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More evidence has emerged on the potentially protective effects of long-chain n-3 fatty acids abundant in fish against such cancers as those of the colon, breast, and prostate.1-4 Those fatty acids include eicosapentaenoic acid (EPA), docosapentaenoic acid (n-3) (DPA), and docosahexaenoic acid (DHA). Regarding their role in the prevention of cancer, however, significant inconsistencies remain among epidemiologic studies.1-4

One of the issues in these studies is the method of assessing the intake of long-chain n-3 fatty acids. Several investigations have adopted the self-reported intake frequency of fish, often assessed with a simple questionnaire, as a surrogate for fatty acid intake.1-3,5 Whether such reported fish consumption reflects the bioavailability of fatty acids, however, remains to be elucidated.

To assess the association of self-reported intake of fish with bioavailability of long-chain n-3 fatty acids, it is useful to examine the correlation between the blood levels of fatty acids and fish consumption.4-11 Nevertheless, only a few such studies10,11 have been conducted in Japan, one of the areas with the highest fish consumption in the world.12

We therefore conducted a cross-sectional study to examine the association between the intake frequency of fish with serum long-chain n-3 fatty acids among control subjects of case-control studies nested in the Japan Collaborative Cohort Study (JACC Study) for Evaluation of Cancer Risk sponsored by the Ministry of Education, Science, Sports and Culture of Japan (Monbusho), a nationwide prospective study.

Study Population and Serum Samples

The potential subjects of this study were 1,319 control subjects, aged 40 to 79 years at baseline, in case-control studies nested in the JACC Study, who were enrolled in 20 study areas. These studies examined associations between the serum levels of fatty acids and the risks for lung, colorectal,11 pancreatic, and biliary tract cancers. At the baseline survey from 1988 through 1990, the subjects completed a self-administered questionnaire on lifestyle factors including dietary habits and also donated blood samples.11 All the subjects were not fasting when blood was drawn.

The dietary component of the questionnaire elicited five possible responses to the subject’s customary intake frequency of 33 items of Japanese foods or dishes: almost never, 1-2 times/month, 1-2 times/week, 3-4 times/week, and almost every day.15 In the questions, we did not specify the time frame such as “during the past one year”, “during the preceding month”, and so on.

For fish and its products, we observed fresh fish, steamed fish paste (‘kamaboko’ in Japanese), and dried or salted fish in the food list. Intake-frequency responses were not elicited in one area for fresh fish, three areas for steamed fish paste, and in two areas for dried or salted fish. Because no information on the intake frequency of fish and its products was available for 62 of the 1,319 potential subjects, we excluded them from the study, leaving 1,257 (631 men and 626 women) eligible for the present analysis.

About half of them (47.2%, n = 593) were participants in the study area where blood was always drawn in a fasting condition. Sera were separated from the blood samples at laboratories in or near the surveyed municipalities as soon as possible after the blood were drawn. The serum of each participant was divided into three to five tubes (100 to 500 µL per tube), which were then stored in deep freezers at -80°C until analyzed in 2001 and 2002.

Informed consent for participation was obtained individually from subjects, except in a few study areas where informed consent was provided at the group level after the aim of the study and confidentiality of the data had been explained to community leaders. The Ethics Committee of Medical Care and Research of Fujita Health University, the Ethical Board of Nagoya University School of Medicine, and the Ethics Committee of Juntendo University School of Medicine approved the protocol of the investigations, including the procedures used to obtain informed consent.

Determination of Serum Fatty Acids

All the samples were analyzed in a single laboratory by a trained staff member. Lipids in 0.2 mL of serum were extracted with Folch’s solution under a nitrogen atmosphere. After methyl esterification by 0.4 M potassium methoxide and 14 weight percentage boron trifluoride methanol, total fatty acids were measured using a gas chromatograph (Shimadzu, GC17A, Kyoto, Japan) equipped with an Omegawax 250 capillary column (30 m x 0.25 mm i.d.; 0.25 µm thickness; Supelco, Bellefonte, PA, USA). Peaks were determined using a flame-ionization detector and were quantified with an electric integrator (Shimadzu, CR-7A, Kyoto, Japan) using pure standard mixtures (Sigma, St. Louis, MO, USA). We adopted the weight percentage of each fatty acid in all detected fatty acids as a measurement value.

Statistical Analysis

We computed the geometric means of serum levels of EPA, DPA, and DHA (weight % of total fatty acids) to summarize the data, since the measurement values showed a distribution skewed to lower values. The 95% confidence intervals of geometric means were estimated based on the standard errors of means for natural logarithms of measurement values. In the analyses of geometric means, the lowest category for the intake frequency of fresh fish (almost never) and the highest one for that of steamed fish paste (kamaboko) (almost every day) were merged into the adjacent category to attain a sufficient sample size. The difference in geometric means between sexes was tested by the t test for loge-transformed values. To examine the associations of intake frequency of fish or its products with serum long-chain n-3 fatty acids, Spearman correlation coefficients were calculated adjusting for age.

Geometric means adjusted for age (40-49, 50-59, 60-69, or 70-79 years) and participating institutions were estimated by applying the results of linear models7 to the logarithms of measurement values. To test for linear trends in crude or adjusted geometric
percentages for EPA, DPA, and DHA was skewed to lower values, particularly for EPA (Figures 1 to 3). The medians were 2.84% (inter-quartile range 2.00%-4.10%) for EPA, 0.86% (0.70%-1.06%) for DPA, and 5.22% (4.38%-6.14%) for DHA in men, while they were 2.41% (1.77%-3.57%), 0.81% (0.68%-0.97%), and 5.08% (4.25%-5.98%), correspondingly, in women. The geometric means of serum levels of the long-chain n-3 fatty acids were slightly higher in men than in women (Table 1). All the means peaked at the fifties and then declined with increasing age.

![Figure 1](image1.png) **Figure 1.** Distribution of serum level of eicosapentaenoic acid (EPA) by sex (weight % of total fatty acids).

![Figure 2](image2.png) **Figure 2.** Distribution of serum level of docosapentaenoic acid (n-3) (DPA) by sex (weight % of total fatty acids).

![Figure 3](image3.png) **Figure 3.** Distribution of serum level of docosahexaenoic acid (DHA) by sex (weight % of total fatty acids).
An increasing trend in the geometric means of serum levels of EPA, DPA, and DHA (adjusted for age and participating institution) was found with an increasing intake frequency of fresh fish and dried or salted fish in both men and women (Table 3). The adjusted geometric means in the highest intake category were higher than those in the lowest by 7% to 40%. The consumption of steamed fish paste (kamaboko) also showed no association with serum EPA, DPA, and DHA levels in the analysis of geometric means. The findings for crude geometric means were not appreciably different from those for adjusted ones (data not shown).

Table 1. Geometric means (GM) of serum levels of long-chain n-3 fatty acids by sex and age (weight % of total fatty acids).

| Sex    | EPA     | DPA     | DHA     |
|--------|---------|---------|---------|
|        | n       | GM      | 95% CI  | GM  | 95% CI  | GM  | 95% CI  |
|        |         |         |         |     |         |     |         |
| Men    | 631     | 2.82    | 2.71 - 2.94 | 0.85 | 0.83 - 0.88 | 5.07 | 4.95 - 5.19 |
| Women  | 626     | 2.47    | 2.37 - 2.58 | 0.80 | 0.79 - 0.82 | 4.93 | 4.81 - 5.04 |
|        | p < 0.001 | p < 0.001 | p = 0.090 |
| Age (years) |        |         |         |     |         |     |         |
| 40-49  | 69      | 2.25    | 1.98 - 2.55 | 0.79 | 0.74 - 0.85 | 4.88 | 4.55 - 5.23 |
| 50-59  | 309     | 2.82    | 2.66 - 3.00 | 0.88 | 0.85 - 0.91 | 5.19 | 5.02 - 5.37 |
| 60-69  | 570     | 2.74    | 2.62 - 2.86 | 0.83 | 0.81 - 0.85 | 5.08 | 4.95 - 5.20 |
| 70-79  | 309     | 2.41    | 2.27 - 2.56 | 0.79 | 0.76 - 0.82 | 4.70 | 4.54 - 4.85 |
|        | Trend p = 0.10 | Trend p = 0.003 | Trend p = 0.002 |

EPA: eicosapentaenoic acid
DPA: docosapentaenoic acid (n-3)
DHA: docosahexaenoic acid
CI: confidence interval

Table 2. Spearman correlation coefficients between intake frequency of fish and serum levels of long-chain n-3 fatty acids (weight % of total fatty acids) by sex.

|          | n    | EPA     | DPA     | DHA     |
|----------|------|---------|---------|---------|
|          |      | GM      | 95% CI  | GM  | 95% CI  | GM  | 95% CI  |
|          |      |         |         |     |         |     |         |
| Men      |      |         |         |     |         |     |         |
| Fresh fish | 621  | 0.16 *** | 0.14 *** | 0.16 *** |
| Steamed fish paste (kamaboko) | 436  | 0.04 | 0.04 | 0.08 |
| Dried or salted fish | 595  | 0.16 *** | 0.11 **  | 0.18 *** |
| Women    |      |         |         |     |         |     |         |
| Fresh fish | 612  | 0.12 **  | 0.05 | 0.05 |
| Steamed fish paste (kamaboko) | 444  | -0.01 | 0.05 | 0.03 |
| Dried or salted fish | 564  | 0.08 * | 0.11 ** | 0.06 |

Adjusted for age.
EPA: eicosapentaenoic acid
DPA: docosapentaenoic acid (n-3)
DHA: docosahexaenoic acid
#: p<0.10; **: p<0.01; ***: p<0.001.
| Intake frequency | Men | | | | Women | | | |
|------------------|-----|-----|-----|-----|-----|-----|-----|-----|
|                  | EPA | DPA | DHA | EPA | DPA | DHA | EPA | DPA | DHA |
| n    | GM  | 95% CI | GM  | 95% CI | GM  | 95% CI | GM  | 95% CI | GM  | 95% CI |
| Fresh fish       |     |       |     |       |     |       |     |       |     |       |
| 2 times/month or less | 57 | 2.48 | 2.18 - 2.83 | 0.83 | 0.77 - 0.90 | 4.79 | 4.45 - 5.15 | 41 | 2.10 | 1.78 - 2.48 | 0.80 | 0.74 - 0.88 | 4.67 | 4.31 - 5.05 |
| 1-2 times/week   | 188 | 2.71 | 2.49 - 2.94 | 0.88 | 0.84 - 0.92 | 5.09 | 4.86 - 5.33 | 195 | 2.33 | 2.13 - 2.55 | 0.80 | 0.77 - 0.84 | 4.96 | 4.75 - 5.18 |
| 3-4 times/week   | 204 | 2.94 | 2.71 - 3.19 | 0.92 | 0.88 - 0.97 | 5.48 | 5.24 - 5.73 | 213 | 2.50 | 2.29 - 2.74 | 0.81 | 0.77 - 0.85 | 4.98 | 4.72 - 5.20 |
| Almost every day | 172 | 3.20 | 2.92 - 3.49 | 0.95 | 0.91 - 1.00 | 5.61 | 5.34 - 5.90 | 163 | 2.82 | 2.55 - 3.12 | 0.86 | 0.82 - 0.91 | 5.21 | 4.96 - 5.47 |
|                  | Trend p < 0.001 | Trend p < 0.001 | Trend p < 0.001 | Trend p < 0.001 | Trend p < 0.001 | Trend p < 0.001 | Trend p < 0.001 | Trend p < 0.001 | Trend p < 0.001 |
| Steamed fish paste (kamaboko) |     |       |     |       |     |       |     |       |     |       |
| Almost never     | 103 | 2.88 | 2.60 - 3.19 | 0.90 | 0.85 - 0.95 | 5.35 | 5.08 - 5.63 | 66 | 2.33 | 2.02 - 2.68 | 0.79 | 0.74 - 0.85 | 4.90 | 4.58 - 5.24 |
| 1-2 times/month  | 148 | 2.87 | 2.61 - 3.16 | 0.87 | 0.82 - 0.91 | 5.28 | 5.03 - 5.54 | 152 | 2.44 | 2.19 - 2.73 | 0.81 | 0.77 - 0.86 | 5.04 | 4.78 - 5.31 |
| 1-2 times/week   | 121 | 2.77 | 2.49 - 3.08 | 0.87 | 0.82 - 0.92 | 5.31 | 5.03 - 5.60 | 144 | 2.44 | 2.18 - 2.72 | 0.83 | 0.78 - 0.87 | 5.07 | 4.81 - 5.35 |
| 3 times/week or more | 64 | 3.03 | 2.67 - 3.44 | 0.91 | 0.85 - 0.97 | 5.59 | 5.25 - 5.95 | 82 | 2.35 | 2.05 - 2.69 | 0.82 | 0.77 - 0.88 | 4.97 | 4.66 - 5.31 |
|                  | Trend p = 0.81 | Trend p = 0.93 | Trend p = 0.34 | Trend p = 0.99 | Trend p = 0.36 | Trend p = 0.72 |   |   |   |
| Dried or salted fish |     |       |     |       |     |       |     |       |     |       |
| Almost never     | 51  | 2.37 | 2.07 - 2.72 | 0.85 | 0.78 - 0.91 | 4.92 | 4.57 - 5.31 | 52 | 1.91 | 1.64 - 2.23 | 0.74 | 0.68 - 0.80 | 4.52 | 4.20 - 4.87 |
| 1-2 times/month  | 132 | 2.86 | 2.60 - 3.15 | 0.90 | 0.85 - 0.95 | 5.24 | 4.96 - 5.53 | 136 | 2.37 | 2.14 - 2.63 | 0.80 | 0.76 - 0.84 | 4.91 | 4.68 - 5.16 |
| 1-2 times/week   | 232 | 2.82 | 2.60 - 3.06 | 0.89 | 0.85 - 0.94 | 5.21 | 4.98 - 5.45 | 202 | 2.64 | 2.41 - 2.89 | 0.83 | 0.79 - 0.87 | 5.07 | 4.86 - 5.29 |
| 3-4 times/week   | 113 | 3.18 | 2.88 - 3.52 | 0.91 | 0.86 - 0.96 | 5.58 | 5.28 - 5.90 | 96  | 2.46 | 2.18 - 2.76 | 0.82 | 0.77 - 0.87 | 4.88 | 4.61 - 5.16 |
| Almost every day | 67  | 3.20 | 2.82 - 3.63 | 0.95 | 0.88 - 1.02 | 5.86 | 5.47 - 6.28 | 78  | 2.68 | 2.37 - 3.04 | 0.89 | 0.83 - 0.95 | 5.33 | 5.02 - 5.66 |
|                  | Trend p < 0.001 | Trend p < 0.001 | Trend p < 0.001 | Trend p < 0.001 | Trend p < 0.001 | Trend p < 0.001 |   |   |   |

*: Adjusted for age and participating institution.  
EPA: eicosapentaenoic acid  
DPA: docosapentaenoic acid (n-3)  
DHA: docosahexaenoic acid  
CI: confidence interval
Fish Intake and Serum EPA, DPA, and DHA

In the present study, we found a clear increasing trend in the geometric means of serum concentrations of EPA, DPA, and DHA with the increasing self-reported intake frequency of fresh fish and dried or salted fish in both sexes. Although the correlations were rather weak, the two intake frequencies significantly correlated with serum concentrations of long-chain n-3 fatty acids, especially in men. The serum levels of EPA, DPA, and DHA in this study are comparable with those reported in another Japanese population.19

The blood levels of long-chain n-3 fatty acids have been related to the dietary intakes of fatty acids assessed using either dietary records20 or long and detailed food frequency questionnaires specifying a portion size.4,5,9,11 The correlations were considerably strong in Norway8,9 and Japan,10,11 where fish consumption is quite high; Pearson's or Spearman's correlation coefficients ranged from 0.3 to 0.6 for EPA, DHA, or total long-chain n-3 fatty acids. Our study demonstrated that the intake frequency of fresh fish and dried or salted fish assessed by responses to simple questions may be associated with blood levels of n-3 long-chain fatty acids at the group level, although the correlations between the intake frequency and serum levels were weaker than those obtained from dietary records or detailed food frequency questionnaires. The intake frequency of steamed fish paste (kamaboko) was not associated with serum long-chain n-3 fatty acids in our study, perhaps because the paste contains much less of these fatty acids than fresh, dried or salted fish.19 In addition, the study participants consumed steamed fish paste much less frequently than they did fresh or dried/salted fish (Table 3), which may also have resulted in the weak association of the paste with the serum fatty acids.

Tokudome et al.19 reported that chicken eggs provide one third of the DHA in middle-aged Japanese men and women, although fish and their products were the most important sources of EPA and DHA, the two major long-chain n-3 fatty acids. The multiple regression analysis by the same researchers, however, showed that the chicken egg was not a major determinant of the inter-individual variation in DHA intake and that more than 90% of the variation (R²) was explained by fish consumption.19 This may indicate that such confounding would be minimal.

To use weight percentage of each fatty acid in serum total fatty acids as a measurement value may have attenuated the correlation between intake frequency of fish and serum n-3 fatty acids, if fish consumption is correlated to serum levels of both total fat and n-3 fatty acid. The intake frequency of fresh fish, steamed fish paste, and dried or salted fish, however, was scarcely correlated with serum total fatty acids irrespective of sex; the age-adjusted Spearman correlation coefficients ranged from -0.08 to 0.03. This indicates that such confounding would be minimal.

When we repeated the analysis using the absolute value of each serum fatty acid, the correlations for fresh fish in both sexes and those for dried or salted fish in men were weakened; the Spearman correlation coefficients for fresh fish (adjusted for age) were 0.13, 0.07, and 0.09 for EPA, DPA, and DHA in men, and 0.09, 0.01, and 0.01 in women, respectively. The coefficients for dried or salted fish in men were 0.12, 0.04, and 0.10 for EPA, DPA, and DHA, respectively. The variations in the conditions and methods for blood sampling between institutions seem to have attenuated the correlations. Although the proportion of each fatty acid among total fatty acids in blood has often been linked to the risk of diseases,20-22 studies relating fish consumption to the absolute value of each fatty acid in blood samples collected in a uniform condition may provide meaningful information.

The strength of our study derives from the use of a biomarker and a large sample size from a population with a high level of fish consumption. Using serum samples allowed for objective measurements of fatty acid intake, taking account of inter-individual variations in bioavailability. Some methodological issues, however, need further elucidation.

First, we could not fully assess fish consumption due to limitations in the food frequency questionnaire, which specified neither the kind of fish nor the serving size. Long-chain n-3 fatty acids are abundant in fatty fish, the consumption of which is correlated with blood levels of EPA and DHA.7 Intakes of long-chain n-3 fatty acids may be more accurately estimated by specifying blue-skinned fish that are rich in the fatty acids, even when simple questions on intake frequency are used.

Second, to evaluate the levels of fatty acids, we used serum samples that were stored at -80°C for 11-14 years. Iso et al. examined 31 serum samples in 1990 and again in 1998.23 They reported no increase or decrease in the compositions of long-chain n-3 fatty acids during those eight years of storage at -80°C. Zeleniuch-Jacquotte and co-workers reported that storage for up to 12 years at -80°C effectively protected polyunsaturated fatty acids from oxidation.24 However, the effects of long-term storage for up to 14 years have not yet been confirmed.

Third, the conditions and procedures for blood sampling varied among participating institutions, e.g., subjects were not always fasting at the time of sampling. We therefore adopted the weight percentage of each fatty acid in serum total fatty acids as a measurement value to best take such a difference into consideration. In fact, no study area showed a distribution of serum levels of long-chain n-3 fatty acids (weight % of total fatty acids) greatly different from any other (data not shown). To assess the effect of the fasting condition, we reanalyzed the data while limiting the subjects to those in the study areas where all participants were fasting at blood draw. In men, the correlation coefficients of intake frequency of steamed fish paste with long-chain n-3 fatty acids increased (age-adjusted Spearman correlation coefficients 0.10, 0.12, and 0.19 for EPA, DPA, and DHA, respectively) compared with those in all subjects. In women, the coefficients of fresh fish increased (0.15, 0.10, and 0.10, correspondingly) while those of steamed fish paste (-0.10, -0.04, and -0.06) and dried or...
salted fish decreased (0.01, 0.03, and -0.09). It is difficult, however, to tell whether the change in correlations is due to the fasting condition of participants or due to the difference in study areas.

Finally, although we used serum total fatty acids as a biomarker, they reflect only a short-term (hours to weeks) intake. The intra-individual variations in fish consumption, therefore, may have attenuated the association between the customary consumption data and serum concentrations of long-chain n-3 fatty acids. Erythrocyte membrane and adipose tissue can be used as alternatives to assess medium- and long-term intakes, respectively. The reference period of the food frequency questionnaire may also affect the correlation between self-reported intake frequency of fish and levels of long-chain n-3 fatty acids in biospecimens.

In conclusion, our study suggests that a population with high self-reported frequency of fish intake, as a group, has higher bioavailability of long-chain n-3 fatty acids than one with low frequency intake, despite the misclassifications at the individual level.

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the European Prospective Investigation into Cancer and Nutrition (EPIC). Tumori 2003; 89: 624-35.
7. Woods RK, Stoney RM, Ireland PD, Bailey MJ, Raven JM, Thien FCK, et al. A valid food frequency questionnaire for measuring dietary fish intake. Asia Pac J Clin Nutr 2002; 11: 56-61.
8. Hjartåker A, Lund E, Bjerre KS. Serum phospholipid fatty acid composition and habitual intake of marine foods registered by a semi-quantitative food frequency questionnaire. Eur J Clin Nutr 1997; 51: 736-42.
9. Andersen LF, Sølvoll K, Drevon CA. Very-long-chain n-3 fatty acids as biomarkers for intake of fish and n-3 fatty acid concentrates. Am J Clin Nutr 1996; 64: 305-11.
10. Kuriki K, Nagaya T, Tokudome Y, Imaeda N, Fujiwara N, Sato J, et al. Plasma concentrations of (n-3) highly unsaturated fatty acids are good biomarkers of relative dietary fatty acid intakes: a cross-sectional study. J Nutr 2003; 133: 3643-50.
11. Kobayashi M, Sasaki S, Kawabata T, Hasegawa K, Tsugane S. Validity of a self-administered food frequency questionnaire used in the 5-year follow-up survey of the JPHC Study Cohort I to assess fatty acid intake: comparison with dietary records and serum phospholipid level. J Epidemiol 2003; 13: S64-S81.
12. Sasaki S, Horacsek M, Kesteloot H. An ecological study of the relationship between dietary fat intake and breast cancer mortality. Prev Med 1993; 22: 187-202.
13. Kojima M, Wakai K, Tokudome S, Suzuki K, Tamakoshi K, Watanabe Y, et al. Serum levels of polyunsaturated fatty acids and risk of colorectal cancer: a prospective study. Am J Epidemiol 2005; 161: 462-71.
14. Ohno Y, Tamakoshi A, the JACC Study Group. Japan Collaborative Cohort Study for Evaluation of Cancer Risk Sponsored by Monbusho (JACC Study). J Epidemiol 2001; 11: 144-50.
15. Date C, Fukui M, Yamamoto A, Wakai K, Ozeki A, Motohashi Y, et al. Reproducibility and validity of a self-administered food frequency questionnaire used in the JACC Study. J Epidemiol 2005; 15(Suppl 1): S9-23.
16. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 1957; 226: 497-509.
17. SAS Institute Inc. SAS/STAT user’s guide, version 8. SAS Institute Inc., Cary, NC, 1999.
18. Resources Council, Science and Technology Agency, Japan. Fatty acids, cholesterol, vitamin E composition table of Japanese foods. Printing Office, Ministry of Finance, Japan, Tokyo, 1989.
19. Tokudome Y, Imaeda N, Ikeda M, Kitagawa I, Fujiwara N, Tokudome S. Foods contributing to absolute intake and variance in intake of fat, fatty acids and cholesterol in middle-aged Japanese. J Epidemiol 1999; 9: 78-90.
20. Albert CM, Campos H, Stampfer MJ, Manson JE, Willett WC, et al. Blood levels of long-chain n-3 fatty acids and the risk of sudden death. N Engl J Med 2002; 346: 1113-8.
21. Saadatian-Elahi M, Toniolo P, Ferrari P, Goudable J, Akhmedkhanov A, Zeleniuch-Jacquotte A, et al. Serum fatty acids and risk of breast cancer in a nested case-control study of the New York University Women’s Health Study. Cancer Epidemiol Biomarkers Prev 2002; 11: 1353-60.
22. Iso H, Sato S, Umemura U, Kudo M, Koike K, Kitamura A, et al. Linoleic acid, other fatty acids, and the risk of stroke. Stroke 2002; 33: 2086-93.
23. Zeleniuch-Jacquotte A, Chajes V, Van Kappel AL, Riboli E, Toniolo P. Reliability of fatty acid composition in human serum phospholipids. Eur J Clin Nutr 2000; 54: 367-72.
24. Arab L, Akbar J. Biomarkers and the measurement of fatty acids. Public Health Nutr 2002; 5: 865-71.