n-6) and C22:6(n-3)/C20:4(n-6) ratios have been demonstrated to be risk factors for early onset of acute coronary syndrome^{17, 18}. It is noteworthy that despite decreases in both C20:4(n-6) and C20:3(n-6) in phospholipids, the C20:4(n-6)/C20:3(n-6) ratio, which serves as an estimate of D5D activities, was increased in this study.

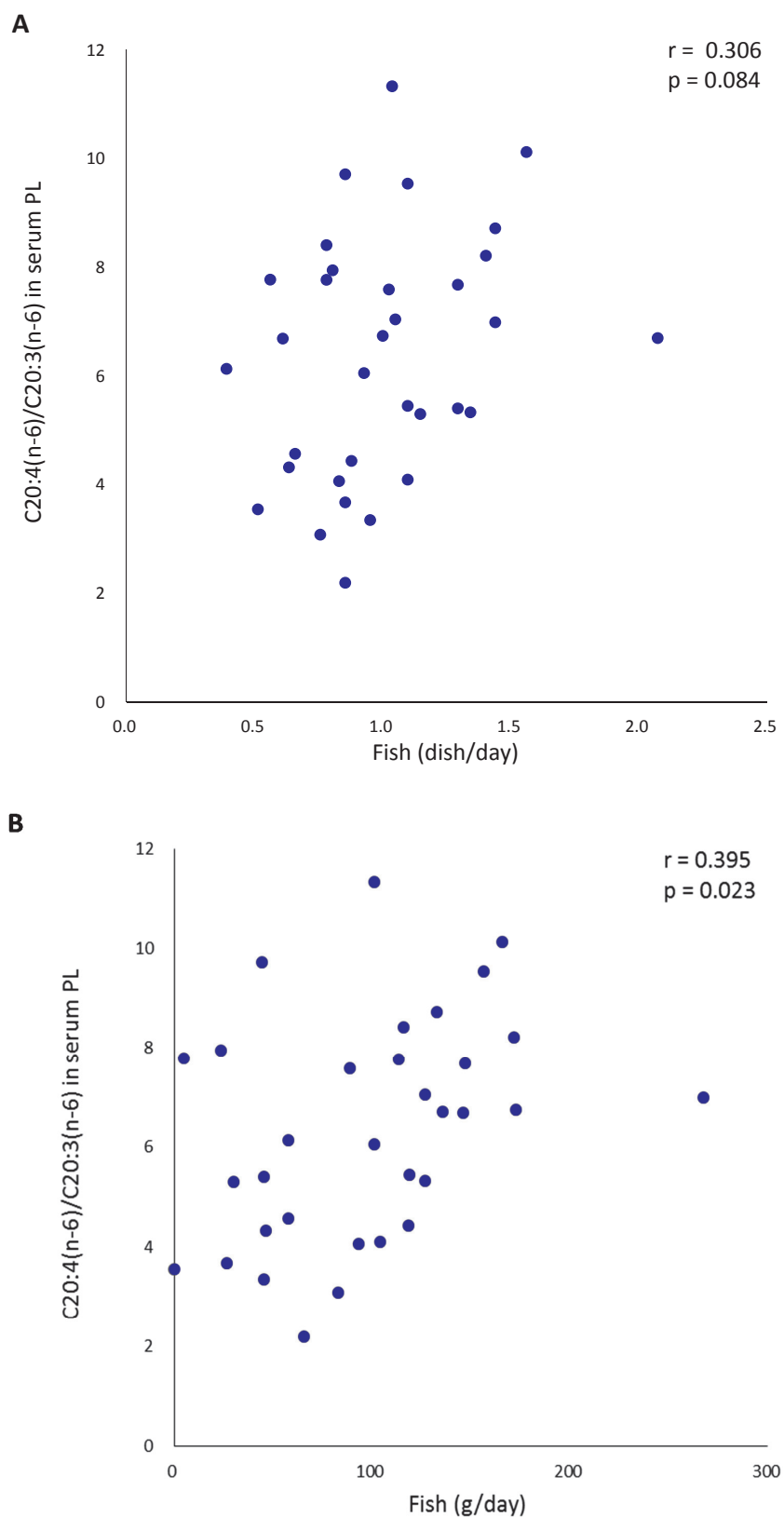


Fig. 2. Simple correlations between fish dish intake (A) and fish intake (B) during the 6 week intervention and C20:4(n-6)/C20:3(n-6) fatty acid ratios in serum phospholipids (PL) at the end of the 6 week intervention

$n = 33$, PL: phospholipid, PUFA: polyunsaturated fatty acid

There was a positive correlation between fish intake and the C20:4(n-6)/C20:3(n-6) ratio. As D5D converts not only C20:3(n-6) to C20:4(n-6) but also C20:4(n-3) to C20:5(n-3), increased C20:5(n-3) from fish may reduce the competitive inhibitory effect on C20:3(n-6) desaturation. Furthermore, changes in the C20:4(n-6)/C20:3(n-6) ratio showed significant correlations with changes in body weight, serum insulin, and lipid concentrations, though no correlations were observed between the change in the C20:5(n-3)/C20:4(n-6) ratio and any of the metabolic markers in this study. Our results suggest the C20:4(n-6)/C20:3(n-6) ratio in phospholipids to be a somewhat more effective index than the C20:5(n-3)/C20:4(n-6) or the C22:6(n-3)/C20:4(n-6) ratios in a dynamic nutritional assessment of atherosclerotic risk.

Maruyama *et al.* reported that estimated D5D in patients with metabolic syndrome was low as compared with that in healthy Japanese subjects¹⁹. A defect in D5D is regarded as being a factor in the initiation and progression of insulin resistance, that is, the metabolic syndrome¹⁹⁻²¹. In Japanese people, a higher serum concentration of C20:3(n-6) and lower estimated D5D activity are reportedly associated with an elevated risk of type 2 diabetes independently of BMI according to a nested case-control study²². In a population-based prospective study, low estimated phospholipid D5D activity was found to be associated with the increased mortality of cardiovascular disease^{23, 24}.

PUFAs exhibit a range of biological effects, many of which are mediated through the formation and actions of lipid mediators such as prostaglandins, leukotrienes, lipoxins, resolvins, and protectins^{25, 26}. Supplementation with fish oil results in decreased production of PGE₂, TXB₂, LTB₄, 5-HETE, and LTE₄ by inflammatory cells²⁷ and marked increase in the abundance of the E-series resolvins precursor, 18-HEPE²⁸. Further study is needed on the production of lipid mediators in response to Japan Diet intake.

In SFAs, the C18:0 percentage was significantly decreased, whereas those of C15:0 and C17:0 were increased. Total SFA did not change in phospholipids, because glycerophospholipids are the major components of serum phospholipids and SFAs attach to carbon number 1, whereas MUFA and PUFA can attach to carbon number 2²⁹. For C18:0 in phospholipids, consistent results have not been obtained. One study showed higher concentrations of C18:0 to be associated with a lower risk of total mortality³⁰, whereas another study reported the proportions of C18:0 to not be associated with any mortality risk²³. Thus, further studies are needed.

The odd-chain fatty acids C15:0 and C17:0, which account for only a small proportion of total SFA in

milk fat and ruminant meat, are accepted biomarkers of dairy fat intake^{31, 32}. On the other hand, some studies report that they can also be synthesized endogenously, for example, from gut-derived C3:0³². To date, it has been reported that increases in C15:0 and C17:0 in serum phospholipids are anti-atherosclerotic; for example, C15:0 and C17:0 concentrations were low in patients with metabolic syndrome^{19, 33, 34}. In this intervention study, although intake volumes of dairy products were unchanged and meat and poultry consumptions were decreased⁴, C15:0 and C17:0 were increased. These results might be caused by the significant decrease in BMI and improvement of insulin resistance. However, the mechanisms underlying these results have not been elucidated, so further studies are needed.

Therefore, this 6 week nutrition education intervention, recommending the Japan Diet, improved fatty acid intakes and fatty acid profiles in phospholipids.

This study has limitations. First, as the study design was single arm, the actual effects on changes in the parameters assessed could not be demonstrated. Second, our subjects were all men in their 30s to 40s not receiving any form of disease treatment. It is necessary to examine effects according to age and gender, as well as to investigate patients with risk factors for atherosclerosis including dyslipidemia. Most other studies used whole blood containing triglycerides to measure circulating fatty acid compositions, but such data reflect the most recent fatty acid intake. Because this study examined fatty acid composition changes in phospholipids reflecting habitual dietary intakes, we cannot simply compare our results with those of studies that examined fatty acid composition changes in whole blood. We did not directly examine vascular function or perform blood vessel imaging studies that depict atherosclerotic change. A potential weakness is the use of fatty acid product: precursor ratio to estimate desaturase activities indirectly, instead of directly measuring the desaturase activity. The use of a product: precursor ratio as a substitute measure is, however, well established, and desaturase activities are commonly estimated from phospholipid fatty acids. The utility of D5D as a comprehensive indicator of the effects of arteriosclerosis prevention and treatment requires detailed evaluation in future studies.

Conclusion

With the nutrition education recommendation to consume the Japan Diet, intakes of meat and poultry and sweets including desserts and snacks decreased, whereas fish, soybean, and seaweed intakes increased. Dietary n-3 PUFA intake increased, but SFA and MUFA decreased and blood phospholipid fatty acid composi-

tions changed to a more anti-atherosclerotic profile. The Japan Diet is effective for rapidly improving fatty acid compositions.

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