

## Serum *n*-3 Fatty Acids, Fish Consumption and Cancer Mortality in Six Japanese Populations in Japan and Brazil

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Japanese people consume significant amounts of long chain *n*-3 polyunsaturated fatty acids (PUFAs) derived from fish, but the association of PUFAs with cancer mortality has not been fully investigated. To study geographic differences in *n*-3 PUFAs intake, we compared serum fatty acid and dietary fish intake among various Japanese populations having different rates of cancer mortality. The subjects were 50 men from each of five regions in Japan and 47 Japanese men from Sao Paulo, Brazil. All were randomly selected and aged 40 to 49 years. Serum fatty acids were measured by gas chromatography and the frequency of fish intake was obtained by a food frequency questionnaire. Significant geographic differences in serum fatty acid levels (% of total fatty acids) and fish intake (days/4 weeks) were observed. The percentages of serum total PUFA were similar in the six regions, though there was an almost three-fold difference in *n*-3 PUFAs content between Brazil (3.9%) and Akita (10.9%). The frequency of total fish intake corresponded to serum *n*-3 PUFAs composition. The relationship between cancer mortality and serum *n*-3 PUFAs levels was not clear, though an inverse association between prostate cancer and serum *n*-3 PUFAs levels appeared to exist. The results suggest that although serum *n*-3 PUFAs varied significantly, the observed geographic difference did not account for the different cancer risks at the population level.

Key words: Serum fatty acids — Fish consumption — Cancer mortality in Japanese

Epidemiological studies have demonstrated that dietary fat consumption modulates the risk of several types of cancer, especially breast, prostate and colorectal cancer.<sup>1,2</sup> The relationship between the intake of specific fatty acids (FA) and risk of cancer in humans has been investigated in several studies.<sup>3,4</sup> Recently, some *in vivo* studies have observed that *n*-3 polyunsaturated fatty acids (PUFAs) inhibit the development of cancer, and that *n*-6 PUFAs promote the development and growth of cancer.<sup>5-7</sup> Some epidemiologic studies have examined the relationship between *n*-3 PUFA intake and the risk of cancer, and also between biomarkers of *n*-3 PUFA intake and the risk of cancer. However, these epidemiologic studies have provided little support for any important relationship between either *n*-3 PUFA intake or biomarkers of *n*-3 PUFA intake and the risk of cancer.

It has been pointed out that Japanese people consume large amounts of long-chain *n*-3 PUFAs because their fish consumption is higher than that of Western populations,<sup>8,9</sup> and the major source of long-chain *n*-3 PUFAs is fish. However, there are no epidemiological studies that describe the relation between *n*-3 PUFAs intake and cancer mortality in Japan, because the Japanese food compo-

sition table was insufficient and the estimation of fatty acids intake has not been accurate.<sup>10</sup> Several case-control studies have discussed possible inverse relationships between fish intake and colon cancer,<sup>11,12</sup> prostate cancer<sup>13</sup> and breast cancer.<sup>14,15</sup> However, the effect of *n*-3 PUFAs on cancer is inconclusive according to the current evidence. The purpose of this study was to investigate geographic differences in fish consumption and serum *n*-3 PUFAs levels, as a biomarker for *n*-3 PUFAs intake, in six Japanese populations in Japan and Brazil. Furthermore, we also studied the relationship with cancer mortality.

### SUBJECTS AND METHODS

**Subjects** Details of the study design and subject characteristics have been described elsewhere.<sup>16,17</sup> Briefly, 880 Japanese males aged 40–49 years were sampled randomly from the general populations of five Public Health Center (PHC) districts (Ninohe, Iwate prefecture; Yokote, Akita prefecture; Katsushika-kita, Tokyo; Saku, Nagano prefecture; and Ishikawa, Okinawa prefecture) in the winters of 1989–91. The response rate was 72% ( $n=634$ ). Fifty males were randomly selected from each area for the analysis of serum fatty acid.

Four hundred and eleven Japanese first- and second-generation male residents in Sao Paulo, Brazil, aged 40 to 69 years were randomly selected in the winter of 1989, for

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a cross-sectional study. A total of 251 (61%) subjects provided blood samples and life style data.<sup>18)</sup> Forty-seven male subjects aged 40 to 49 years were used in this study.

Age-adjusted mortality rates to the world population (AAMRjpn)<sup>19)</sup> per 100,000 population during the five years from 1985 to 1989 in each PHC district<sup>16)</sup> are shown in Table I with respect to the major causes of death, stomach cancer, colon cancer, rectum cancer, breast cancer and prostate cancer. The rates for Brazil (AAMRbrz) in Table I were estimated from the AAMR of Japan in 1987 (AAM-Rjpn) and the standardized mortality ratios of Japanese Brazilians (SMRbrz) in 1979–81 for AAMR of Japan in 1980<sup>20, 21)</sup> as follows;  $AAMRbrz = SMRbrz * AAMRjpn$ . The mortality rate from all cancers ranged from 115.9 in Okinawa to 169.4 in Tokyo.

Table II shows the background characteristics of the study subjects. The subjects in Tokyo tended to be slightly older while those in Iwate tended to be younger. Body mass index (BMI) was higher in Brazil and Okinawa. Total cholesterol and triglycerides were the highest in Bra-

zil, and HDL-cholesterol was higher in Tokyo and Akita.

**Food frequency questionnaire** The questionnaire included a total of 38 food items. Trained dietitians or public health nurses interviewed the subjects to determine the average number of days per week during the past month. Four frequency categories were as follows: less than once a week, 1–2 days/week, 3–4 days/week, and almost every day.

Since information on the food portion size was not specified or asked, we calculated the monthly frequency of fish intake as four times the scores assigned to the four weekly frequency categories (less than once a week=0, 1–2 days/week=1.5, 3–4 days/week=3.5, and almost every day=6). The details of fish consumption were classified according to the following seven categories: (1) river fish including sweetfish, carp, loach, and crucian carp; (2) blue fish including horse mackerel, mackerel, sardines, and pacific saury; (3) red fish including salmon, tuna, and skipjack; (4) white fish including flatfish and cod; (5) squid and octopus; (6) crab and shrimp; (7) shellfish.

Table I. Mortality Profiles of Six Study Regions. Age-adjusted Mortality Rates of Men (Excluding Breast Cancer) in 1985–1989 per 100,000 Population

ICD 9	Cause of death	Regions						
		Japan (1987)	Iwate (Ninohe)	Akita (Yokote)	Tokyo (Katsushika-kita)	Nagano (Saku)	Okinawa (Ishikawa)	Brazil <sup>b)</sup> (Sao Paulo)
	All causes	527.7	630.4	544.3	579.5	475.4	458.2	480.2
410–414	Ischemic heart disease	31.8	34.1	24.3	54.9	16.7	23.6	63.8
420–429	Other heart disease	58.1	95.0	62.8	33.0	55.7	43.4	20.9
430–438	Cerebrovascular disease	72.2	100.5	92.0	78.9	70.0	35.3	53.4
140–208	All cancers	150.8	127.1	154.7	169.4	130.9	115.9	122.2
151	Stomach	38.0	29.3	49.1	49.1	38.3	17.3	32.7
153	Colon	8.1	6.7	9.9	9.9	6.9	7.0	8.2
154	Rectum	6.2	8.0	5.8	7.3	4.8	4.4	1.7
177	Breast <sup>a)</sup>	5.9	5.4	3.9	7.8	4.0	4.2	4.2
185	Prostate	3.6	4.5	2.7	6.2	5.2	3.2	7.8

a) Calculated from SMR for Japan 1980.

b) Age-adjusted mortality rates of women.

Table II. Background Characteristics of Study Subjects

Parameter	Regions					
	Iwate (Ninohe) Mean (SD)	Akita (Yokote) Mean (SD)	Tokyo (Katsushika-kita) Mean (SD)	Nagano (Saku) Mean (SD)	Okinawa (Ishikawa) Mean (SD)	Brazil (Sao Paulo) Mean (SD)
No. of subjects	50	50	50	50	50	47
Age [y]	43.5 (2.5)	44.3 (3.2)	45.3 (2.8)	44.6 (3.1)	44.3 (3.1)	44.5 (3.1)
Body mass index [kg/m <sup>2</sup> ]	23.2 (2.5)	23.6 (2.7)	23.3 (2.8)	23.5 (3.3)	24.6 (3.1)	24.9 (3.4)
Total cholesterol [mg/dl]	186.8 (36.1)	197.9 (35.7)	206.1 (31.4)	190.2 (32.4)	204.9 (35.5)	213.0 (46.9)
HDL-cholesterol [mg/dl]	45.5 (9.4)	51.8 (14.6)	52.0 (10.8)	48.2 (12.5)	50.3 (13.4)	46.3 (12.8)
Triglycerides [mg/dl]	121.4 (84.3)	168.8 (189.7)	119.7 (71.4)	124.2 (53.6)	149.5 (93.4)	214.2 (158.4)

The questionnaire used in Sao Paulo was translated into Portuguese. Trained dietitians or nurses interviewed the subjects in a similar manner to that used in Japan.

**Blood collection and analysis** Peripheral venous blood was obtained after fasting for at least 5 h. After leaving the blood for an hour at room temperature to facilitate clotting, the serum was separated by centrifugation. Serum was stored frozen in an ice box with sufficient dry ice until they were sent to the laboratories, where they were stored at  $-80^{\circ}\text{C}$  until analysis.

For fatty acid analysis, all samples were assayed blindly at the same time and in random order with regard to area in a commercial laboratory (SRL Co., Tokyo). In the laboratory, total fat was extracted from serum using the Folch method<sup>22)</sup> and dried with  $\text{N}_2$  gas. The residue was hydrolyzed into free fatty acids with 0.5 M HCl. The free fatty acids were extracted with chloroform and then dried with  $\text{N}_2$  gas. The residue was methyl-esterified with 0.4 M potassium methoxide/methanol and 14% boron trifluoride-methanol. Samples were then checked by gas chromatography.

The composition of serum, including 21 specific fatty acids, was measured as the mean weight percent (SD) of each fatty acid per total fatty acids. The analytical coefficients of variation (CV) of three reference samples for eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and total *n*-3 PUFA were 4.6, 4.9, 4.7, respectively. **Statistical analysis** We calculated the mean levels of serum fatty acids and fish intake between the six regions and their 95% confidence intervals. To assess the associations between the mortality rate and serum fatty acid composition (%), Pearson's correlation coefficients were computed. Correlations were also computed with absolute values ( $\mu\text{g}/\text{ml}$ ) of serum fatty acid. We used the SAS program package<sup>23)</sup> for statistical analysis.

**RESULTS**

Table III shows the means and 95% confidence intervals of serum fatty acid composition in the 6 regions. Saturated fatty acid (SFA) composition was the highest and PUFA composition was the lowest in Akita. Regional differences

Table III. Serum Fatty Acid Composition in Japanese Men in Six Regions in Japan and Brazil (%)

	Ninohe, Iwate (n=50) Mean (95%CI)	Yokote, Akita (n=50) Mean (95%CI)	Saku, Nagano (n=50) Mean (95%CI)	Katsushika-kita, Tokyo (n=50) Mean (95%CI)	Ishikawa, Okinawa (n=50) Mean (95%CI)	Sao Paulo, Brazil (n=47) Mean (95%CI)
SFA	32.9 (32.2–33.6)	33.5 (32.9–34.1)	32.3 (31.6–33.0)	32.2 (31.6–32.7)	31.9 (31.2–32.7)	32.1 (31.4–32.8)
MUFA	21.7 (20.9–22.6)	22.9 (21.8–24.1)	22.6 (21.8–23.5)	22.8 (21.9–23.7)	22.7 (21.8–23.5)	22.2 (20.9–23.5)
PUFA	45.3 (43.9–46.8)	43.6 (42.0–45.2)	45.1 (43.8–46.3)	45.1 (43.8–46.3)	45.4 (44.0–46.8)	45.7 (44.0–47.4)
$\alpha$ -Linolenic acid	0.9 (0.8–1.0)	0.8 (0.7–0.9)	0.9 (0.8–0.9)	0.7 (0.7–0.8)	0.9 (0.8–1.0)	0.8 (0.7–0.9)
EPA	3.5 (3.0–4.0)	3.6 (3.1–4.1)	2.6 (2.2–3.0)	2.7 (2.2–3.1)	1.4 (1.2–1.6)	0.6 (0.5–0.7)
DHA	5.3 (5.0–5.7)	5.6 (5.2–5.9)	4.7 (4.4–5.1)	4.8 (4.4–5.2)	3.5 (3.2–3.7)	2.0 (1.7–2.3)
<i>n</i> -3 PUFAs	10.7 (9.9–11.5)	10.9 (10.1–11.8)	9.0 (8.2–9.7)	8.9 (8.1–9.8)	6.4 (5.9–6.8)	3.9 (3.5–4.3)
<i>n</i> -6 PUFAs	34.7 (33.0–36.3)	32.6 (31.1–34.2)	36.1 (34.6–37.5)	36.1 (34.8–37.5)	39.0 (37.6–40.5)	41.8 (40.2–43.5)
<i>n</i> -3/ <i>n</i> -6	0.32 (0.29–0.36)	0.35 (0.31–0.38)	0.26 (0.23–0.29)	0.26 (0.22–0.29)	0.17 (0.15–0.18)	0.09 (0.08–0.11)

SFA, total saturated fatty acids; MUFA, total monounsaturated fatty acids; PUFA, total polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

Table IV. Frequencies of Monthly Fish Consumption in Japanese Men in Japan and Brazil

	Ninohe, Iwate (n=50) Mean (95%CI)	Yokote, Akita (n=50) Mean (95%CI)	Saku, Nagano (n=50) Mean (95%CI)	Katsushika-kita, Tokyo (n=50) Mean (95%CI)	Ishikawa, Okinawa (n=50) Mean (95%CI)	Sao Paulo, Brazil (n=47) Mean (95%CI)
River fish	1.5 (0.6–2.3)	2.0 (0.9–3.1)	1.3 (0.6–2.0)	0.6 (0.0–1.3)	0.1 (–0.1–0.4)	0.9 (0.1–1.7)
Blue fish	10.2 (8.3–12.1)	7.6 (5.8–9.5)	9.9 (8.3–11.5)	8.8 (7.0–10.7)	5.8 (3.7–7.8)	4.5 (3.0–6.0)
White fish	6.8 (5.5–8.1)	4.8 (3.8–5.8)	4.7 (3.6–5.8)	3.4 (2.1–4.7)	4.5 (2.6–6.4)	3.9 (2.3–5.4)
Red fish	6.8 (5.5–8.2)	8.9 (7.3–10.5)	8.4 (7.0–9.8)	5.7 (4.4–7.0)	7.6 (5.5–9.6)	3.1 (1.5–4.7)
Squid, octopus	5.8 (4.1–7.4)	5.5 (4.4–6.7)	4.7 (3.6–5.8)	3.6 (2.5–4.6)	5.4 (3.5–7.2)	0.4 (–0.1–1.2)
Crab, shrimp	2.2 (1.2–3.3)	3.9 (2.9–4.9)	2.4 (1.3–3.4)	2.9 (1.7–4.0)	2.2 (1.2–3.3)	0.6 (–0.1–1.2)
Shellfish	5.1 (4.0–6.3)	3.6 (2.7–4.6)	4.3 (3.0–5.5)	4.2 (2.6–5.8)	2.0 (1.0–3.0)	0.0
Total	38.4 (33.3–43.5)	36.5 (31.6–41.4)	35.7 (31.1–40.3)	29.2 (23.7–34.8)	27.6 (21.0–34.1)	13.4 (8.5–18.3)

For the description of fish, see "Subjects and Methods."

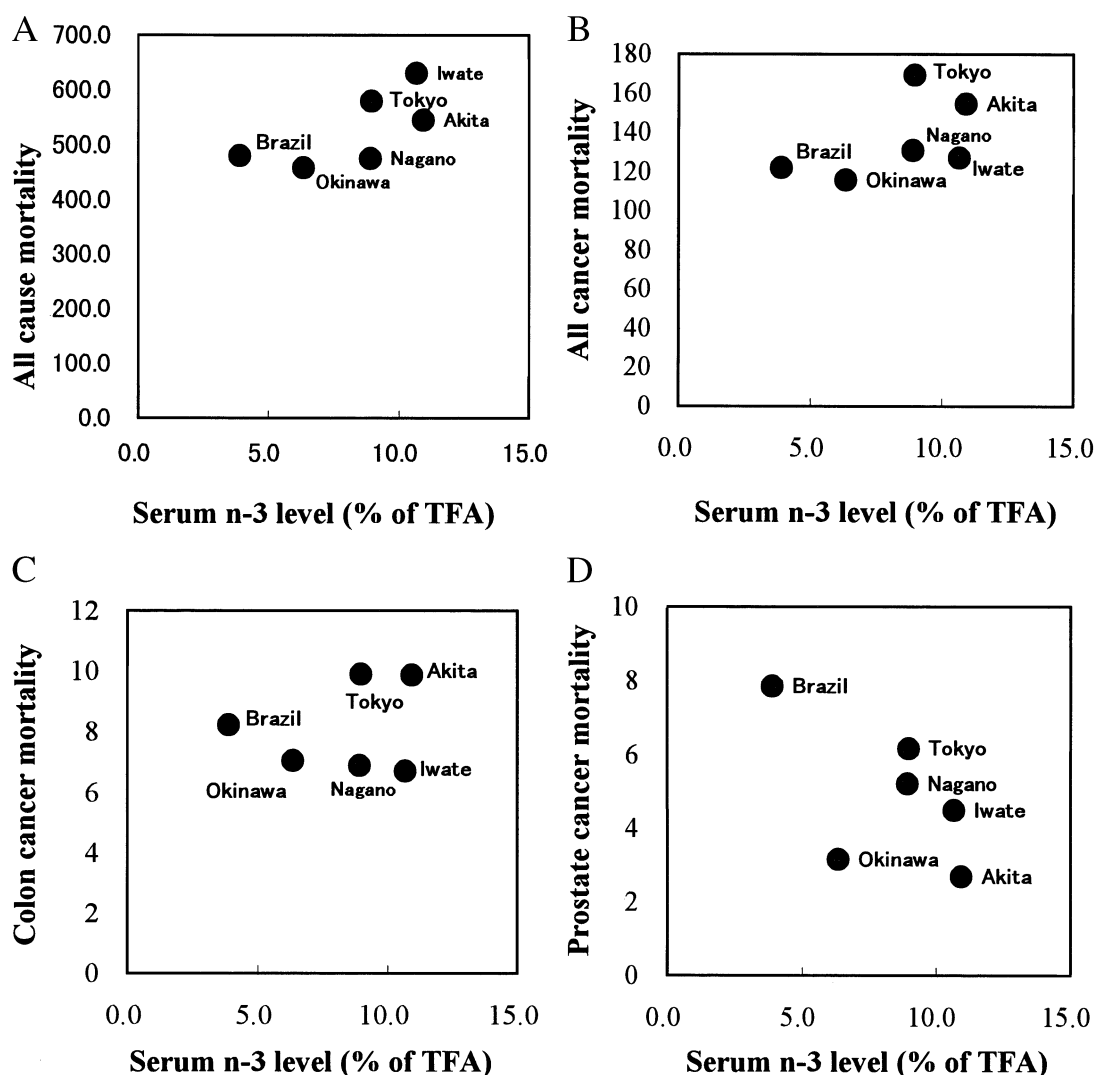


Fig. 1. Relation between (A) all cause mortality ( $r=0.68$ , 95%CI 0.46–0.90), (B) all cancer mortality ( $r=0.52$ , 95%CI 0.14–0.89), (C) colon cancer mortality ( $r=0.16$ , 95%CI –0.44–0.75), (D) prostate cancer mortality ( $r=-0.59$ , 95%CI –1.20–0.02) and serum *n*-3 level in Japanese men in six areas in Japan and Brazil.

were not observed for monounsaturated fatty acid (MUFA). For *n*-3 PUFAs, no regional differences were seen for  $\alpha$ -linolenic acid (ALA), although DHA and total *n*-3 PUFAs were the highest in Akita (5.6%, 10.9%), followed by Iwate (5.3%, 10.7%), being almost three-fold higher than for Brazil (2.0%, 3.9%). EPA was the highest in Akita (3.6%), followed by Iwate (3.5%), and there was an almost six-fold difference compared with Brazil (0.6%). The opposite trend was seen in total *n*-6 PUFAs (32.6% in Akita, 41.8% in Brazil). Similar geographic differences were observed for absolute values ( $\mu\text{g}/\text{ml}$ ) of each FA. For example, total *n*-3 PUFAs were the highest

in Akita (367.3  $\mu\text{g}/\text{ml}$ ), being about 2.5-fold greater than in Brazil (150.0  $\mu\text{g}/\text{ml}$ ).

Table IV shows the means and 95% confidence interval of frequencies of monthly fish consumption in the six regions. Geographic differences were seen for all kinds of fish consumption. The difference was especially large for blue fish, which contain large amounts of *n*-3 PUFAs (4.5 days/month in Brazil, 10.2 days/month in Iwate). Total fish consumption was almost three-fold greater in Iwate (38.4 days/month) than in Brazil (13.4 days/month).

Fig. 1 presents the Pearson correlation coefficients between the serum total *n*-3 PUFAs composition and mor-

tality rates from all causes, all cancers, colon cancer and prostate cancer in the six regions. The mortality rates from all causes and all cancers were positively correlated with serum total *n*-3 PUFA composition (Fig. 1, A and B). The mortality rates from colon cancer were not correlated with serum total *n*-3 PUFA composition (Fig. 1C). The mortality rates from prostate cancer were inversely correlated with total *n*-3 PUFA composition ( $r=-0.59$ , 95%CI  $-1.20-0.02$ ), although not significantly (Fig. 1D). Similar findings were observed for the association between the absolute values ( $\mu\text{g/ml}$ ) of serum FA and the prostate cancer mortality rate ( $r=-0.67$ , 95%CI  $-1.21-0.13$ ). We also observed a correlation between the serum *n*-3 PUFAs composition and ischemic heart disease (IHD) ( $r=-0.53$ , 95%CI  $-1.09-0.04$ ) or cerebrovascular disease (CVD) ( $r=0.84$ , 95%CI  $0.75-0.93$ ) mortality rate. In addition, we observed a correlation between the fish intake and cancer mortality rates. The mortality rate from prostate cancer was inversely correlated with fish intake ( $r=-0.61$ , 95%CI  $-1.12-0.11$ ). However, no clear associations were observed for other cancers.

## DISCUSSION

This study is the first to demonstrate geographic variations in serum *n*-3 PUFAs composition and *n*-6 PUFAs composition in Japanese in six regions using random sampling methods. The differences in serum *n*-3 PUFAs composition were opposite to the differences in serum *n*-6 PUFAs composition and the differences in serum *n*-3 PUFAs composition corresponded to the differences in fish consumption in the six regions. We also observed an association between cancer mortality and serum *n*-3 PUFAs composition. There was a possible inverse association between prostate cancer and serum *n*-3 PUFAs levels.

This study has several limitations. First, there are the issues of ecological bias, confounding and temporal ambiguity, which are difficult to control.<sup>24</sup> Second, we had only six observational districts. This small number of districts may not only attenuate the reliability of the relation between serum *n*-3 PUFAs and cancer mortality rates, but also make it impossible to calculate multivariate regression coefficients and to account for other independent variables. Therefore, we were unable to take into account many dietary confounding factors. For instance, concerning rectum cancer, there may be an association between blue fish and dietary fiber intake. Thirdly, using incidence rates is preferable to mortality for cancers with a relatively good prognosis. However, because it was impossible to obtain the incidence rates for the five Japanese PHC districts, we used the mortality rates and assumed that the prognoses were comparable. Fourthly, in this study, serum *n*-3 PUFAs composition (%) and fish consumption were at relatively high levels and comparable in the Japanese

regions with the exception of Okinawa. This finding may attenuate the relation between serum *n*-3 PUFAs composition and cancer mortality. Fifthly, serum fatty acid may not reflect long-term dietary intake patterns because of the short half-life times of the FA in sera.

Previous reports in Japan have shown differences in serum and plasma *n*-3 PUFAs composition (%) between rural and urban areas<sup>25, 26</sup> and between fishing and farming villages.<sup>27, 28</sup> In this study, serum EPA and DHA composition (%) ranged from 2.6–3.6 and 4.7–5.6, respectively, excluding Okinawa and Brazil, levels which are similar to those of other studies in Japanese.<sup>25, 27–29</sup> The serum EPA and DHA compositions (%) of Japanese in Sao Paulo were 0.6 and 2.0, respectively, values that are similar to those of Caucasian Americans in previous reports.<sup>25, 28</sup> Serum EPA and DHA composition (%) in Okinawa were halfway between areas in Japan and Sao Paulo. From the observed geographic differences of serum fatty acid composition, the number of subjects seems to be enough to obtain the means of serum *n*-3 PUFAs in each area.

Japanese serum ALA composition (%) was reported to be about 1.0 and Caucasian American ALA composition (%) to be about 0.6.<sup>25, 28</sup> In this study, serum ALA composition (%) was in the range of 0.7–0.9 and there were no geographic variations in serum ALA composition (%).

Total fish consumption was almost three-fold higher in Iwate, Akita and Nagano than Brazil. The Organization for Economic Co-operation and Development (OECD) described Japanese fish consumption as being higher than in Western countries.<sup>8</sup> Dietary intake levels of *n*-3 fatty acid have been shown to be reflected in serum or plasma *n*-3 PUFAs levels,<sup>30–32</sup> while fish consumption is reflected in serum *n*-3 PUFAs levels.<sup>33</sup> In this study, the difference between areas with low levels of fish consumption, i.e. Brazil and Okinawa, and the other areas with higher levels, reflected the difference in serum *n*-3 PUFAs composition. However, among the other four regions whose fish consumption was relatively high, the differences of fish consumption could not explain the differences of serum *n*-3 PUFAs composition.

It has been reported that the growth of prostate cancer cells is inhibited by the consumption of long-chain *n*-3 PUFA in animal experiments.<sup>34, 35</sup> Although the mechanism by which *n*-3 PUFA influences prostate cancer development and progression is not well understood, prostate cancer cell lines are suppressed by inhibitors of eicosanoid biosynthesis and long-chain *n*-3 PUFA *in vitro*.<sup>36</sup> Ecological studies have not shown any association between *n*-3 PUFAs and prostate cancer,<sup>37</sup> though one case-control study reported an inverse risk association with arachidonic acid (AA)/EPA ratio.<sup>37</sup> We found no association between the serum AA/EPA ratio and prostate cancer mortality rates, although there was an inverse correlation between *n*-3 PUFAs composition and prostate cancer mortality rates.

In addition, a comparable relationship was observed between the absolute value of serum *n*-3 PUFAs and fish intake. It has also been reported that the risk of prostate cancer is positively correlated with ALA intake<sup>37,38)</sup> and plasma ALA acid levels.<sup>39)</sup> We observed no significant association between serum ALA levels and prostate cancer mortality rates in this ecological study, partly because there were no variations in serum ALA levels.

International comparisons have suggested positive associations between total or saturated fat intake and risk of breast cancer.<sup>15)</sup> Further, *n*-3 PUFAs may inhibit the enhancement of the promotional phase of experimental mammary carcinogenesis both *in vitro* and in animal studies.<sup>15)</sup> However, the involvement of dietary fatty acids in the etiology of human breast cancer remains controversial, and may involve hormones, eicosanoids and gene expression. Although several case-control studies have reported a relationship between *n*-3 PUFAs in adipose tissue and breast cancer,<sup>40–42)</sup> these reports provide weak evidence for protection by *n*-3 PUFAs against invasive breast cancer in women. A recent EURAMIC (the European Community Multicenter Study on Antioxidants, Myocardial Infarction, and Cancer) study has suggested that the balance between *n*-3 and *n*-6 PUFAs of adipose tissue may play a role in breast cancer.<sup>43)</sup> The epidemiologic evidence does not support an association between *n*-3 PUFAs intake and risk of breast cancer.<sup>44)</sup> In this study, although the subjects were men, the regional difference of the serum FA in men is expected to be comparable to that of women. Therefore we calculated the association between both serum *n*-3 PUFAs composition and the serum *n*-3 to *n*-6 PUFAs ratio of these subjects and the women's breast cancer mortality rate in each area. No clear relationship between breast cancer mortality rate and serum *n*-3 PUFAs ( $r=0.20$ , 95%CI  $-0.20-0.60$ ) or the serum *n*-3 to *n*-6 PUFAs ratio ( $r=0.16$ , 95%CI  $-0.28-0.60$ ) was found.

Several experimental studies have indicated that *n*-3 PUFAs alter a variety of biological intermediates and inhibit colonic tumorigenesis; this is probably mediated via the eicosanoid pathway.<sup>45)</sup> Case-control and cohort studies do not generally support the hypothesis that *n*-3 PUFAs inhibit colon carcinogenesis.<sup>46)</sup> Regarding fish consumption, some case-control<sup>47,48)</sup> and cohort<sup>49–53)</sup> studies have reported inverse associations. In this study, no relationship between serum *n*-3 PUFAs levels and colon cancer mortality rates was observed. We observed a narrow

range for the colon cancer mortality rate and wide ranges for the rectum and prostate cancer mortality rates. These differences in ranges may attenuate individual correlations between mortality rates and serum *n*-3 PUFAs.

There are very few reports on the relation between risk of other cancers and *n*-3 PUFAs in previous epidemiologic studies. Only one case-control study reported an inverse association between AA and DHA serum concentrations and thyroid cancer risk,<sup>54)</sup> while one nested case-control study reported an inverse association between dihomo-gammalinolenic acid serum concentration and lung cancer risk.<sup>55)</sup> In the present study, the correlations between rectum cancer and either *n*-3 FA or *n*-3/*n*-6 FA and between lung cancer and *n*-3 FA were significantly positive. Furthermore, we observed a significant positive association between serum *n*-3 PUFAs and the CVD mortality rate. However, it is difficult to speculate on the biological mechanisms.

In summary, we found geographic differences in the compositions of serum *n*-3 FA and *n*-6 FA and fish intake in the six regions studied. Although a relationship between prostate cancer mortality rate and serum *n*-3 FA levels was suggested, the results for other cancer mortality rates were not clear. To determine the relationship between specific fatty acids and cancer risk, further research is needed to estimate other food or nutrient intakes and to take them into account. A population-based prospective study is under way in the same regions in Japan as used in this study. It is hoped this prospective study will clarify the relationship between the risk of cancer and specific fatty acids at the individual level.

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