n-3 and n-6 Fatty Acid Intake and Serum Phospholipid Fatty Acid Composition in Middle-Aged Women Living in Rural and Urban Areas in Okayama Prefecture

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Summary Dietary fatty acids and serum lipids were evaluated in 68 middle-aged women living in the northern, rural area of Okayama Prefecture, and were compared with the values obtained from 65 urban women from the southern part of this prefecture. A higher level in HDL cholesterol and a lower atherogenic index were observed in the rural women. The percent of energy intake as fat was lower (20.4±0.8% vs. 23.2±0.7%) and that of carbohydrate was greater in the rural group. Eicosapentaenoic (EPA, 0.41±0.04 g/day) and docosahexaenoic acid (DHA, 0.70±0.08 g/day) intakes were significantly higher in the rural subjects than in the urban group. Significantly higher DHA levels and n-3/n-6 fatty acid ratios in serum total phospholipids were found in rural women in their fifties and the sixties compared to urban women. Dietary linoleic acid (LA) amounts were positively correlated with LA (p<0.05), and negatively with the EPA (p<0.05) and DHA (p<0.01) contents of serum total phospholipids. These results suggest that the traditional Japanese diet, containing little fat but enriched in complex carbohydrates and n-3 fatty acids of marine origin, may be related to the low atherogenic index in this rural area.

Key Words dietary fatty acid, serum fatty acid, serum phospholipid, n-6 fatty acid, n-3 fatty acid, n-3/n-6 ratio, middle-aged Japanese women

High-fat diets have been implicated in coronary heart disease, atherosclerosis and several types of cancers (1). On the other hand, highly unsaturated n-3 fatty acids of marine origin, such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), are thought to have cardiovascular protective and
anti-inflammatory effects (2), due to their ability to modify prostanoid synthesis (3) and to reduce platelet aggregation (4). Fish oil has been shown to have an antitumor effect on one type of colon cancer (5). Epidemiological studies have shown that the incidence of cardiovascular disease and several types of cancers is lower in the Japanese than in Western populations. In the Japanese diet, dietary fats make up 25.5% of the total energy intake, and habitual fish intake is an important characteristic of these diets (6). Recently, the Japanese diet has shifted to a Western-style diet, resulting in an increase in daily fat consumption from 24.7 g in 1960 to 58.4 g in 1992. Daily polyunsaturated fatty acid intake did not change from 1971 to 1990, whereas during this same period, consumption of some food groups showed some changes (7). However, there are some differences in habitual food intake among the elderly and the young, and rural and urban populations, and these differences may have some effects on the incidence of several diseases.

In this study, we examined the dietary fatty acids and the fatty acid composition of serum phospholipids in middle-aged women living in a rural area of Okayama Prefecture, which has a lower mortality rate from cardiovascular disease than the average in Japan, and compared these measurements with those obtained from women living in an urban area which has a higher mortality rate from cardiovascular disease.

SUBJECTS AND METHODS

Subjects. Sixty-eight women average age, 54.3 years old (y) (range 40–68 y) living in a northern rural area and 65 women with an average age of 54.1 y (range 40–69 y) living in a southern urban part of Okayama Prefecture were studied in 1990 and in 1992, respectively. They were volunteers attending an annual health education meeting under the auspices of the local government. All the women were instructed how to record their food intakes during this 3-day period. Informed consent was obtained from all participants.

For each participant, triceps and subscapular skin folds were measured using a calliper (MK-60, Yagami, Nagoya, Japan). Blood pressure was measured and approximately 5 ml of blood was withdrawn, centrifuged, and the resulting serum separated for phospholipid and cholesterol analyses. All measurements and blood sampling were done between 8:30–10:00 am in the non-fasting subjects. None of the subjects were habitual drinkers or smokers. Body fat composition was determined by bioelectrical impedance analysis (BIA) systems using a tetra polar electrode method, with a localized 88 mA and 50 kHz current injection (Selco SIF-881, Yagami, Nagoya).

Dietary intakes were recorded for 3 consecutive days by each participant. These were converted to nutrient and fatty acid intake by a computerized database (NUT version 2.0, Human Science Laboratory, Nagahama) based on The 4th Revised Edition of The Standard Table of Food Composition in Japan (8) and The Table of Fat-soluble Components in Japanese Foods (9).
Laboratory methods. Total cholesterol and HDL-cholesterol in serum were determined enzymatically using kits (Wako Pure Chemical Industries, Ltd., Tokyo). Total lipids were extracted from 0.5 ml of serum with chloroform-methanol (2:1, v/v) (10). Total phospholipids were isolated from crude lipid extracts by thin-layer chromatography (Silica Gel 60 F_{254}, Merck, Darmstadt) using a solvent system of petroleum ether/ethyl ether/acetic acid (75:25:1, v/v/v). The plates were sprayed with 0.1% 8-anilino-1-naphthalenesulfonic acid ammonium salt, viewed under ultraviolet light, and the spots corresponding to phospholipid fractions were extracted with methanol. Methyl esters were prepared by treating the extracts with a 15% boron trifluoride methanol solution (Tokyo Kasei Kogyo Co., Ltd., Tokyo). After extraction with n-hexane, the methylated fatty acids were analyzed using a gas-liquid chromatograph (GLC-14A, Shimadzu, Kyoto) equipped with a 25 m × 0.5 mm capillary column (HR-SS-10, Shinwa Chemical Industries, Ltd., Kyoto). The initial oven temperature was 150°C for these runs; after 1 min, this temperature was increased by 3°C/min to a final temperature of 210°C for 25 min. Nitrogen was used as the carrier gas with a flow rate of 1 ml/min. The temperatures at the injection port and the hydrogen flame ionization detector were 250°C. Peaks were identified by authentic standards (Sigma Chemical Co., St. Louis, MO). Tricosanoic acid was used as an internal standard for quantitation of fatty acids.

Statistics. Data are expressed as M±SE. Student’s t-test was used to compare the mean values of urban and rural subjects. Analysis of variance was used to examine age differences. Pearson product-moment correlations were used to confirm the relationships between dietary fatty acid and serum fatty acid composition.

RESULTS

Characteristics of the subjects

The subjects living in the rural and urban areas were classified according to age into three groups, 40–49 (forties), 50–59 (fifties) and 60–69 y (sixties) (Table 1). Twenty-three of the rural women and 22 of the urban women in their fifties and all subjects in their sixties were postmenopausal. There were no significant differences in body mass index (BMI) among these groups. However, body fat % (weight %) in the rural women was significantly lower than that in the urban women. Skinfold thickness for all the age groups of rural women was less than the values given in the Annual reports of nutritional status in Japanese (11). The skinfold thickness and body fat % increased as a function of age in the urban women but not in the rural women. Nine of the rural women were classified as obese according to their skinfold thickness (>50 mm) and body fat indices (>30%), whereas, 21 of the 65 urban women were classified as obese. Systolic blood pressure in the 50–59 y rural women were significantly higher than those of the same age group in the urban women.
Table 1. Characteristics of the subjects.

| Area of residence | Age (y) | Total for all subjects |
|-------------------|---------|-----------------------|
|                   | 40-49   | 50-59     | 60-69     |         |
| Number of subjects| Rural   | 21        | 25        | 22       | 68       |
|                   | Urban   | 18        | 25        | 22       | 65       |
| BMI (kg/m²)       | Rural   | 22.6±0.1  | 22.2±0.2  | 23.1±0.1 | 22.6±0.0 |
|                   | Urban   | 22.7±0.2  | 23.7±0.1  | 23.6±0.1 | 23.4±0.0 |
| Skinfold thickness (mm)* | Rural | 32.0±0.4  | 33.8±0.5  | 32.3±0.4* | 32.8±0.2 |
|                   | Urban   | 34.1±0.5  | 36.5±0.4  | 39.3±0.6  | 36.1±0.2 |
| Body fat (%)      | Rural   | 20.9±0.2* | 23.3±0.2  | 21.4±0.2**| 21.9±0.1**|
|                   | Urban   | 24.5±0.3  | 26.3±0.2  | 27.5±0.3  | 26.1±0.1 |

M±SE. *Skinfold thickness represents the sum of triceps and subscapular measurements. Significantly different from urban area, **p<0.01, *p<0.05.

Table 2. Serum lipid contents and atherogenic indices.

| Serum lipids (mg/100ml) | Area of residence | Age (y) | Total for all subjects |
|-------------------------|-------------------|---------|-----------------------|
|                         | 40-49 | 50-59   | 60-69     |         |
| Total cholesterol (A)   | Rural  | 179±9   | 188±7*   | 222±10   | 196±5   |
|                         | Urban  | 187±7   | 214±6    | 219±9    | 207±5   |
| HDL-cholesterol (B)     | Rural  | 60±2**  | 58±2     | 58±3     | 59±1*   |
|                         | Urban  | 49±3    | 59±3     | 58±3     | 54±2    |
| Atherogenic index (A-B/B)| Rural | 1.9±0.1** | 2.5±0.2 | 2.8±0.2** | 2.4±0.1** |
|                         | Urban  | 2.9±0.2 | 3.1±0.2  | 3.6±0.3  | 3.2±0.1 |

M±SE. Significantly different from urban area, **p<0.01, *p<0.05.

Serum total-cholesterol and HDL-cholesterol levels and the atherogenic index ((total cholesterol−HDL cholesterol)/HDL cholesterol) are shown in Table 2. Serum total cholesterol levels in the 50–59y rural women were significantly lower than those of 50–59y urban women. Total serum cholesterol values for rural women in their forties and fifties were lower than the mean levels reported for age-matched Japanese women (11). The HDL-cholesterol level of rural women in their forties was markedly higher than that of urban women of the same age. The atherogenic index in the rural women was significantly lower than that in the urban women.

**Dietary intake**

The calculated nutrient intakes based on the 3-day dietary records taken by
Table 3. Dietary intake of energy and nutrients.

| Dietary intake         | Area of residence | Age (y) | Total for all subjects |
|------------------------|-------------------|---------|------------------------|
|                        | Urban             | 40-49   | 50-59                  | 60-69                  |                      |
| Energy (kcal/day)      | Rural             | 2,083±94| 2,028±84               | 1,955±46**             | 2,021±45*            |
|                        | Urban             | 1,911±102| 1,855±76              | 1,790±101              | 1,852±52             |
| Protein (g/day)        | Rural             | 75±4    | 77±4                   | 77±3                   | 76±2                 |
|                        | Urban             | 78±4    | 75±3                   | 78±6                   | 77±3                 |
| Animal (%)             | Rural             | 44.8±2.8| 47.5±1.9               | 45.5±2.3               | 46.0±1.3             |
|                        | Urban             | 52.7±1.9| 48.5±1.8               | 51.6±2.0               | 50.7±1.1             |
| Fat (g/day)            | Rural             | 54±6    | 47±3                   | 45±2                   | 48±2                 |
|                        | Urban             | 47±4    | 51±4                   | 49±3                   | 49±2                 |
| Cholesterol (mg/day)   | Rural             | 425±55  | 417±52                 | 440±57                 | 427±31               |
|                        | Urban             | 316±28  | 340±19                 | 367±41                 | 343±17               |
| Vitamin E (mg/day)     | Rural             | 9.3±0.8 | 8.9±0.6                | 8.5±0.7                | 8.9±0.4              |
|                        | Urban             | 7.7±0.8 | 9.9±0.9                | 9.4±1.1                | 9.0±0.4              |
| Crude fiber (g/day)    | Rural             | 4.8±0.4 | 5.1±0.3                | 5.5±0.4                | 5.1±0.2              |
|                        | Urban             | 4.1±0.4 | 4.6±0.5                | 4.6±0.5                | 4.4±0.3              |
| Salt (g/day)           | Rural             | 11.9±0.6| 11.6±0.5               | 12.2±0.8               | 12.0±0.4             |
|                        | Urban             | 9.7±0.8 | 10.1±0.6               | 12.4±1.0               | 10.8±0.5             |

Percent of energy as:

| Protein                | Area of residence | 40-49     | 50-59     | 60-69     | Total for all subjects |
|------------------------|-------------------|-----------|-----------|-----------|-----------------------|
| Rural                  | 14.0±0.5          | 14.9±0.5  | 14.9±0.5  | 14.6±0.3  |
| Urban                  | 16.1±0.5          | 15.8±0.5  | 17.2±0.6  | 16.4±0.3  |
| Carbohydrate           | Rural             | 64.1±2.0* | 65.0±1.3* | 66.7±1.6* | 65.2±0.9**            |
|                        | Urban             | 61.8±1.1  | 59.5±1.4  | 56.9±1.6  | 59.3±0.8              |
| Fat                    | Rural             | 21.6±1.7  | 20.1±1.0  | 19.4±1.2* | 20.4±0.8*             |
|                        | Urban             | 21.4±0.9  | 23.7±1.3  | 24.0±1.2  | 23.2±0.7              |
| n-6 PUFA               | Rural             | 5.0±0.4   | 4.2±0.4   | 4.6±0.6   | 4.6±0.2               |
|                        | Urban             | 4.4±0.2   | 4.8±0.3   | 4.8±0.3   | 4.7±0.2               |
| n-3 PUFA               | Rural             | 1.1±0.1   | 1.1±0.1   | 1.1±0.2   | 1.1±0.1               |
|                        | Urban             | 1.0±0.1   | 1.1±0.1   | 1.1±0.1   | 1.1±0.1               |

M±SE. Significantly different from urban area, **p<0.01, *p<0.05.

the subjects are summarized in Table 3. Daily total energy intakes decreased with age in both the rural and urban groups. The percent of energy intake as fat continued to be lower in all age categories of the rural group in contrast with the urban group and a significantly lower level was recognized in the rural subjects. Forty-seven percent of rural women and 26.2% of urban women had a lower fat energy % than the levels stated in Recommended Dietary Allowances for the Japanese (12). In contrast, 35.4% of the urban women and 19.1% of the rural women had a higher fat energy % than the recommended levels. The percent of energy as carbohydrate was significantly higher in rural women than urban women in all age groups. Cholesterol intakes were higher in the rural group compared to the urban group at all ages studied although these differences were not significant. Vitamin E intakes were similar for all age categories of the rural and urban women’s groups, and paralleled the fat intakes.
Table 4. Intake of selected fatty acids in women living in rural and urban areas.

| Fatty acid (g/day) | Area of residence | Age (y) | Total for all subjects |
|-------------------|-------------------|---------|------------------------|
|                   |                   | 40-49   | 50-59      | 60-69      |                     |
| 18:2n-6           | Rural             | 11.3±0.8| 9.1±0.8    | 10.4±1.3  | 9.9±0.5             |
|                   | Urban             | 9.0±0.7 | 9.8±0.8    | 10.0±1.0  | 9.6±0.5             |
| 18:3n-3           | Rural             | 1.4±0.1 | 1.1±0.1    | 1.6±0.4   | 1.2±0.1             |
|                   | Urban             | 1.1±0.2 | 1.2±0.1    | 1.3±0.1   | 1.2±0.1             |
| 20:4n-6           | Rural             | 0.21±0.02| 0.19±0.02 | 0.20±0.03 | 0.20±0.02           |
|                   | Urban             | 0.17±0.01| 0.17±0.01 | 0.16±0.02 | 0.17±0.01           |
| 20:5n-3           | Rural             | 0.35±0.04| 0.44±0.10 | 0.43±0.12 | 0.41±0.04*          |
|                   | Urban             | 0.27±0.04| 0.36±0.06 | 0.22±0.03 | 0.28±0.03           |
| 22:6n-3           | Rural             | 0.58±0.06| 0.73±0.13 | 0.78±0.19 | 0.70±0.08*          |
|                   | Urban             | 0.52±0.07| 0.59±0.07 | 0.45±0.06 | 0.52±0.04           |
| Total SFA         | Rural             | 17.3±1.9| 15.7±1.1  | 14.1±1.1  | 15.7±0.8            |
|                   | Urban             | 14.3±1.3| 16.0±1.4  | 15.5±1.2  | 15.3±0.7            |
| Total MUFA        | Rural             | 18.0±1.9| 15.5±1.2  | 15.2±1.6  | 16.2±0.9            |
|                   | Urban             | 15.5±1.3| 17.6±1.7  | 17.7±1.6  | 17.0±0.9            |
| Total n-6         | Rural             | 11.5±0.6| 9.4±0.8   | 10.5±1.3  | 10.5±0.6            |
|                   | Urban             | 9.2±0.7 | 10.0±0.8  | 10.2±1.0  | 9.9±0.5             |
| Total n-3         | Rural             | 2.3±0.2 | 2.5±0.3   | 2.8±0.4   | 2.6±0.2             |
|                   | Urban             | 2.1±0.3 | 2.3±0.2   | 2.1±0.2   | 2.2±0.1             |
| n-3/n-6           | Rural             | 0.27±0.05| 0.30±0.05 | 0.32±0.05 | 0.29±0.03           |
|                   | Urban             | 0.26±0.04| 0.25±0.02 | 0.22±0.02 | 0.24±0.02           |

M±SE. Significantly different from urban women, *p < 0.05.

Dietary fatty acid intake of the subjects is shown in Table 4. The major fatty acids ingested by all subjects in the rural and urban areas were oleic acid (18:1n-9) (31.5±0.5 wt% vs. 33.6±0.4 wt%), LA (22.5±0.9 wt% vs. 22.3±0.5 wt%) and palmitic acid (16:0) (20.4±0.3 wt% vs. 20.1±0.3 wt%), respectively. The amounts of EPA and DHA ingested by rural women were significantly higher than intakes of these fatty acids by the urban women. The n-3/n-6 fatty acid ratio increased with age in the rural women and decreased in the urban women. Daily intakes of fish were 82±6 and 88±7 g for the rural and urban women, respectively.

Fatty acid compositions of serum total phospholipids

Fatty acid compositions (mol%) of serum total phospholipids are shown in Table 5. Significantly higher DHA levels and n-3/n-6 ratios were found in the 50–59 and 60–69 age groups in the rural area compared to the urban area. Serum LA levels in the 50–59 y rural women were lower than those obtained in the same age group in the urban women. Significant differences among age groups were observed in 16:0, 18:0, 18:1n-9, LA and total n-6 fatty acid levels in the rural area; however, the age difference was recognized only in the DHA level in the urban area. Changes in the LA levels of serum phospholipids in the rural area were positively correlated (r = 0.285, p < 0.05) to the LA intakes found in this area. Dietary LA

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Table 5. Levels of selected fatty acids in serum phospholipid of rural and urban women.

| Fatty acid | Area of residence | Age (y) | Total for all subjects | p* |
|------------|------------------|---------|------------------------|----|
|            | Rural | Urban | 40-49 | 50-59 | 60-69 |                  |
| 16 : 0     | 35.9 ± 0.7**     | 36.6 ± 0.3** | 36.7 ± 0.8 | 37.3 ± 0.6 | <0.05 |
| 16 : 1n-7  | 12.2 ± 0.1      | 1.7 ± 0.2 | 1.6 ± 0.2 | 1.5 ± 0.1 | 1.3 ± 0.1 |
| 18 : 0     | 13.5 ± 0.4      | 15.8 ± 0.5** | 15.1 ± 0.3** | 14.9 ± 0.3 | <0.01 |
| 18 : 1n-9  | 12.4 ± 0.3      | 10.4 ± 0.5** | 12.2 ± 0.4 | 11.6 ± 0.3 | <0.01 |
| 18 : 2n-6  | 19.0 ± 0.6      | 15.1 ± 0.9* | 16.8 ± 0.7 | 16.8 ± 0.5 | <0.01 |
| 18 : 3n-3  | 17.6 ± 0.9      | 17.9 ± 0.6 | 18.6 ± 0.6 | 18.1 ± 0.4 |
| 20 : 3n-6  | 1.5 ± 0.2       | 1.5 ± 0.1 | 1.6 ± 0.1 | 1.5 ± 0.1 |
| 20 : 4n-6  | 6.0 ± 0.5       | 6.4 ± 0.4 | 5.9 ± 0.3 | 6.1 ± 0.2 |
| 20 : 5n-3  | 5.3 ± 0.2       | 5.6 ± 0.2 | 6.0 ± 0.3 | 5.6 ± 0.1 |
| 22 : 6n-3  | 4.5 ± 0.3       | 4.7 ± 0.3** | 4.6 ± 0.3** | 4.6 ± 0.2** |
| Total n-6  | 27.2 ± 0.7*     | 23.9 ± 0.6 | 24.9 ± 0.8 | 25.2 ± 0.5 | <0.05 |
| Total n-3  | 24.4 ± 1.0      | 24.9 ± 0.7 | 25.3 ± 0.6 | 24.9 ± 0.4 |
| n-3/n-6    | 9.0 ± 0.6       | 9.2 ± 0.6 | 9.0 ± 0.5** | 9.0 ± 0.3** |
|            | 7.6 ± 0.4       | 7.7 ± 0.5 | 7.3 ± 0.2 | 7.5 ± 0.2 |
|            | 0.33 ± 0.02     | 0.40 ± 0.03* | 0.37 ± 0.02** | 0.37 ± 0.01** |
|            | 0.33 ± 0.03     | 0.32 ± 0.02 | 0.29 ± 0.01 | 0.31 ± 0.01 |

M ± SE. *Significant difference among age groups. **Data given as molar percent of total fatty acids. Significantly different from urban area, **p < 0.01, *p < 0.05.

levels were negatively correlated with the n-3/n-6 ratio ($r = -0.467, p < 0.01$) in the serum total phospholipids. Although there was no significant correlation between dietary EPA and DHA intakes and the mol% of these two fatty acids in the serum phospholipid fractions, significantly higher DHA level in the serum phospholipid was recognized in the rural subjects, who ingested larger amounts of DHA and EPA than urban subjects.

**DISCUSSION**

A changing dietary pattern has been observed in the Japanese population (13). However, in the present study, data obtained from 3-day dietary records indicate that women living in a rural area retain the traditional Japanese dietary habits,
including a high percentage of energy intake as carbohydrate and a low fat intake. On the other hand, in urban women, the habitual food intake has shifted to a Western-style diet. However, the percentage of energy as fat was less than the levels reported for Western countries (14).

Egusa et al. (15) have reported that the incorporation of Westernized food habits into the Japanese diet has contributed to the increased concentrations of serum total cholesterol and triglycerides and to the prevalence of type IIa and IIb hyperlipidemia. The higher total cholesterol level in urban women in their fifties observed in the present study may be partly due to their Westernized dietary habits. Although energy intake was higher in rural, compared to urban women, the body fat ratio was smaller in the rural women, possibly because of the heavy physical labor associated with farming. The physical activity and the low body fat ratio may be related to the low atherogenic index observed in these women.

It is suggested in the Recommended Dietary Allowance for Japanese (12) that the ratio of n-6 to n-3 fatty acids should be maintained about 4:1. The Canadian Nutrition Recommendations also suggest that the n-3 fatty acids should be at least 0.5% of energy with a ratio of n-6 to n-3 fatty acids in the range of 4:1 to 10:1 (16). The ratio of the n-3/n-6 fatty acids in the diets of urban women (0.24) is close to the level recommended for Japanese (0.25) but is higher (0.29) in rural women. The calculated intakes of n-6 and n-3 fatty acids in the rural women are the same as the amounts calculated from the model menu for men in their forties (17). Energy intake from EPA and DHA in the rural women in the present study are similar to those reported in the Tromsø study (18) in which n-3 fatty acid intake in Norwegian fish eaters were examined. The decreased n-3/n-6 fatty acid ratios, seen in rural women in their forties are similar to urban women in the same age group, suggesting that the consumption of fish, particularly fatty fish is declining in the younger, rural women.

The n-6 fatty acid compositions of serum total phospholipids in the rural women are similar to results reported in male Japanese farmers by Yamori et al. (19), although the levels of DHA are lower and saturated fatty acids are higher than the values obtained from the men.

James et al. (20) reported a strong correlation between the LA contents of plasma and neutrophil total phospholipids and dietary LA, which in his subjects ranged from 2.5 to 17.5% of energy. In the present study, the LA contents in serum phospholipids also appeared to reflect the dietary LA contents in all the age groups of rural women. Furthermore, when expressed as a percent of the total daily fatty acid intake, the LA intake showed a positive correlation with serum levels of this fatty acid and a negative correlation with the serum levels of EPA and DHA. These data agree with those of Houwelingen et al. (21) who reported a negative association between LA intake and serum amounts of EPA in elderly men. Furthermore, Chan et al. (22) reported that the ratio of LA to α-LnA may have important effects on the levels of the longer chain n-3 fatty acids, especially EPA, in platelet and plasma phospholipids.
Many studies have shown an association between the quality of fat and serum total cholesterol (23, 24). Fumeron et al. (25) and Harris et al. (26) reported that n-3 PUFA increase LDL and HDL2 levels in healthy young men and in hypertriglyceridemic patients, although serum triglyceride levels are decreased by n-3 PUFA supplementation in hypertriglyceridemic patients. Recently, it has been reported that the incorporation of fish into a 30% fat diet reduces LDL-cholesterol and triglyceride levels and increases HDL2-cholesterol (27). In our study, there was no correlation between n-3 PUFA intake and total and HDL-cholesterol levels, although low total cholesterol and high HDL-cholesterol levels were found in rural women consuming high amounts of EPA and DHA with a low percentage of energy as fat.

Kromhout et al. reported that consuming as little as 30g of fish per day reduces the incidence of heart disease in men (28, 29). Our rural subjects consumed 81g of fish per day or 2.7 times the amount recommended by Kromhout et al. However, the amount of fish consumed by our subjects is still lower than the mean consumption of Japanese (96.8 g/day) in 1992 (6). In rural areas, the standardized mortality rates from cardiovascular disease are lower than the mean level in Japan (60.9 vs. 100), and are higher in the urban areas (110.2) although in urban areas the daily fish intake is 87.6 g/day. Dietary factors such as overeating, a high percent of energy as fat and low dietary fiber intake also affect the incidence of heart disease (30).

In conclusion, the traditional Japanese food habits followed by the rural middle-aged women led to low levels of serum n-6 PUFA, high levels of DHA and high n-3/n-6 fatty acid ratios. Also, low body fat contents resulting from daily physical activity may be related to the low atherogenic index in this region. A longer period of observation in both the rural and urban women may throw light on the desirable fatty acid intake needed for a good health in middle-aged women.

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