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Relation of long chain n-3 polyunsaturated fatty acid intake to serum high density lipoprotein cholesterol among Japanese men in Japan and Japanese—American men in Hawaii: the INTERLIPID study

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Abstract

Epidemiologic evidence shows an inverse relationship between fish consumption and coronary heart disease (CHD) mortality. Associations between dietary intake of long chain n-3 polyunsaturated fatty acids (PUFA) and serum high density lipoprotein (HDL) cholesterol concentration are unknown. In this study, the association between n-3 PUFA (eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA)) intake and serum HDL cholesterol among Japanese men and women in Japan and Hawaii was examined. The study

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population consisted of Japanese ancestries from five research centers of the International Study of Macronutrients and Blood Pressure (INTERMAP) study, in Japan and Hawaii (672 men and 676 women), surveyed between 1996 and 1998. Four 24-h dietary recalls and one set of serum lipid measurements were performed. For men, n-3 PUFA intake and HDL cholesterol were higher in Japan than in Hawaii (n-3 PUFA: 1.32 g/day versus 0.47 g/day, $p < 0.001$). For women, n-3 PUFA intake was higher in Japan than in Hawaii ($p < 0.001$) but HDL cholesterol was not significantly different ($p = 0.752$). After adjustment for age, body mass index, physical activity, number of cigarettes per day, alcohol intake, and hormone replacement therapy (for women), n-3 PUFA intake was positively associated with serum HDL cholesterol in men (4.6 mg/dl higher HDL cholesterol with 1% kcal higher n-3 PUFA intake, $p = 0.011$). This association was not observed in women. This positive association of dietary n-3 PUFA with serum HDL cholesterol may partially explain the low mortality from CHD among Japanese men.

Keywords

Coronary disease; Diet; Fish oils; HDL cholesterol; Omega-3 fatty acids; Population study; Japan

1. Introduction

The low coronary heart disease (CHD) mortality in Japan, where fish is an important component of the diet, suggests that consumption of fish may be protective against the atherosclerotic diseases [1]. An inverse association between fish intake and risk of death from CHD has been found in prospective studies in Western countries [2], but no data on this matter have been reported for Japan.

The putative beneficial effect has been attributed to the long chain n-3 polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) for which the main dietary sources are fish, shellfish, and marine mammals [3], and fatty fish intake has been reported to be protective against CHD [4]. In many n-3 PUFA supplementation studies, significant lowering of triglycerides was observed [5–7], but the effect on high density lipoprotein (HDL) cholesterol has been reported variously to be decrease [5], increase [5,7] and none [6,8]. Moreover, studies concerning associations between dietary fish intake and HDL cholesterol of individuals are sparse and their findings inconclusive [9,10].

In Japan, westernization of dietary habits and population average serum total cholesterol level have continued to increase [11]. Recently, mean cholesterol level in young adults in Japan was found to be similar to that of persons of the same age in the U.S. [12]. However, age-adjusted mortality from CHD remains significantly lower in Japan than in the U.S. and other Western countries [12]. Although smoking rates remain high, low levels of low density lipoprotein (LDL) cholesterol, body mass index (BMI) and high HDL cholesterol among Japanese have been suggested to contribute to the low CHD mortality [13]. It is known that BMI and smoking habit correlate negatively with HDL cholesterol level, while alcohol intake and physical activity correlate positively [10,14]. In women, menopause [15] and hormone replacement therapy [16,17] are also reported to have effects on HDL cholesterol

concentration. But the influence of most specific dietary factors on HDL cholesterol level is unclear. High fish consumption in Japan might be expected to have a favorable effect on HDL cholesterol level.

In the International Study of Macronutrients and Blood Pressure (INTERMAP), dietary surveys were conducted with a highly standardized protocol in random samples in 17 centers in four countries (Japan, People's Republic of China, U.K. and U.S.) [18]. INTERLIPID was an INTERMAP ancillary study of CHD risk factors in four Japanese population samples in Japan and a Japanese-American population sample in Hawaii [19], stimulated in part by earlier data indicating that Japanese-Americans had higher total fat and saturated fatty acids intake and lower fish consumption [20] than Japanese in Japan. The objective of this study was to examine the association in these five samples between long chain n-3 PUFA intake and serum HDL cholesterol level taking age, BMI, physical activity, smoking, hormone replacement therapy in women and intake of other nutrients and alcohol into consideration as potential confounding variables.

2. Materials and methods

Detailed methods of the INTERMAP study have been described [18,21]. They are summarized briefly here. In the INTERMAP study, blood pressure measurements were made on four different days; medical and lifestyle information, four 24-h dietary recalls, and two 24-h urine collections were obtained for each participant. In addition, non-fasting blood was drawn from INTERLIPID participants [19]. Data based on specific analytes measured in these samples, as well as, dietary data and other information obtained from INTERMAP, were used in analyses here.

2.1. Participants

The INTERLIPID participants were from five INTERMAP research centers: four in Japan and one in Hawaii [19]. Participants ages 40–59 years were recruited by the research centers. The five populations were: (1) Japanese factory workers in Toyama, central Japan (149 men and 150 women); (2) Japanese factory workers in Sapporo, northern Japan (149 men and 148 women); (3) Japanese residents in Aito-town, a rural town in Shiga prefecture, central Japan (129 men and 129 men); (4) Japanese factory workers in Wakayama, central Japan (145 men and 143 women); and (5) third and fourth generation offsprings of Japanese emigrants living in Honolulu, Hawaii (100 men and 106 women).

The ethical committees of the Shiga University of Medical Science, the Sapporo Medical University, the Kanazawa Medical University, the Wakayama Medical University, the Pacific Health Research Institute and Northwestern University approved the study protocol. Written informed consent was obtained from all participants.

2.2. Anthropometric and lifestyle assessment

The participants visited clinics four times on two pairs of consecutive days approximately 3 weeks apart. Height and weight with light clothes were measured at each visit, four times. Using a questionnaire, trained observers inquired about physical activity, smoking status, previous medical history of cerebro-cardiovascular diseases and use of medication.

To evaluate physical activity, questions were posed about number of hours per day spent in heavy activity, moderate activity, slight activity, watching TV, other sedentary and no activities (sleeping); the interviewer ensured that the total time added up to 24 h. A physical activity index was calculated by multiplying the time spent in different activities by corresponding weighting factors that parallel the increased rate of oxygen consumption associated with increasingly more intense physical activity; the procedure used in the Framingham off spring study [22] was followed. In the calculation, hours of watching television were designated sedentary activity. Menopausal status and use of hormone replacement were also asked of women.

2.3. Dietary assessment

Four 24-h dietary recalls were conducted with each participant at the four visits by specially trained dietary interviewers. Prior to the data collection a supervising nutritionist in each country trained all interviewers and certified that they had the appropriate skills to conduct dietary interviews and handle dietary data using computers. Standardized quality control procedures were adopted to ensure the quality of dietary data throughout data collection [21]. Standard Tables for Food Composition in Japan, 4th edition, with matched fatty acids values and micro-nutrients were used to calculate Japanese nutrient intakes. In the U.S., dietary assessment was performed using the Nutrition Data System, Nutrition Coordinating Center, University of Minnesota. The Nutrition Coordinating Center in cooperation with the country nutritionists was responsible for completion of the country specific databases on nutrient composition of all foods consumed by INTERMAP participants, and for assuring quality and comparability of these nutrient data [21,23].

2.4. Serum lipid measurements

For the INTERLIPID study, non-fasting blood was drawn on the second day of the first two-day visit. Serum and plasma were obtained by centrifugation within 30 min of blood drawing and stored immediately under refrigeration. All specimens were frozen and stored locally at -70°C “for long term preservation”. Serum lipids were measured in a central laboratory approximately 6–12 months after the samples were frozen. Samples from Hawaii and the Japanese centers were shipped to the central laboratory in Japan on dry ice for analysis. Individual samples from the five centers were randomly allocated for analysis to avoid systematic measurement bias in assigned order in the autoanalyzer. The central laboratory was standardized using the Lipid Standardization Program, Centers for Disease Control and Prevention, Atlanta, GA, U.S., and successfully met the criteria of precision and accuracy of control measurements [24]. The laboratory is currently a member of the Cholesterol Reference Method Laboratory Network (CRMLN) [25]. Serum concentrations of total, HDL and LDL cholesterol, triglycerides and lipoprotein(a) (Lp(a)) were directly measured by enzymatic methods on an auto-analyzer (Hitachi 7107, Tokyo, Japan) [26]. We included standard sera of known values with each batch; there were no significant differences in standard serum levels among the batches. The analytical coefficients of variation (CV) were less than 3% for total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides and Lp(a).

2.5. Data analyses

For each person, the mean of individual nutrients from the four 24-h dietary recalls was used in analyses. Data are presented as the contribution to total energy intake from total carbohydrates, total fat, monounsaturated (MUFA), saturated (SFA), polyunsaturated (PUFA), long chain n-3 polyunsaturated (PUFA) fatty acids and alcohol. In INTERMAP, intakes of EPA, DHA and docosapentaenoic acid (DPA; 22:5n-3, another long chain n-3 PUFA originating mainly from fish) were estimated and the long chain n-3 PUFA intake was calculated as the total EPA, DHA and DPA intake. EPA, DHA and DPA intakes of individuals were highly correlated (Pearson correlation coefficients between any two of the three fatty acids greater than 0.9) and results from analyses using any combination of these n-3 PUFAs were similar. n-3 PUFA, EPA, DHA and DPA intakes are also presented in grams. Keys score was calculated as $1.35 \times (2S - P) + 1.5 \times C^{1/2}$, where S was the percentage of calories from saturated fatty acids, P was percentage of calories from polyunsaturated fatty acids and C was dietary cholesterol (in mg/1000kcal). BMI was calculated as weight divided by height squared (kg/m^2). Average number of cigarettes per day was calculated including and excluding non-smokers at baseline. Percentage of hormone replacement use was calculated for postmenopausal women. Analysis of variance was used to compare means of Japanese samples; t -test, to compare means of Japanese and Japanese-Americans. Chi-squared tests were used to compare smoking rates, use of lipid lowering drug, menopausal status and use of hormone replacement therapy.

To assess within sample relationships between long-chain n-3 PUFA and HDL cholesterol, gender-specific multiple linear regression analysis was used with long chain n-3 PUFA intake (%kcal), age (years), BMI (kg/m^2), physical activity index, number of cigarettes per day, hormone replacement therapy (for women, 0 = no and 1 = yes) and alcohol intake (%kcal) as independent variables and serum HDL cholesterol concentration (mg/dl) as dependent variable. Before the regression analyses, statistical distributions of variables were checked; n-3 PUFA intake for both genders, alcohol intake and number of cigarettes per day for women were skewed with right tails. Results were similar in analyses without and with these variables transformed to logarithms to adjust for skewness. We, therefore, show results from the analyses without this adjustment. Within sample regression coefficients for HDL cholesterol and the dependent variables were averaged (pooled) to yield estimates for the five samples combined, with each sample coefficient weighted by the inverse of its variance [27].

To explore possible dietary confounding factors of n-3 PUFA and HDL cholesterol relation, correlation coefficients between other nutrient factors and n-3 PUFA were checked. Nutrients for which sizable correlations were found ($|r| \geq 0.4$) were added to the multiple linear regression analyses. To explore interaction effect of gender and n-3 PUFA intake on HDL cholesterol, multivariate analyses for the two genders combined (controlled for gender), adding the interaction term (gender $\times$ n-3 PUFA intake), were performed. Significance was set at $p < 0.05$.

SPSS for Windows 9.00 (SPSS Inc., Chicago) was used for the statistical analysis.

3. Results

For both genders, there was no significant difference in height between Japanese in Japan and Japanese in Hawaii, but body weight and BMI were significantly higher in Hawaii than in Japan ($p < 0.001$ for both variables, Table 1). For both genders, smoking rate was higher in Japan than in Hawaii ($p < 0.001$) and male smokers in Japan smoked significantly greater numbers of cigarettes per day than those in Hawaii ($p = 0.006$). Lipid lowering drugs were used by a significantly greater percentage of men and women in Hawaii than in Japan ($p < 0.001$). A significantly greater percentage of women in Japan had reached menopause ($p = 0.004$). A total of 62.5% of postmenopausal women in Hawaii received hormone replacement therapy, whereas only 3.6% of postmenopausal women in Japan did ($p < 0.001$).

Nutrient intakes are shown in Table 2. For men, intakes of total energy, protein and total fat were higher in Hawaii than in Japan ($p = 0.022$, $p < 0.001$ and $p < 0.001$, respectively); men in Japan had higher carbohydrate intake than in Hawaii ($p < 0.001$). For women, there were no significant differences in total energy and protein intake between Japan and Hawaii, but women in Japan consumed more carbohydrate ($p < 0.001$) and less total fat ($p < 0.001$) than women in Hawaii. Both genders in Japan consumed more EPA, DHA, DPA, n-3 PUFA and alcohol and less SFA, MUFA and total PUFA than in Hawaii ($p < 0.001$ for all variables). Keys score was significantly higher in Hawaii than in Japan ($p = 0.003$) for men; there was no significant difference for women ($p = 0.226$).

For further analyses, 67 of the 1348 participants were excluded because they reported taking lipid lowering drugs. Results for serum lipids are shown in Table 3. For men, serum total cholesterol, LDL cholesterol and triglycerides were higher in Hawaii than in Japan ($p < 0.001$ for all variables); HDL cholesterol was higher in Japan than in Hawaii ($p = 0.036$). For women, serum total cholesterol, LDL cholesterol and triglycerides were higher in Hawaii than in Japan ($p = 0.019$, $p < 0.001$ and $p < 0.001$, respectively); there was no significant difference in HDL cholesterol ($p = 0.752$). There was no significant difference in Lp(a) concentration between Japan and Hawaii for either gender.

Results of within sample multiple linear regression analyses with HDL cholesterol as a dependent variable and age, BMI, physical activity index, number of cigarettes per day, alcohol intake, hormone replacement (for women) and n-3 PUFA intake as independent variables are shown in Table 4. There were inverse associations between BMI and HDL cholesterol for all samples. Signs of regression coefficients between physical activity index and HDL cholesterol were positive in all male subgroups and two female subgroups. Number of cigarettes per day and HDL cholesterol were inversely related for all samples. Hormone replacement therapy was positively associated with HDL cholesterol level in three of four samples. Positive associations between alcohol intake and HDL cholesterol were observed for all samples. For men, signs of regression coefficients between n-3 PUFA intake and HDL cholesterol were positive for all samples; for women, positive for two samples and inverse for three.

Pooled regression coefficients from the data for all five samples, separately for men and women, are shown in Table 5. There was a significant inverse relation of BMI with HDL cholesterol for both genders ($p < 0.001$). For men, a border-line positive association ($p = 0.052$) between physical activity index and HDL cholesterol was observed; this association was not observed in women. The relationship between number of cigarettes per day and HDL cholesterol was significantly inverse for both men and women ($p < 0.001$ and $p = 0.014$). There was no significant association between hormone replacement therapy and HDL cholesterol. Alcohol intake was significantly positively correlated with HDL cholesterol for both genders ($p < 0.001$). The relationship of n-3 PUFA intake to HDL cholesterol was significantly positive for men ($p = 0.011$); with n-3 PUFA intake higher by 1% of kcal, e.g., 1.5%kcal versus 0.5%kcal, HDL cholesterol was higher by an estimated 4.6 mg/dl. No association was observed for women ($p = 0.944$).

In the multivariate analyses for the two genders combined (controlled for gender), an overall significant relation between n-3 PUFA intake and HDL cholesterol was observed and there was no significant effect of the gender $\times$ n-3 PUFA intake interaction term (data not shown).

Heterogeneity was tested for all variables included in the multiple linear regression analysis. Significant ($p < 0.05$) heterogeneity for the BMI-HDL cholesterol relation for men was observed in separate regression coefficients across the five samples.

Some fatty acids intakes (eicosenoic acid: 20:1, docosenoic acid: 22:1, 18:1 *trans-fatty* acid, 18:2 *trans-fatty* acid, total *trans-fatty* acid) showed sizable correlation with n-3 PUFA intake ($|r| \geq 0.4$); relation between n-3 PUFA intake and HDL cholesterol did not change when these fatty acids were added to the multiple linear regression analyses.

4. Discussion

The present study found a significant positive relationship between long chain n-3 PUFA intake and serum HDL cholesterol concentration among middle-aged men of Japanese ancestry with adjustment for age, BMI, physical activity index, number of cigarettes per day, and intake of other nutrients and alcohol. In accordance with previous findings [10,14], BMI and cigarette smoking showed significant inverse relations to HDL cholesterol while alcohol intake and physical activity were positive related. For women, neither n-3 PUFA intake nor physical activity index were related to serum HDL cholesterol. However, for women positive significant relationship with alcohol intake and inverse relationships with BMI and cigarettes were similar to the findings for men.

Previous within population cross-sectional studies of fish intake and HDL cholesterol yielded inconclusive findings [10,14,28]. Fehily estimated fish consumption from 7-day weighed dietary records [28]; Yano et al. [14] and Bolton-Smith et al. [10] used the food frequency questionnaire method to estimate fish intake. We assess the dietary data from the present study to be among the most reliable available, derived from perhaps the only dietary database developed to maximize comparability of nutrient data between Japan and the U.S. Four highly standardized 24-h dietary recalls were performed for each participant with multiple quality control procedures [21]. Consistent with our findings for men, several cross-

sectional studies [29,30] reported positive associations between fish intake and serum n-3 PUFA concentration, and serum n-3 PUFA concentration and HDL cholesterol.

Physical activity index was not related to HDL cholesterol in women. Physical activity index was calculated from the answers to questions about number of hours per day spent at six levels of physical activity. Most of the female participants were employed [80.9% and 89.6% in Japan and Hawaii, respectively, either employees or self-employed (data not shown)]. However, women, especially in Japan, are frequently responsible for the majority of cleaning and other such household work. Household work includes many activities that may vary daily, while occupational activities, which may be more likely to be the main activity for male participants, are often more likely to be relatively constant. Thus, there is the possibility that level of physical activity for individual women was difficult to assess accurately by the questions used in this study and a relationship between physical activity and HDL cholesterol was missed. More detailed questionnaires and direct measurement of activity with a pedometer or accelerometer, might aid in examining this relation.

HDL cholesterol was reported to decrease within a few years after menopause in Western countries [15], but this association has not been reported for Japanese women. In the present study, there were no significant associations between age and HDL cholesterol for the Japanese samples (Table 4), where more than 95% of postmenopausal women did not receive hormone replacement. Nor was there any significant difference between average HDL cholesterol levels of pre- and postmenopausal women based on analysis of covariance (data not shown). In Hawaii, where more than half of postmenopausal women received hormone replacement (Table 1), the regression coefficient between age and HDL cholesterol was significantly positive ($p = 0.043$, Table 4), although hormone replacement therapy did not affect HDL cholesterol level ($p = 0.826$, Table 4). Chiba [31] reported that menopause had no effect on HDL cholesterol level in Japanese women. Hormone replacement therapy was found to increase HDL cholesterol in clinical trials both in the U.S. [16] and Japan [17]. Other factors including hormonal changes and dietary factors may have worked to keep HDL cholesterol level relatively stable in perimenopausal women in Japan.

Results of previous studies from Western countries suggest that a small amount of fish intake (one or two meals of fish per week) affords considerable protection from death due to CHD [2] but no similar observations have been reported in Japan where people consume more fish than meat. INTERMAP data suggest that middle-aged Japanese men in Japan consume about 116 g of fish and 63 g of meat per day [26], levels equal to the average intakes of Japanese adult men reported in the National Nutrition Survey in Japan (2001) [32]. Japanese men in INTERMAP obtained 0.45 g of EPA and 0.75 g of DHA from their daily diet (Table 2), less than the amount used in many fish oil supplementation studies [5,7]. Our present findings suggest that moderate n-3 PUFA intake from daily consumption of fish, which is a major feature of the Japanese diet, has a putatively beneficial effect on the serum lipid profile of men. No significant effects of n-3 PUFA on HDL cholesterol were seen in women. However, multivariate analyses for the two genders combined (controlled for gender), indicated an overall significant relation between n-3 PUFA intake and HDL cholesterol. Addition of an interaction term (gender $\times$ n-3 PUFA) yielded evidence for no significant interaction across the two genders. Thus, the non-significant result for women may be a

chance finding. CHD mortality for Japanese women continues to be lower than for women in Western countries [12]. The effects of n-3 PUFA intake on CHD risk factors in women should be further explored.

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Anthropometry measurement and number of cigarettes per day. Mean (S.D.): INTERLIPID study, Japan and Hawaii, 1996–1998

Table 1

	Toyama	Sapporo	Aito-Town	Wakayama	^a <i>p</i>	Japan total	Hawaii	^b <i>p</i>
Men	<i>n</i> = 149	<i>n</i> = 149	<i>n</i> = 129	<i>n</i> = 145		<i>n</i> = 572	<i>n</i> = 100	
Age (year)	49.7 (4.8)	49.5 (5.1)	49.4 (6.1)	49.4 (5.3)	0.951	49.5 (5.3)	50.6 (5.2)	0.073
Height (m)	1.67 (0.06)	1.69 (0.06)	1.67 (0.06)	1.69 (0.05)	0.001	1.68 (0.06)	1.69 (0.06)	0.307
Weight (kg)	65.5 (8.9)	69.0 (8.6)	65.4 (9.0)	67.4 (8.3)	0.001	66.9 (8.8)	80.0 (14.7)	<0.001
BMI (kg/m ²)	23.6 (2.8)	24.1 (2.5)	23.4 (2.7)	23.7 (2.7)	0.102	23.7 (2.7)	28.5 (4.6)	<0.001
Smokers (%)	59.1	38.9	50.0	58.9	0.001	51.7	13.2	<0.001
Number of cigarettes (per day) in smokers	21.4 (9.3)	19.7 (7.9)	24.2 (9.3)	24.9 (10.0)	0.003	22.7 (9.4)	14.2 (10.6)	0.006
Number of cigarettes (per day) including non-smokers	12.6 (12.7)	7.7 (10.8)	12.2 (13.8)	14.6 (14.5)	<0.001	11.7 (13.2)	1.4 (5.3)	<0.001
Physical activity index	31.8 (6.1)	27.7 (2.4)	37.4 (12.2)	31.3 (4.7)	<0.001	31.9 (0.3)	32.0 (6.9)	0.894
Lipid lowering drug use (%)	2.7	2.7	4.6	2.7	0.750	3.1	18.4	<0.001
Women	<i>n</i> = 150	<i>n</i> = 148	<i>n</i> = 129	<i>n</i> = 143		<i>n</i> = 570	<i>n</i> = 106	
Age (year)	49.1 (4.6)	49.6 (5.2)	49.9 (6.2)	48.4 (5.2)	0.134	49.2 (5.3)	49.8 (4.9)	0.331
Height (m)	1.54 (0.05)	1.54 (0.06)	1.54 (0.06)	1.56 (0.06)	0.001	1.55 (0.06)	1.54 (0.05)	0.062
Weight (kg)	55.6 (8.0)	55.5 (8.2)	55.6 (7.9)	55.4 (8.1)	0.997	55.5 (8.0)	60.8 (12.6)	<0.001
BMI (kg/m ²)	23.4 (3.2)	23.2 (3.0)	23.4 (3.0)	22.7 (3.0)	0.131	23.2 (3.1)	25.7 (5.4)	<0.001
Smokers (%)	3.3	18.9	1.6	9.7	<0.001	8.6	4.6	0.082
Number of cigarettes (per day) in smokers	3.2 (2.0)	13.8 (6.3)	2.0 (1.4)	11.2 (6.7)	0.002	11.5 (7.0)	13.0 (7.6)	0.646
Number of cigarettes (per day) including non-smokers	0.1 (0.7)	2.6 (6.0)	0.0 (0.3)	1.1 (3.9)	<0.001	1.0 (3.8)	0.6 (3.1)	0.343
Physical activity index	32.4	28.6	45.3	30.9	0.002	33.9	29.4	0.263
Lipid lowering drug use (%)	2.7	6.1	3.9	0.7	0.075	3.3	9.9	0.001
Postmenopause (%)	45.3	49.3	49.6	33.3	0.018	44.3	30.5	0.004
Hormone replacement use (%)	4.4	8.2	0.0	0.0	0.031	3.6	62.5	<0.001

BMI, body mass index.

^a Obtained by analysis of variance among four Japanese centers.

^b Obtained by *t*-test between Japan and Hawaii.

Table 2

Nutrient intake from dietary survey, mean of four 24-h dietary recalls. Mean (S.D.): INTERLIPID study, Japan and Hawaii, 1996–1998

	Toyama	Sapporo	Aito-Town	Wakayama	^a <i>p</i>	Japan total	Hawaii	^b <i>p</i>
Men	<i>n</i> = 149	<i>n</i> = 149	<i>n</i> = 129	<i>n</i> = 145		<i>n</i> = 572	<i>n</i> = 100	
Total energy (kcal/day)	2286.8 (414.7)	2176.7 (373.8)	2395.3 (517.3)	2272.0 (378.0)	<0.001	2278.8 (427.3)	2427.1 (612.6)	0.022
Protein (%kcal)	15.8 (2.3)	16.5 (2.2)	15.4 (2.3)	15.4 (2.2)	<0.001	15.8 (2.3)	17.2 (2.9)	<0.001
Carbohydrate (%kcal)	53.3 (7.1)	50.5 (7.8)	56.0 (7.6)	49.8 (6.9)	<0.001	52.3 (7.7)	48.1 (7.6)	<0.001
Total fat (%kcal)	23.6 (5.1)	24.6 (4.8)	21.7 (4.6)	24.5 (3.9)	<0.001	23.7 (4.8)	31.9 (6.3)	<0.001
SFA (%kcal)	5.9 (1.5)	6.3 (1.8)	5.6 (1.5)	6.4 (1.3)	<0.001	6.1 (1.6)	9.1 (2.2)	<0.001
MUFA (%kcal)	8.3 (2.2)	9.1 (2.2)	7.7 (1.9)	9.1 (1.7)	<0.001	8.6 (2.1)	11.9 (2.8)	<0.001
PUFA (%kcal)	6.6 (1.6)	6.2 (1.5)	5.8 (1.4)	6.2 (1.4)	<0.001	6.2 (1.5)	7.5 (1.8)	<0.001
n-3 PUFA (%kcal) ^c	0.54 (0.3)	0.60 (0.3)	0.45 (0.2)	0.49 (0.2)	<0.001	0.52 (0.3)	0.18 (0.17)	<0.001
n-3 PUFA (g/day)	1.37 (0.88)	1.43 (0.88)	1.22 (0.72)	1.24 (0.66)	0.067	1.32 (0.8)	0.47 (0.45)	<0.001
EPA (g/day)	0.47 (0.34)	0.50 (0.33)	0.41 (0.27)	0.41 (0.25)	<0.001	0.45 (0.30)	0.14 (0.15)	<0.001
DHA (g/day)	0.78 (0.47)	0.80 (0.47)	0.70 (0.38)	0.71 (0.36)	0.135	0.75 (0.43)	0.27 (0.27)	<0.001
DPA (g/day)	0.12 (0.09)	0.13 (0.09)	0.11 (0.07)	0.12 (0.07)	0.481	0.12 (0.1)	0.06 (0.05)	<0.001
Keys score	27.5 (5.6)	30.4 (6.6)	27.5 (6.1)	29.4 (5.0)	<0.001	28.7 (5.9)	31.2 (7.6)	0.003
Alcohol (%kcal)	7.3 (6.7)	8.3 (7.8)	6.8 (6.3)	10.2 (7.4)	<0.001	8.2 (7.2)	2.8 (4.6)	<0.001
Women	<i>n</i> = 150	<i>n</i> = 148	<i>n</i> = 129	<i>n</i> = 143		<i>n</i> = 570	<i>n</i> = 106	
Total energy (kcal/day)	1813.9 (296.5)	1732.9 (320.5)	1878.7 (367.1)	1771.0 (293.0)	0.001	1796.7 (322.7)	1765.3 (391.0)	0.437
Protein (%kcal)	16.1 (1.9)	16.4 (2.6)	16.1 (2.3)	15.9 (2.3)	0.270	16.1 (2.3)	16.6 (3.0)	0.171
Carbohydrate (%kcal)	57.8 (5.2)	54.1 (7.4)	58.3 (5.8)	54.6 (5.6)	<0.001	56.2 (6.4)	50.9 (8.7)	<0.001
Total fat (%kcal)	25.2 (4.3)	26.8 (5.4)	24.8 (4.6)	27.7 (4.6)	<0.001	26.1 (4.9)	31.9 (7.7)	<0.001
SFA (%kcal)	6.6 (1.5)	7.2 (2.0)	6.7 (1.7)	7.8 (1.8)	<0.001	7.1 (1.8)	9.4 (2.7)	<0.001
MUFA (%kcal)	8.8 (1.9)	9.8 (2.4)	8.8 (2.0)	10.2 (2.0)	<0.001	9.4 (2.2)	11.7 (3.6)	<0.001
PUFA (%kcal)	6.9 (1.4)	6.5 (1.5)	6.4 (1.2)	6.6 (1.5)	0.023	6.6 (1.4)	7.5 (2.3)	<0.001
n-3 PUFA (%kcal)	0.50 (0.3)	0.55 (0.3)	0.47 (0.3)	0.43 (0.3)	0.006	0.49 (0.3)	0.14 (0.2)	<0.001
n-3 PUFA (g/day)	1.00 (0.57)	1.07 (0.73)	0.98 (0.61)	0.84 (0.52)	0.016	0.97 (0.6)	0.26 (0.3)	<0.001
EPA (g/day)	0.34 (0.21)	0.37 (0.28)	0.32 (0.22)	0.27 (0.19)	0.003	0.32 (0.23)	0.08 (0.09)	<0.001
DHA (g/day)	0.57 (0.31)	0.61 (0.39)	0.57 (0.34)	0.50 (0.29)	0.037	0.56 (0.33)	0.15 (0.16)	<0.001
DPA (g/day)	0.09 (0.06)	0.10 (0.08)	0.09 (0.06)	0.08 (0.05)	0.091	0.09 (0.07)	0.03 (0.03)	<0.001

	Toyama	Sapporo	Aito-Town	Wakayama	^a <i>p</i>	Japan total	Hawaii	^b <i>p</i>
Keys score	28.4 (5.5)	32.6 (6.8)	30.3 (6.6)	32.7 (6.1)	<0.001	31.0 (6.5)	32.2 (9.5)	0.226
Alcohol (%kcal)	0.8 (2.0)	2.6 (4.8)	0.7 (1.7)	1.9 (2.8)	<0.001	1.5 (3.2)	0.6 (1.8)	<0.001

SFA, saturated fatty acids; MUFA, mono unsaturated fatty acids; PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA docosapentaenoic acid.

^aObtained by analysis of variance among four Japanese centers.

^bObtained by *t*-test between Japan and Hawaii.

^cCalculated as total EPA, DHA and DPA.

Table 3

Serum lipids. Mean (S.D.): INTERLIPID study, Japan and Hawaii, 1996–1998

	Toyama	Sapporo	Aito-Town	Wakayama	^a <i>p</i>	Japan total	Hawaii	^b <i>p</i>
Men	<i>n</i> = 145	<i>n</i> = 145	<i>n</i> = 123	<i>n</i> = 141		<i>n</i> = 554	<i>n</i> = 81	
Total cholesterol (mg/dl)	198.7 (29.3)	196.5 (26.6)	202.1 (28.5)	197.7 (28.3)	0.420	198.6 (28.2)	211.6 (29.3)	<0.001
HDL cholesterol (mg/dl)	54.3 (13.3)	52.9 (14.8)	54.0 (13.9)	54.5 (12.7)	0.758	53.9 (13.7)	51.2 (10.4)	0.036
LDL cholesterol (mg/dl)	122.5 (28.6)	109.8 (26.5)	124.6 (27.1)	125.6 (28.0)	<0.001	120.4 (28.2)	137.9 (25.8)	<0.001
Triglycerides (geometric mean, mg/dl)	132.2 (1.7)	125.6 (1.7)	138.6 (1.8)	132.5 (1.8)	0.554	131.9 (1.7)	186.6 (1.8)	<0.001
Lp(a) (geometric mean, U/l)	8.3 (2.3)	8.4 (2.2)	9.5 (2.2)	9.9 (2.2)	0.185	9.0 (2.2)	8.6 (2.6)	0.729
Women	<i>n</i> = 146	<i>n</i> = 139	<i>n</i> = 124	<i>n</i> = 142		<i>n</i> = 551	<i>n</i> = 95	
Total cholesterol (mg/dl)	200.3 (33.5)	202.2 (28.7)	203.4 (30.2)	202.7 (31.7)	0.853	202.1 (31.1)	210.2 (31.5)	0.019
HDL cholesterol (mg/dl)	59.5 (12.2)	59.9 (15.1)	61.1 (16.3)	60.6 (13.4)	0.791	60.2 (14.2)	59.7 (13.5)	0.752
LDL cholesterol (mg/dl)	125.3 (30.3)	115.6 (29.6)	123.8 (28.5)	129.9 (29.8)	0.001	123.7 (30.0)	136.1 (33.4)	<0.001
Triglycerides (geometric mean, mg/dl)	94.2 (1.6)	97.0 (1.7)	95.3 (1.6)	100.2 (1.6)	0.725	96.7 (1.6)	133.8 (1.7)	<0.001
Lp(a) (geometric mean, U/l)	9.6 (2.3)	10.7 (2.3)	11.7 (2.3)	9.7 (2.4)	0.193	10.3 (2.3)	11.8 (2.4)	0.161

Blood was drawn in non-fasting state. HDL cholesterol, high density lipoprotein cholesterol; LDL cholesterol, low density lipoprotein cholesterol; Lp(a), lipoprotein(a).

^aObtained by analysis of variance among: four Japanese centers.^bObtained by *t*-test between Japan and Hawaii.

Table 4

Results of multiple linear regression analysis with HDL cholesterol (mg/dl) as dependent variable and age, BMI, physical activity index, number of cigarettes per day, hormone replacement, alcohol intake, and n-3 PUFA intake as independent variables in five centers, unstandardized regression coefficients and standard errors. INTERLIPID study, Japan and Hawaii, 1996–1998

	Age (years) B (S.E.) ^a <i>p</i>	BMI (kg/m ²) B (S.E.) ^a <i>p</i>	Physical activity index B (S.E.) ^a <i>p</i>	Num of cigarettes (per day) B (S.E.) ^a <i>p</i>	Hormone replacement (no = 0, yes = 1) B (S.E.) ^a <i>p</i>	Alcohol intake (%kcal) B (S.E.) ^a <i>p</i>	n-3 PUFA ^a (%kcal) B (S.E.) ^a <i>p</i>
Men							
Toyama	-0.005 (0.184) 0.978	-1.738 (0.310) <0.001	0.134 (0.149) 0.368	-0.112 (0.072) 0.124	-	0.971 (0.138) <0.001	5.966 (2.991) 0.048
Sapporo	0.104 (0.232) 0.656	-2.130 (0.457) <0.001	0.892 (0.461) 0.055	-0.179 (0.104) 0.088	-	0.431 (0.146) 0.004	2.244 (3.499) 0.522
Aito-town	0.066 (0.197) 0.739	-1.833 (0.438) <0.001	0.086 (0.096) 0.370	-0.113 (0.086) 0.191	-	0.732 (0.189) <0.001	5.626 (5.146) 0.277
Wakayama	-0.167 (0.185) 0.370	-1.353 (0.361) <0.001	0.091 (0.227) 0.688	-0.191 (0.070) 0.007	-	0.599 (0.134) <0.001	5.091 (4.182) 0.226
Hawaii	0.054 (0.226) 0.814	-0.661 (0.237) 0.007	0.189 (0.167) 0.262	-0.341 (0.215) 0.117	-	0.760 (0.223) 0.001	3.012 (7.446) 0.687
Women							
Toyama	-0.018 (0.232) 0.937	-1.134 (0.342) 0.001	-0.199 (0.239) 0.406	-1.714 (1.505) 0.257	18.835 (7.948) 0.019	0.637 (0.493) 0.199	4.478 (3.931) 0.257
Sapporo	0.164 (0.241) 0.496	-1.519 (0.432) 0.001	0.222 (0.461) 0.631	-0.266 (0.203) 0.193	0.482 (5.906) 0.935	1.026 (0.255) <0.001	-4.472 (3.628) 0.220
Aito-town	-0.048 (0.240) 0.842	-1.142 (0.587) 0.054	-0.010 (0.018) 0.591	-5.927 (5.187) 0.255	-	0.173 (0.856) 0.841	9.614 (5.495) 0.083
Wakayama	-0.275 (0.211) 0.193	-0.954 (0.345) 0.007	-0.448 (0.292) 0.128	-0.555 (0.274) 0.044	-0.209 (8.717) 0.811	1.782 (0.389) <0.001	-3.402 (4.123) 0.411
Hawaii	0.601 (0.293) 0.043	-1.111 (0.230) <0.001	0.192 (0.384) 0.617	-0.243 (0.364) 0.506	0.723 (3.286) 0.826	2.075 (0.654) 0.002	-6.281 (7.799) 0.423

PUFA, polyunsaturated fatty acids; B, unstandardized regression coefficient; S.E., standard error.

^aCalculated as total EPA, DHA and DPA.

Table 5

Results from pooled regression analysis with HDL cholesterol (mg/dl) as dependent variable and age, BMI, physical age, BMI, physical activity index, number of cigarettes per day, hormone replacement, alcohol intake, and n-3 PUFA intake as independent variables: INTERLIPID study, Japan and Hawaii, 1996–1998

	Age (year)	BMI (kg/m ²)	Physical activity index	Num of cigarettes (per day)	Hormone replacement (no = 0, yes = 1)	Alcohol (%kcal)	n-3 PUFA ^a (%kcal)
Men (<i>n</i> = 635)							
Regression coefficient (S.E.)	−0.003 (0.090)	−1.308 (0.147)	0.132 (0.068)	−0.154 (0.039)	–	0.690 (0.070)	4.595 (1.807)
<i>z</i> -score	0.030	8.897	1.936	3.919		9.836	2.543
<i>p</i>	0.976	<0.001	0.052	<0.001		<0.001	0.011
Women (<i>n</i> = 646)							
Regression coefficient (S.E.)	0.028 (0.107)	−1.138 (0.150)	−0.012 (0.018)	−0.366 (0.148)	2.339 (2.580)	1.183 (0.183)	−0.140 (2.004)
<i>z</i> -score	0.265	7.584	0.656	2.470	0.907	6.466	0.070
<i>p</i>	0.795	<0.001	0.516	0.014	0.368	<0.001	0.944

BMI, body mass index; PUFA, polyunsaturated fatty acids; S.E., standard error.

^aCalculated as total EPA, DHA and DPA.