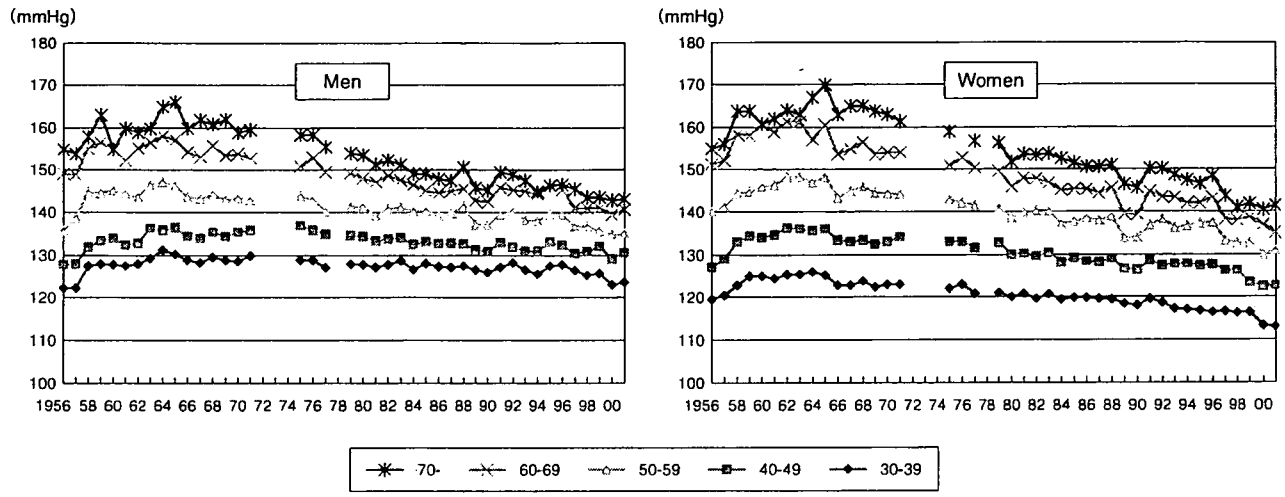

Fig. 5. Trends in systolic and diastolic blood pressures for men and women in Japan, 1956-2001.

Systolic and diastolic blood pressure levels for men and women have declined substantially since around 1965, whereas the rate of stroke mortality was highest in Japan. In particular, those in men and women aged over 60 years were much higher and evident compared to those for men and women in their 30s to 50s. The lowering trend in systolic and diastolic blood pressure has slowed down since around 1990 and is compatible with that of the similar slowdown phenomenon of stroke mortality.

rate in Japan would result in about a 1.3% reduction in stroke[21]; therefore, it is reasonably concluded than the recent decline in the smoking rate in men contributed to the decline in stroke mortality and incidence.

Serum total cholesterol is not a risk factor for stroke, either cerebral hemorrhage or cerebral infarction, in Japan[29, 33-37] because most incidents of cerebral infarction in Japan are caused by hypertension and smoking[11, 21-30, 33]. Although atherosclerosis of large vessels in the brain is caused by hypercholesterolemia[38], the proportion caused by hypercholesterolemia is quite low in Japan[33].

The most important risk factors for CHD are hypertension, hypercholesterolemia, smoking and diabetes mellitus[21, 29]. These are no different from the findings in the USA and European countries[39].

Trends in Population Blood Pressure Level and the Prevalence of Hypertension

The National Nutrition Survey of Japan has been conducted since the Second World War for the purpose of monitoring the population's nutrition intake through the random selection of families in government statistical areas throughout Japan. Blood pressure measurement was also introduced in 1956 in this National Nutrition Survey of about 10,000 men and women in randomly selected families[40]. Japan is an exceptional country in the world for monitoring its population's blood pressure level as well as for the prevalence of high blood pressure during the long period since 1956[3-5, 40].

Fig. 5 shows the trend in systolic blood pressure (SBP) level for men and women in 10-year age groups. The figures for both men and women show that SBP levels have declined since around 1965 for men aged 50 years and older and for women in all age groups. The level in men aged 40-49 and 30-39 has also declined since 1965. These declining trends are compatible with the declining trends in age-adjusted stroke mortality, that is, stroke mortality has also declined since 1965[4] (**Fig. 1**). For men aged 60-69, SBP declined by around 15 mmHg during 1965-1990[5].

The prevalence of severe hypertension, defined as SBP≧180 mmHg, in men and women has also declined since 1965 and shows almost a similar pattern to that of SBP[4]. For example, the prevalence of severe hypertension in men aged 60-69 was around 21% in 1965 and only 4% in 1990.

Trends in Smoking Rate

The average smoking rate in men was 82.3% in 1965, which had decreased by 45.5% in 2005[41]. The absolute decline in the smoking rate in men during 1965-1990 was around 20% (**Fig. 6**). The smoking rate in men in all age groups is continuously declining. In particular, the smoking rate in elderly men aged 60 years and over has declined considerably from 74.6% in 1965 to 49.4% in 1990 and to 31.4% in

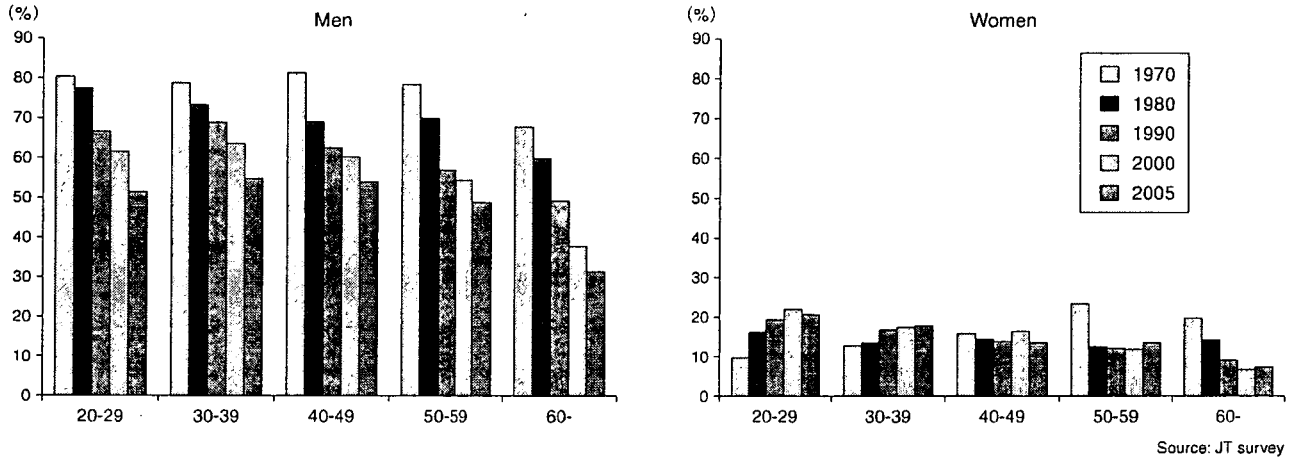

Fig. 6. Trend in smoking rate for men and women by 10-year age groups in Japan, 1970-2005.

The smoking rate in Japanese men was very high at around 80%, except for men aged 60 years and older, in 1970, while that of Japanese women was far lower. The high smoking rate in men has declined considerably, especially in men aged 60 years and over. On the other hand, that for young women aged 20-29 years has increased to 20%.

2005[41]. For women, the smoking rate is generally low in all age groups and that of elderly women aged 60 years and older was 23.0% in 1965, 9.4% in 1990 and 5.5% in 2005[41]. Therefore, the absolute decline in the smoking rate in men and women aged 60-69 during the years 1965-1990 was 15.2% and 13.6%, respectively.

Trends in Serum Total Cholesterol Level

Serum total cholesterol increased substantially in Japan following an increase in dietary fat intake. The daily dietary fat intake for adults in 1950 was around 10% kcal of total energy intake; however, it increased greatly to around 25% kcal in 1990[3, 4, 40, 42].

The Seven Countries Study recorded an average serum total cholesterol level of around 160 mg/dL in 1956 for men in Ushibuka and Tanushimaru in Kyushu, and around 250 mg/dL for men in Kuopio, Finland[43, 44]. The Cerebro-Cardiovascular Survey in 1980 and 1990 of a representative Japanese population showed a serum total cholesterol of around 190 mg/dL and 200 mg/d, respectively, in middle-aged men[5, 42] (**Fig. 7**). These national trends were compatible with the findings of a review paper on serum total cholesterol for many cohort studies in Japan[45]. The increasing trend in serum total cholesterol stems from the increase in dietary fat intake in populations since Keys's dietary factor[46] defined by fat intake in Japanese populations was confirmed to be well correlated with serum total cholesterol in populations[46]. Furthermore, the recent slowdown of dietary fat intake in

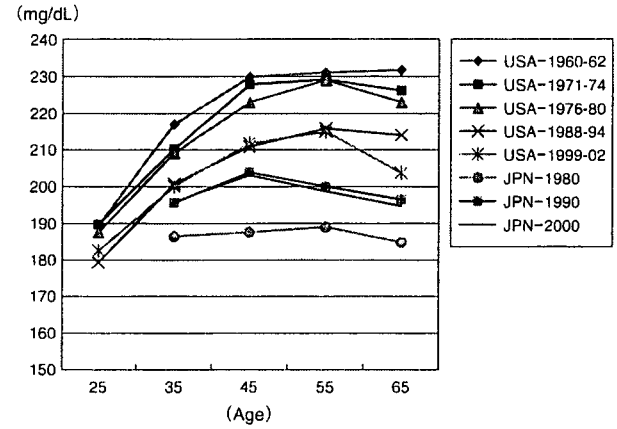

Fig. 7. Trend in serum total cholesterol for Japanese men compared with Americans.

Serum total cholesterol levels in Japan increased during 1980-1990, while those in America decreased gradually during around 1960-1980. In the 1980s, there was a 40 mg/dL difference between Japanese and American men aged 50-59 years, but this difference had lowered to 15 mg/dL by 1990. For men aged 30-49 years, the difference in serum total cholesterol level was around 5-8 mg/dL. There were no changes in Japanese men and American men during 1990-2000, except for American men aged 60 years and over. The data on American men aged 65 is for men aged 60 years and older, but for Japanese men it is for 60-69 years.

Japanese populations[42] is also compatible with the halt in the increase of serum total cholesterol level with some effect from treatment for hypercholesterolemia in Japanese populations[5].

Possible Explanation for the Decline in Stroke and Coronary Heart Disease

Hypertension and smoking are potent risk factors for CHD and stroke[11, 21-37]. The higher the population blood pressure, the higher the risk of CHD and stroke[21-24, 29]. As estimated in "Health Japan 21", if the population SBP were lowered by 2 mmHg, the estimated reduction in CHD and stroke would be 4.8% and 6.4%, respectively, based on Japanese cohort studies[21]. Similarly, a 1% reduction in the smoking rate is estimated to result in a 1.3% decrease in CHD and stroke[21].

Therefore, it is reasonably concluded that a reduction in population blood pressure level and also a substantial reduction in the prevalence of severe hypertension contributed greatly to the decline in CHD mortality as well as stroke[3-5]. Since the average blood pressure reduction in men aged 30-69 was around 7.4 mmHg and smoking rate reduction was approximately 20% during the years 1965-1990, it is expected that CHD and stroke reduction would be 44% and 50%, respectively. The actual reduction in CHD and stroke mortality for men aged 30-69 was 51% and 79%, respectively, during the same period. Therefore, more than 80% of the observed reduction in CHD mortality for men aged 30-69 can be explained by the decrease in population blood pressure level and smoking rate. Similarly, 63% of the reduction in stroke mortality can be explained by the same factors.

It is true in Japanese populations that serum total cholesterol is a risk factor for CHD[21, 29, 35, 36]; therefore, the increase in population serum total cholesterol level directly contributes to increasing CHD in the Japanese population. However, the increase in serum total cholesterol appears mainly in young to middle-aged populations[5] (**Fig. 7**), and its adverse effects may surface in later years. In contrast, elderly people as a high risk group for CHD continue to maintain a level lower than 200 mg/dL, similar to their lower level in the past[5]. In addition, there was also a 5-15 mg/dL difference in serum total cholesterol level between middle-aged to elderly men in Japan and the USA[5] (**Fig. 7**); therefore, the adverse influence of raised total cholesterol on CHD in the elderly is considered to be overcome by the reduction in both population blood pressure and smoking rate[3, 5]. In addition, the increase in serum total cholesterol is not related to the risk of stroke[21, 29, 35, 36]; it had no effect on stroke mortality reduction. It is also worth noting that the elderly in the USA in 1990-2000 had a higher serum total cholesterol level when they were younger in 1960-70 than when they were older (**Fig. 7**).

Trends in Diabetes Mellitus

The trend in the prevalence of diabetes mellitus (DM) is not precisely known, especially for age-adjusted and/or age-specific data. DM prevalence was estimated for half the subjects of the National Nutrition Survey in 1997 and 2002[47]. Age-specific prevalence of DM did not differ greatly except for elderly people aged 70 years and over; however, with the rapidly aging Japanese population, we have not determined the extent to which the prevalence of DM in the age group of 70 years and older increased in the past 5 years[47]. Although conclusive data are not available to confirm any trend in DM prevalence over the past three decades, it is reasonably estimated that DM prevalence in Japan increased somewhat following BMI increase in men and elderly women. It is well known that DM and glucose intolerance are a risk factor for stroke and CHD in Japan; i.e., relative risk is 2-3[29, 48-51]. Therefore, the increasing prevalence of DM and glucose intolerance may contribute in part to an increased adverse influence on stroke and CHD.

Conclusions

A significant reduction in stroke mortality and incidence has been achieved since 1965 as well as the prevention of increased CHD mortality and incidence. This phenomenon stems from the reduction in population SBP and the smoking rate. On the other hand, although the serum cholesterol level in Japanese people in both genders and all age groups increased greatly following the increase in dietary fat intake, it is reasonably concluded that its adverse effects on CHD were overcome by the decline in SBP level and the smoking rate in men and women. Nevertheless, the present younger generation with a higher serum total cholesterol level compared to that of past young generations, may face higher CHD incidence and mortality in the future. Therefore, we should carefully monitor the new generations as well as the general population for CHD incidence[14-16, 51, 52].

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Original Article

Association of Lipoprotein-associated Phospholipase A2 with Coronary Calcification among American and Japanese Men

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BACKGROUND: We have previously reported that the prevalence of coronary artery calcification (CAC) was substantially lower among Japanese than American men despite a less favorable profile of many traditional risk factors in Japanese men. To determine whether lipoprotein-associated phospholipase A2 (Lp-PLA2) levels are related to the difference in the prevalence of CAC between the two populations.

METHODS: A total of 200 men aged 40-49 years were examined: 100 residents in Allegheny County, Pennsylvania, United States, and 100 residents in Kusatsu City, Shiga, Japan. Coronary calcium score (CCS) was evaluated by electron-beam tomography, Lp-PLA2 levels, nuclear magnetic resonance (NMR) lipoprotein subclasses, and other factors were assessed in 2001-2002.

RESULTS: Lp-PLA2 levels were higher among American than Japanese men (Mean ± standard deviation 301.7 ± 82.6 versus 275.9 ± 104.7 ng/mL, respectively, p=0.06). Among all Japanese men and those with low density lipoprotein (LDL) cholesterol ≥130 mg/dL, there was an inverse association of the prevalence of CCS>0 with the tertile groups of Lp-PLA2 levels (p=0.08 and p=0.03, respectively). American men did not have any association between CCS>0 with the tertile groups of Lp-PLA2 (p=0.62). Although Lp-PLA2 among both populations correlated positively with LDL and total cholesterol, American and Japanese men had different correlations with NMR lipoprotein subclasses. Reported high odds ratio for CCS>0 among American compared to Japanese men was not reduced after adjusting for Lp-PLA2 levels.

CONCLUSION: Lp-PLA2 may have different mechanisms of action among American and Japanese men. Lp-PLA2 levels can not explain the observed CAC differences between the two populations.

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Key words: Atherosclerosis, 1-Alkyl-2-acetylglycerophosphocholine Esterase, Coronary Arteriosclerosis, Calcification, Asian Continental Ancestry Group, European Continental Ancestry Group.

Lipoprotein-associated phospholipase A2 (Lp-PLA2) is a subtype of the phospholipase A2 superfamily, a family of enzymes that hydrolyze phospholipids, and is secreted by cells of the monocyte–macrophage series, T-lymphocytes, and mast cells. The biological role of Lp-PLA2 has been controversial with seemingly contradictory pro-atherogenic and anti-atherogenic[1-3] functions being proposed.

Identifying racial differences in the association of Lp-PLA2 with coronary artery calcification (CAC) or coronary artery disease (CAD) can help to identify populations who can potentially reduce future cardiovascular events by therapeutically lowering baseline Lp-PLA2 levels or by inhibiting Lp-PLA2 activity.

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Among the Japanese, an inherited deficiency of Lp-PLA2 was reported to be associated with CAD and stroke[4-7]. However, this mutation has not been found among Caucasians.[8] Among Caucasians, Lp-PLA2 was reported to be an independent risk factor for CAD and stroke in many,[9-14] but not all, studies.[15-17] Data assessing the relationship of CAC with Lp-PLA2 are very limited among Caucasians[18,19] and there are no data, to our knowledge, among the Japanese.

We have previously reported that the prevalence of CAC was significantly lower among Japanese than white American men aged 40-49 (13% versus 47%, P<0.001), despite a less favorable profile of many traditional risk factors in the Japanese,[20] which might imply that there are strong protective factors against atherosclerosis among the Japanese. This Japanese paradox is most probably unexplained by genetic differences between the Japanese and Americans as rates of CHD among Japanese Americans living in the United States (US) are much higher than among the Japanese living in Japan.[21] We examined 200 American and Japanese men. The aim of the study was to compare CAC and risk factors between American men (a high CHD population) and Japanese men (a low CHD population) born after World War II who adopted a western lifestyle. We hypothesized that (1) levels of Lp-PLA2 are much higher among American men, and (2) the higher Lp-PLA2 levels among American men partly explain the higher prevalence of CAC among American men compared to Japanese men.

METHODS

Study Population

After obtaining informed consent forms, a total of 200 men aged 40-49 years were examined between 2001 and 2002 as previously described:[20] 100 American residents in Allegheny County, Pennsylvania, US, and 100 residents in Kusatsu City, Shiga, Japan.

In Allegheny County, subjects voluntarily participated in the study after it was announced with the eligibility criteria through the University of Pittsburgh Medical Center (UPMC) Health Plan. Out of the 100 American subjects, 99 were Caucasians. In Kusatsu City, subjects were randomly selected from the Basic Residents' Register. The participation rate was 49%. Exclusion criteria were (1) clinical cardiovascular disease, (2) type 1 diabetes, (3) cancer except skin cancer in the past two years, (4) renal failure, and (5) genetic familial hyperlipidemias. The study protocol was approved by the Institutional Review Boards (IRB) of the University of Pittsburgh, Pittsburgh, Pennsylvania, US, and Shiga University of Medical Science, Otsu, Japan.

CAC and Lp-PLA2 Assessment

As previously described,[20] electron-beam tomography (EBT) scanning was done using a GE-Imatron C150 Electron Beam Tomography scanner (GE Medical Systems, South San Francisco, US) at both sites. Readings of the scanning were done centrally at the Cardiovascular Institute, Pittsburgh, Pennsylvania, US, using the widely accepted Agatston scoring method.[22] The reproducibility of the EBT scans had an intraclass correlation of 0.99.

Lp-PLA2 levels among both populations were centrally measured using an enzyme-linked immunoabsorbent assay (PLAC test®, diaDexus Inc, South San Francisco, California) on fasting plasma samples that were stored frozen at -80℃. The lowest standard limit of detection is 1.3 ng/mL. The inter-assay coefficient of variation was 6.3% and the intra-assay coefficient of variation was 4.3%.

Assessment of Other Risk Factors

A self-administered questionnaire was used to obtain information on demography, smoking habits, alcohol consumption, and other factors. Body mass index (BMI) was calculated as weight (kg)/height squared (m^2). Waist circumference was measured at the level of the umbilicus while participant was standing erect. Blood pressure was measured in the right arm of seated participants after the participant emptied his bladder and sat quietly for 5 minutes using a standard mercury sphygmomanometer.

Analysis of frozen samples from the US and Japan were done centrally at US laboratories. Fasting serum lipids, glucose, and insulin were measured at the Heinz Laboratory, Department of Epidemiology, University of Pittsburgh. Serum lipids were measured with standardized methods according to the Centers for Disease Control and Prevention. C-reactive protein (CRP) was measured at the Laboratory for Clinical Biochemistry Research, University of Vermont, by a calorimetric competitive enzyme-linked immunosorbent assay. Lipoprotein subclasses, sizes, and the particle numbers were determined by nuclear magnetic resonance (NMR) spectroscopy at Lipo-Scienec Laboratory, Raleigh, North Carolina.

Statistical Analysis

One Japanese participant with an almost zero level of Lp-PLA2 was excluded from the analysis due to a possible genetic Lp-PLA2 deficiency. Two Japanese participants without appropriate EBT images and four Japanese participants without Lp-PLA2 data were also excluded from the analysis, resulting in 100 Americans and 93 Japanese. Normally distributed continuous variables were expressed as means ± standard deviation (SD) and were compared using a two-sample t test. Highly skewed continuous variables (CRP and triglycerides) are expressed as median and inter-quartile range and were compared using the Mann-Whitney U test. Categorical data were expressed in percentages and were compared using the Chi-square statistic (or Fisher's exact test when indicated). Correlations of Lp-PLA2 with other continuous variables were calculated using Pearson's (normal data) or Spearman's correlation (skewed data). Correlations of Lp-PLA2 with NMR variables were calculated using Spearman's correlation. All tests were two-tailed and were considered significant if $p<0.05$.

Because the distribution of coronary calcium score (CCS) was

very skewed, the data were analyzed in a categorical form (0 and >0). To compare CAC between American and Japanese men by Lp-PLA2 level, the prevalent CCS>0 was compared among race-specific Lp-PLA2 tertile groups. To test whether Lp-PLA2 levels can explain the observed differences in CAC prevalence between American and Japanese men, odds ratios for CCS>0 among American compared to Japanese men were calculated using logistic regression models after adjustment for age (model 1), age and Lp-PLA2 levels (model 2), age and conventional risk factors (model 3), and age, conventional risk factors, and Lp-PLA2 levels (model 4). SPSS® software (release 14.0, SPSS Inc., Chicago, US) was used for all statistical analyses.

RESULTS

Lp-PLA2 levels were higher among American men (301.7 ±82.6 ng/mL) than Japanese men (275.9 ± 104.7 ng/mL); the difference was marginally significant (p=0.060, Table 1).

Lp-PLA2 levels were negatively correlated to waist circumferences in both American and Japanese men but significant only in American men. Lp-PLA2 levels among both populations had significant positive correlations with both total cholesterol (Americans r=0.37, p<0.001; Japanese r=0.30, p=0.004) and low density lipoprotein (LDL) cholesterol (Americans r=0.44, p<0.001; Japanese r=0.30, p=0.003). However, high density lipoprotein (HDL) cholesterol levels did not correlate with Lp-PLA2 levels among either American (p=0.37) or Japanese men (p=0.50). Total and small very low density lipoprotein (VLDL) cholesterol concentrations positively and significantly correlated with Lp-PLA2 levels among American men. Large LDL concentration and LDL size also positively and significantly correlated while both medium small LDL and medium HDL concentrations negatively and significantly correlated with Lp-PLA2 levels among American men. Only large LDL concentration positively and significantly correlated with Lp-PLA2 levels among Japanese men. Neither CRP nor fibrinogen correlated with Lp-PLA2 levels in either American or Japanese men (Table 2).

Japanese men had a trend of inverse association of the prevalence of CCS>0 with the tertile groups of Lp-PLA2 levels (p=0.08); the association was significant among Japanese men with LDL?130 mg/dL (N=49, p=0.03). American men did not have any significant associations of the prevalence of CCS>0 with the tertile groups of Lp-PLA2 (p=0.62) (Figures 1 and 2).

Age-adjusted odds ratios for CCS>0 among American compared to Japanese men were highly significant before (6.0, 95% CI 2.9, 12.2, p<0.001) and after (6.4, 95% CI 3.0, 13.3, p<0.001) adjusting for Lp-PLA2 levels. Similarly, odds ratios for CCS>0 among American compared to Japanese men adjusted for multiple risk factors (age, systolic blood pressure, blood glucose, smoking, alcohol, BMI, waist circumference, CRP, total, LDL and HDL, triglycerides, and lipid medication) were highly significant before (7.6 95% CI 2.4-23.8%, p=0.001) and after (8.7 95% CI 2.5-29.7%, p=0.001) adjusting for Lp-PLA2 levels.

Table 1. Comparison of major characteristics between the American and Japanese men.†

	Americans (n=100)	Japanese (n=93)	p-value
Age (year)	44.6 ±2.9	44.7 ±2.8	0.785
Waist girth (cm)	96.4 ±9.8	84.7 ±8.5	<0.001
Body mass index (kg/m²)	27.0 ±3.3	23.3 ±3.1	<0.001
Systolic blood pressure (mmHg)	113.7 ±9.6	122.8 ±14.1	<0.001
Diastolic blood pressure (mmHg)	78.4 ±5.8	78.7 ±10.4	0.785
Total cholesterol (mg/dL)	192.8 ±31.3	221.4 ±37.6	<0.001
Triglycerides (mg/dL) ††	116.0 (79.0-167.5)	131.0 (90.0-181.0)	0.172
HDL cholesterol (mg/dL)	45.9 ±11.6	54.8 ±14.9	<0.001
LDL cholesterol (mg/dL)	119.7 ±30.0	136.3 ±39.1	0.001
Fasting glucose (mg/dL)	95.3 ±9.1	103.6 ±8.7	<0.001
Fasting insulin (μIU/mL)	12.5 ±6.6	8.2 ±3.9	<0.001
C-reactive protein (mg/L) ††	0.91 (0.45-2.20)	0.43 (0.21-0.86)	<0.001
Coronary calcium score ≥0 (%)	47%	13%	<0.001
Lipid medication (%)	8	3.1	0.134
Current cigarette Smoking (%)	15	48.5	<0.001
Alcohol drinking everyday (%)	16	46.4	<0.001
Lp-PLA2 (ng/mL)	301.7 ±82.6	275.9 ± 104.7	0.06

†: Mean and standard deviation unless mentioned otherwise

††: Median and inter-quartile range

Lp-PLA2: lipoprotein-associated phospholipase A2

Table 2. Correlation† of lipoprotein-associated phospholipase A2 (Lp-PLA2) level with coronary risk factor and nuclear magnetic resonance (NMR) lipoprotein subclasses among American and Japanese men.

	Americans (n=100)	Japanese (n=93)
Age (year)	0.02	-0.13
Waist girth (cm)	-0.20	-0.16
Body mass index (kg/m²)	-0.15*	-0.12
Systolic blood pressure (mmHg)	0.01	-0.08
Diastolic blood pressure (mmHg)	0.01	-0.12
Total cholesterol (mg/dL)	0.37***	0.30**
Triglycerides (mg/dL)	0.06	-0.07
HDL cholesterol (mg/dL)	-0.09	0.07
LDL cholesterol (mg/dL)	0.44***	0.30**
Fasting glucose (mg/dL)	0.02	-0.20*
Fasting insulin (μIU/mL)	-0.11	-0.09
C-reactive protein (mg/L)	0.11	0.04
Total VLDL particles (nmol/L)	0.27**	0.02
Large VLDL/chylomicrons (nmol/L)	0.01	-0.07
Medium VLDL (nmol/L)	0.16	-0.07
Small VLDL (nmol/L)	0.32**	0.17
Total LDL particles (nmol/L)	0.14	0.08
IDL (nmol/L)	-0.07	-0.01
Large LDL (nmol/L)	0.52**	0.22*
Small LDL (total) (nmol/L)	-0.17	-0.05
Medium small LDL (nmol/L)	-0.21*	-0.06
Very small LDL (nmol/L)	-0.16	-0.05
Total HDL particles (nmol/L)	-0.10	-0.01
Large HDL (nmol/L)	0.11	0.11
Medium HDL (nmol/L)	-0.25*	-0.12
Small HDL (nmol/L)	-0.10	-0.06
VLDL size (nm)	-0.12	-0.15
LDL size (nm)	0.35**	0.10
HDL size (nm)	0.12	0.18

†: Pearson's rho with normal data or Spearman's rho with skewed data.
*: <0.05, **: <0.01, ***: <0.001
LDL: low density lipoprotein
HDL: high density lipoprotein
IDL: intermediate density lipoprotein
VLDL: very low density lipoprotein

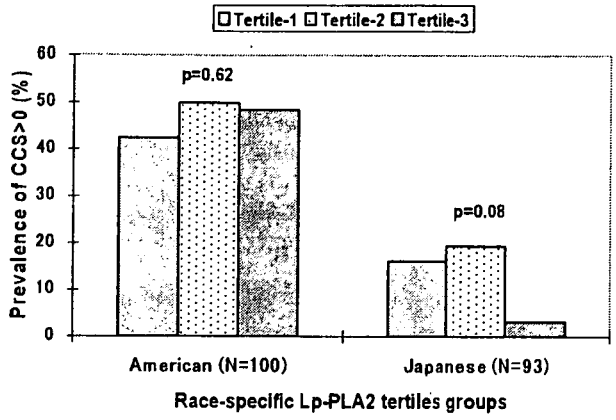

Figure 1 (All participants)

Figure 1. Prevalence of those with coronary calcium score (CCS) > 0 among the 2 populations by tertile group of lipoprotein-associated phspholipase A2 (Lp-PLA2): all participants.

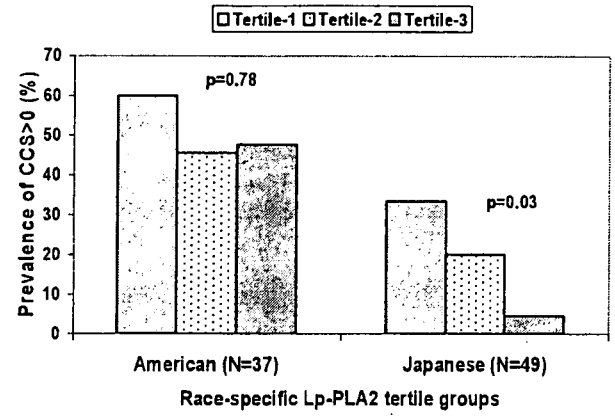

Figure 2 (Those with LDL-C≥130 mg/dL)

Figure 2. Prevalence of those with coronary calcium score (CCS) > 0 among the 2 populations by tertile group of lipoprotein-associated phspholipase A2 (Lp-PLA2): those with low density lipoprotein cholesterol ≥ 130 mg/dL.

DISCUSSION

The current data showed marginally significantly higher levels of Lp-PLA2 among American compared to Japanese men. There was no significant association between Lp-PLA2 levels with the prevalence of CAC among American men while there appeared to be a negative association between Lp-PLA2 levels with the prevalence of CAC among Japanese men, especially among those with LDL ≥ 130 mg/dL.

With few exceptions,[15-17] the majority of clinical and epidemiologic studies among western populations suggested that Lp-PLA2 is an independent predictor of cardiovascular events (CAD and stroke).[9-14] Whether Lp-PLA2 is an independent predictor of CAC or not is less clear. Only two studies examined the association between coronary calcification and Lp-PLA2 levels among white populations with inconsistent findings.[18,19] In a recent report from the Rotterdam Study,[10] Lp-PLA2 activity measured concurrently to EBT scanning was not associated with coronary calcification in either men or women. This finding was in contrast to previous findings from the same population that showed Lp-PLA2 as a predictor of CHD and stroke.[10] In the Coronary Artery Risk Development in Young Adults (CARDIA) study,[18] however, Lp-PLA2 level had a significant and independent association with CAC among young white and black American men and women.

The negative association between Lp-PLA2 levels and the prevalence of CAC among Japanese men supports the suggested anti-atherogenic effect of Lp-PLA2 among Japanese.[23] The anti-atherogenic properties of Lp-PLA2 are thought to be due to the degradation of the potent pro-inflammatory phospholipid platelet-activating factor (PAF), possibly through its partial association with HDL cholesterol, as well as the enzymatic catabolism of biologically active oxidized phospholipids in LDL cholesterol.[1-3] Moreover, deficiency of enzymatic activity resulting from single nucleotide polymorphism in the Lp-PLA2 gene was reported to be associated with increased risk of developing atherosclerosis and its clinical manifestations.[4-7]

In this report, the risk of having CAC was several times higher in American men compared to Japanese men before and after adjusting for Lp-PLA2 levels. The findings suggest that the higher Lp-LPA2 levels among American compared to Japanese men cannot explain the higher prevalence of CAC among American men. In this study, among both populations, neither CRP nor fibrinogen were correlated with Lp-PLA2 levels, which is in accordance with previous studies.[9,10,16,18]

One major strength of this study is the ability to examine the association between Lp-PLA2 levels and NMR lipoprotein subclasses, which could help with understanding the Lp-PLA2 mechanism of action. This is because the majority of circulating Lp-PLA2 in plasma is associated with LDL particles.[11] Although Lp-PLA2 among both populations in this report correlated positively with LDL and total cholesterol as measured by traditional chemical methods, lipoprotein particle sizes and concentrations as measured by NMR spectroscopy correlated differently with Lp-PLA2 levels among Japanese and American men, suggesting that Lp-PLA2 could have different mechanisms of action. Other strengths include comparing two populations with different cardiovascular risk,[20] the lack of interference of treatments (only 5.7% of all participants were using cholesterol lowering agents), and the availability of multiple established cardiovascular risk factors. There are several limitations in this study: (1) the small sample size of this study; however, the data may serve as a pilot study for future large scale prospective studies comparing Japanese-white differ-

ences in the relation between Lp-PLA2 and CAC or CAD; (2) the observational and cross-sectional nature of the data, precluding causal inferences; and (3) the fact that American subjects were volunteers and not a randomly selected population.

In summary, Lp-PLA2 levels cannot explain the observed CAC differences between the two populations. Lp-PLA2 was not associated with CAC among American men and appeared to be negatively associated with CAC among Japanese men. American and Japanese men had different correlations with lipoprotein subclasses, suggesting different Lp-PLA2 mechanisms of action among the two populations. These data need to be confirmed by large scale prospective studies before any clinical implications are anticipated.

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Food Omega-3 Fatty Acid Intake of Individuals (Total, Linolenic Acid, Long-Chain) and Their Blood Pressure

INTERMAP Study

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Abstract—Findings from short-term randomized trials indicate that dietary supplements of omega-3 polyunsaturated fatty acids (PFA) lower blood pressure of hypertensive persons, but effect size in nonhypertensive individuals is small and nonsignificant. Data are lacking on food omega-3 PFA and blood pressure in general populations. The International Study of Macro- and Micro-nutrients and Blood Pressure (INTERMAP) is an international cross-sectional epidemiologic study of 4680 men and women ages 40 to 59 from 17 population-based samples in China, Japan, United Kingdom, and United States. We report associations of food omega-3 PFA intake (total, linolenic acid, long-chain) of individuals with blood pressure. Systolic and diastolic blood pressure were measured 8 times at 4 visits. With several models to control for possible confounders (dietary, other), linear regression analyses showed inverse relationship of total omega-3 PFA from food (percent kilocalories, from four 24-hour dietary recalls) to systolic and diastolic blood pressures. With adjustment for 17 variables, estimated systolic blood pressure/diastolic blood pressure differences with 2 standard deviation higher (0.67% kcal) omega-3 PFA were −0.55/−0.57 mm Hg (Z-score −1.33, −2.00); for 2238 persons without medical or dietary intervention, −1.01/−0.98 mm Hg (Z −1.63, −2.25); for 2038 nonhypertensive persons from this sub-cohort, −0.91/−0.92 mm Hg (Z −1.80, −2.38). For linolenic acid (largely from vegetable foods), blood pressure differences were similar, eg, for the 2238 "nonintervened" individuals, −0.97/−0.87 mm Hg (Z −1.52, −1.95); blood pressure differences were −0.32/−0.45 mm Hg for long-chain omega-3 PFA (largely from fish). In summary, food omega-3 PFA intake related inversely to blood pressure, including in nonhypertensive persons, with small estimated effect size. Food omega-3 PFA may contribute to prevention and control of adverse blood pressure levels. (***Hypertension*. 2007;50:313-319.**)

Key Words: blood pressure ■ nutrition ■ food ■ omega-3 polyunsaturated fatty acids ■ population study

Unclarity persists concerning efficacy of omega-3 (ω-3) polyunsaturated fatty acid (PFA) intake for prevention and control of the cardiovascular diseases (CVD) and their major risk factors. This is particularly the case for population-wide ω-3 PFA from foods. As to ω-3 PFA supplements for secondary prevention of CVD, recent reviews/meta-analyses come to diverse conclusions.[1,2] Inconsistencies also prevail on influences of supplemental ω-3 PFA on blood pressure (BP). Meta-analyses of randomized clinical trials (RCTs) on ω-3 PFA supplements reported significant BP reduction overall and in hypertensive participants; significant heterogeneity in systolic BP (SBP) outcomes across trials; only small nonsignificant systolic and diastolic BP (DBP) lowering in nonhypertensive individuals.[3–8] Almost no population-based observational data exist on relation of food ω-3 PFA of individuals to their BP.[9]

Possible reasons for the heterogeneous RCT findings on ω-3 PFA supplements and BP are: actual effect size is small, particularly in nonhypertensive individuals, hence false-negative findings are probable unless sample sizes are large, and BP is measured repeatedly by high-quality techniques.

In observational studies, data on nutrient intakes and other variables must be extensive and high-quality, enabling characterization of ω-3 PFA intake by individuals and control for

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multiple possible confounders. The population-based International Study of Macro- and Micro-nutrients and Blood Pressure (INTERMAP) Study on nutrients and blood pressure was designed to cope with such problems.[10–15] Its basic premises are: multiple nutrients have small independent influences on BP of individuals that in combination yield sizable effects. To detect impact of single nutrients on BP of individuals, it is essential to collect standardized, high-quality data on large samples of diverse populations. Accordingly, INTERMAP surveyed in-depth 4680 men and women ages 40 to 59 from 17 population samples in Japan, People's Republic of China, United Kingdom, United States, enabling it to address main unanswered questions on ω-3 PFA intake and BP: (1) Does food ω-3 PFA intake of individuals relate independently to their SBP/DBP? (2) Is this the case throughout the population, including nonhypertensive individuals? (3) Are both linolenic and long-chain ω-3 PFA intake independently associated with their SBP/DBP? INTERMAP hypothesized that dietary ω-3 PFA intake of individuals is inversely related to their blood pressure.[10] Findings on food ω-3 PFA and BP are reported here.

Methods

Population Samples, Field Methods (1996–1999)

INTERMAP included men and women ages 40 to 59 years from population random samples in Japan (4 samples), People's Republic of China (PRC, 3), United Kingdom (UK, 2), and United States (US, 8).[10] Staff were trained and certified for BP measurement by international/national senior colleagues based on a common standardized protocol.[10] Each participant attended 4 times, visits 1 and 2 on consecutive days, visits 3 and 4 on consecutive days on average 3 weeks later. For BP measurement, each participant—having emptied his/her bladder—was seated comfortably for 5 minutes, feet flat on the floor, in a quiet room, with no physical activity in the preceding half hour. Korotkoff sounds I and V were criteria for SBP and DBP. BP was measured twice at each visit with a random zero sphygmomanometer; BP at each visit was the average of the 2 readings. Measurements of height and weight, and questionnaire data on daily alcohol consumption over the previous 7 days were obtained at 2 visits. Dietary data were collected at each visit by a trained interviewer with use of the in-depth multi-pass 24-hour recall method.[11] All foods and drinks consumed in the previous 24 hours, including dietary supplements, were recorded. Questionnaire data were obtained on demographic and other possible confounders. Quality control was extensive.

Each participant provided 2 24-hour urine collections, start and end timed at the research center (visits 1 to 2 and 3 to 4); measurements included urinary volume, sodium, potassium, creatinine, urea[10]; 10% of samples were split locally and sent to the Central Laboratory with different identification number to estimate technical error.

Individuals were excluded if they did not attend all 4 visits; diet data were considered unreliable; energy intake from any 24-hour dietary recall was below 500 or greater than 5000 kcal/d for women, 8000 kcal for men; 2 urine collections were not available; data on other variables were incomplete or indicated protocol violation (total exclusions: 215 people). For each exclusion, a supplementary participant was recruited.

The study received institutional ethics committee approval for each site; all participants gave written consent; all the procedures followed were in accordance with institutional guidelines.

Statistical Methods

Food data of individuals were converted into nutrient intakes (83 nutrients) with use of enhanced country-specific food tables, standardized across countries by the Nutrition Coordinating Center, University of Minnesota.[11,12] For nutrients supplying energy, intake was calculated as percent total energy; for others, as intake/1000 kcal; nutrients were calculated also as amounts/24 hours. Food data were used to estimate main food groups supplying ω-3 PFA—total, linolenic acid (largely from vegetable sources), long-chain ω-3 PFA (largely from fish; eicosapentaenoic acid [EPA], docosahexaenoic acid [DHA], docosapentaenoic acid [DPA]). Urinary values/24 hours were calculated as products of urinary concentrations and timed volume standardized to 24 hours. Measurements/person were averaged, for BP and nutrient variables, across the 4 visits; for the urinary excretions, across the 2 24-hour collections. For descriptive statistics, means, standard deviations, numbers, and percentages were calculated by country and study-wide. Reliability of SBP, DBP, and ω-3 PFA intakes from the mean of the 4 visits was estimated from the formula $1/[1+(\text{ratio}/4)]\times 100$, where the ratio is intraindividual variance/interindividual variance, estimated separately for 8 gender/country strata and pooled by weighting each stratum-specific estimate by (sample size minus one). This gives a first approximation of reliability, ie, an estimate of the size of an observed coefficient as a per cent of the true coefficient in a univariate regression analysis.[16,17]

Associations among nutritional variables were explored by partial correlation, adjusted for sample, age, gender; pooled across countries, weighted by sample size. Multiple regression analyses were used to examine relationships of food ω-3 PFA (percent kcal) of individuals—total, linolenic acid, long-chain—to their SBP and DBP. These analyses were done for four cohorts: all 4680 participants; 2238 "nonintervened" persons not on a special diet, not consuming nutritional supplements, not with diagnosed CVD/diabetes (DM); not taking medication for high BP, CVD, diabetes; ie, exclusion of people whose data might bias the food ω-3 PFA-BP relationship; nonhypertensive individuals—SBP <140, DBP <90 mm Hg, not taking antihypertensive medication—from the total cohort (n=3671) and from the "nonintervened" subcohort (n=2038). Adjustment for confounders was done sequentially: for sample, age, gender, weight, height (Model 1); plus reported special diet, dietary supplement intake, moderate/heavy physical activity (hours/d), history of CVD/DM, family history of hypertension (Model 2); plus 24-hour urinary sodium, potassium, (or urinary sodium/creatinine, potassium/creatinine) and 7-day alcohol intake (Model 3); plus dietary cholesterol, saturated fatty acids (SFA), calcium (Model 4); plus dietary fiber or magnesium or phosphorus, separately because of collinearity (Models 5a, 5b, 5P).

Regression models were fitted by country and coefficients pooled across countries, weighted by inverse of variance, to estimate overall association; cross-country heterogeneity was tested; interactions were assessed for age, gender, and body mass index (BMI, weight/height^2 [kg/m^2]). Regression coefficients were expressed as mm Hg for 2 standard deviation (SD) higher food ω-3 PFA, from pooled within-country standard deviations weighted by sample size.

Sensitivity analyses involved: inclusion of energy intake with nutrient densities; use of g/d intakes adjusted for energy; addition to regression models of other nutrients (monounsaturated fatty acids, oleic acid, ω-6 PFA, linoleic acid, arachidonic acid, trans fatty acids, vegetable protein, animal protein, estimated total sugars, vitamin E); exclusion from the "nonintervened" subcohort of people taking nonsteroidal antiinflammatory drug (NSAID); exclusion of people with marked intraindividual variability in nutrient intake or SBP, DBP.

Analyses were with SAS version 8.02 by Q.C. and I.J.B.

Results

Descriptive Statistics

Detailed data are tabulated in the online supplement to this paper (please see Table S1 at http://hyper.ahajournals.org). Average SBP ranged from 117.2 (Japan) to 121.3 mm Hg (PRC); average DBP, from 73.2 (PRC) to 77.3 (UK) mm Hg. Mean BMI and energy intake were lower for Japanese and PRC participants, highest for American. Mean total ω-3 PFA

TABLE 1. Estimated Mean Difference in Blood Pressure (mm Hg), Dietary Total ω-3 PFA (% kcal) Higher by Two Standard Deviations,* Sequential Regression Models, All Men and Women (n=4680)

		Systolic Blood Pressure		Diastolic Blood Pressure	
Model	Other Variables, Added Sequentially†	Difference mm Hg	Z-Score	Difference mm Hg	Z-Score
1	Sample, age, gender, height, weight	−0.54	−1.32	−0.63‡	−2.23
2	Special diet, supplement intake, CVD-DM diagnosis, physical activity, family history of high BP	−0.58	−1.44	−0.61‡	−2.16
3	Urinary Na, urinary K, alcohol	−0.38	−0.94	−0.48‡	−1.71
4	Dietary cholesterol, SFA, calcium	−0.56	−1.38	−0.55	−1.94
5a	Dietary fiber or,	−0.53	−1.30	−0.54	−1.90
5b	Dietary magnesium, or	−0.51	−1.23	−0.54	−1.90
5P	Dietary phosphorus	−0.55	−1.33	−0.57	−2.00

Units are mmol/24 hours (urinary Na, urinary K), g/24 hours (alcohol), mg/1000 kcal (calcium, magnesium, phosphorus, cholesterol), %kcal (ω-3 PFA, SFA).

Special diet: Weight loss, weight gain, vegetarian, salt reduced, diabetic, fat modified, or any other diet.

CVD-DM: History of heart attack, other heart disease, stroke, or diabetes.

Supplement Intake: Taking any dietary supplement at time of the study.

PFA indicates polyunsaturated fatty acids; Na, sodium; K, potassium; SFA, saturated fatty acids.

All nutrients are from foods only, exclusive of amounts from dietary supplements.

Z-score ≥1.96: uncorrected $P \leq 0.05$; ≥2.58: uncorrected $P \leq 0.01$.

*Two standard deviation difference is 0.669 %kcal for total omega 3 PFA; as grams/24 hours, 1.929.

†Variables listed are added to each prior model, so that for example, Model 5P contains all variables listed in Models 1–4 and dietary phosphorus.

‡P value for cross-country heterogeneity ≤0.05.

intake from foods (g/24 hours and % kcal) was highest in Japan (1.35% kcal), lowest in China (0.55% kcal); linolenic acid (ALA) was about 78% of all ω-3 PFA overall (60% for Japanese, 98% for Chinese). EPA+DHA constituted most long-chain ω-3 PFA. Main food groups supplying long-chain ω-3 PFA were—for all 4680 participants—fish and fish products (77.3% of all EPA+DHA); shellfish and shellfish products (4.9%); red meats, poultry, eggs and their products (19.9%). This pattern prevailed for participants from Japan (90.4% of EPA+DHA from fish, shellfish, and their products) and from the US (78.0%), to a lesser extent for those from the UK (56.0%).

Univariate estimates for reliability of the per person values of ω-3 PFA intake based on means of 4 24-hour recalls were for all 4680 participants: total ω-3 PFA (%kcal)—observed coefficient 45.6% of true coefficient; linolenic acid, 53.3%; EPA, 31.0%; DHA, 31.0%; DPA, 27.6%; EPA+DHA, 35.3%; EPA+DHA+DPA, 35.3%. Subcohort data on reliability were similar to the foregoing. These estimates varied across countries, eg, for total ω-3 PFA intake (all 4680 participants): Japan 28.6%; PRC 79.3%; UK 40.8%; US 55.2%. BP reliability estimates were 94.3% for SBP and 93.0% for DBP.

Partial Correlation Data

Food total ω-3 PFA (% kcal) was correlated directly with food linoleic acid (partial r=0.48) and total ω-6 PFA (0.48), arachidonic acid (0.23), total monounsaturated fatty acids (MFA; 0.34), oleic acid (0.27), Vitamin E (0.35); inversely with total available carbohydrate (−0.34) and total sugars (−0.21). ALA was correlated similarly with the foregoing variables; it was not correlated with EPA, DHA, DPA, or their sums. EPA, DHA, DPA were highly intercorrelated (r values 0.65 to 0.84). Sum EPA+DHA was correlated with total protein (0.30), arachidonic acid (0.36), phosphorus (0.18), vitamin E (0.16), as was sum EPA+DHA+DPA.

Relation of Food Total Omega-3 PFA to Blood Pressure

All 4680 Participants

Consistently, dietary total ω-3 PFA was inversely related to SBP and DBP (Table 1). With 2 standard deviation higher total ω-3 PFA (0.669% kcal=about 1.9 g/d), estimated difference in SBP was about −0.4 to −0.6 mm Hg; in DBP, about −0.5 to −0.6 mm Hg (DBP Z-scores −1.71 to −2.23). For all models on the relation of ω-3 PFA to BP, there was no statistically significant interaction with age, gender, or BMI. The inverse ω-3 PFA-BP relation was nonsignificantly stronger for US participants, eg, for model 5P, estimated SBP difference of −1.26 mm Hg with 2 SD higher ω-3 PFA, DBP −0.80 mm Hg; for PRC participants, DBP difference −1.73 mm Hg. Analyses with control for urinary Na/creatinine and K/creatinine (instead of 24-hour Na and K excretion) yielded similar findings, eg, with Model 5P DBP difference −0.56 mm Hg (Z -1.96) with ω-3 PFA intake 2 SD higher.

"Nonintervened" Subcohort (n=2238)

Percentage of persons with untreated high BP in this subcohort was: 11.8% for men, 5.7% for women. With 2 SD higher ω-3 PFA intake, estimated SBP and DBP differences were consistently greater than for all participants, eg, with model 5P, SBP difference −1.01 and DBP difference −0.98 with 2 SD higher ω-3 PFA (Table 2). These differences were nonsignificantly larger for PRC and US participants, SBP −1.28 and −2.22 mm Hg, DBP −2.12 and −2.03 mm Hg.

Nonhypertensive Persons (n=3671 and 2038)

For the 3671 nonhypertensive persons from the total cohort, SBP and DBP differences with 2 SD higher dietary ω-3 PFA

TABLE 2. Estimated Mean Difference in Blood Pressure (mm Hg), Dietary Total ω-3 PFA (% kcal) Higher by 2 Standard Deviations,* Model 5P, All Persons, "Nonintervened" Persons, Nonhypertensive Persons

Group	No. of Persons	Systolic Blood Pressure		Diastolic Blood Pressure	
		Difference mm Hg	Z-Score	Difference mm Hg	Z-Score
All persons	4680	−0.55	−1.33	−0.57	−2.00
"Nonintervened" persons	2238	−1.01	−1.63	−0.98	−2.25
Nonhypertensive persons from total cohort	3671	−0.74	−2.05	−0.72	−2.63
Nonhypertensive persons from "Nonintervened" subcohort	2038	−0.91	−1.80	−0.92	−2.38

PFA indicates polyunsaturated fatty acids.

"Nonintervened" persons: individuals not on a special diet, not consuming nutritional supplements, not with diagnosed CVD/DM, not taking medication for high BP/CVD/DM.

Other variables in Model 5P: Sample, age, gender, height, weight, physical activity, family history of high BP, urinary sodium and potassium, alcohol intake, dietary cholesterol, saturated fatty acids, calcium, phosphorus; also, for all persons and all nonhypertensive persons, special diet, supplement intake, CVD/DM diagnosis.

Nonhypertensive persons: individuals with SBP <140 mm Hg and DBP <90 mm Hg and not reporting use of medication for high BP.

All tests for cross-country heterogeneity were nonsignificant.

*Two standard deviation difference is 0.669 %kcal for total ω-3 PFA for all 4680 participants, similar for the 3 subcohorts; the 2 SD difference as grams/24 hours is 1.929.

were greater than for all 4680 participants, eg, SBP difference −0.74 mm Hg and DBP difference −0.72 mm Hg (Model 5P; Table 2). SBP differences were nonsignificantly larger for UK and US participants, −0.89 mm Hg and −1.66 mm Hg; DBP differences, larger for PRC and US participants, −1.22 mm Hg and −1.14 mm Hg. Findings were similar for nonhypertensive persons from the subcohort of "nonintervened" individuals.

Sensitivity Analyses

Multiple other regression models yielded results qualitatively similar to the foregoing, eg, modifications A-D of Model 5P (Table 3). With Model D, excluding people with high day-to-day variability in SBP, DBP, or nutrient intakes, BP differences, and Z-scores were greater than for all 4680 persons.

Relation of Linolenic Acid and Long-Chain ω-3 PFA From Foods to Blood Pressure

For all 4680 participants and the 3 subcohorts, relations of linolenic acid and long-chain ω-3 PFA to BP were similar in models where ALA and sum, EPA+DHA (or sum, EPA+DHA+DPA) were or were not considered together.

ALA Intake From Foods and BP

Consistently, ALA was inversely related to SBP; thus, in Model 5P estimated SBP differences with 2 SD higher intake (0.566% kcal, = about 1.6 g/d) ranged from −0.60 mm Hg (all 4680 participants) to −0.97 mm Hg (2238 "nonintervened" persons; Table 4). Repeatedly, data for US participants showed nonsignificantly larger estimated SBP differences, eg, with Model 5P differences of −1.50 mm Hg to −1.92 mm Hg across the 4 groups.

The relation of food ALA to DBP was also inverse, with evidence of significant cross-country heterogeneity, eg, Model 5P (Table 4). For the 4 countries separately, estimated DBP differences varied in sign and amount, eg, Model 5P for all 4680 persons: Japan +0.52 mm Hg, PRC −1.52 mm Hg, UK +2.46 mm Hg, US −0.97 mm Hg; similarly for the "nonintervened" subcohort (n=2238): +0.32, −1.90 (Z=−2.35), +2.20, −1.83 (Z=−2.21).

TABLE 3. Sensitivity Analyses: Estimated Mean Difference in Blood Pressure (mm Hg), Dietary Total ω-3 PFA Intake (%kcal) Higher by Two Standard Deviations,* Men and Women Combined

Modification of Model 5P	No. of Persons	Systolic Blood Pressure		Diastolic Blood Pressure	
		Difference mm Hg	Z-Score	Difference mm Hg	Z-Score
A. %kcal with inclusion of energy intake (kcal/24 hours)	4680	−0.53	−1.30	−0.57	−1.99
B. g/24 hours adjusted for energy intake (kcal/24 hours)	4680	−0.80	−1.61	−0.57	−1.65
C. %kcal with exclusion from the "Nonintervened" subcohort of people taking NSAID	2131	−0.90	−1.42	−0.82	−1.85
D. %kcal with exclusion of people with high day-to-day variability of SBP, DBP, and/or nutrient intakes	3473	−0.76	−1.63	−0.67	−2.08

All tests for cross-country heterogeneity were nonsignificant.

*2 SD difference in dietary total ω-3 PFA is 0.669 %kcal (analyses A, C, D) or 1.929 g/24 hours (analysis B).

TABLE 4. Estimated Mean Difference in Blood Pressure (mm Hg), Dietary Linolenic Acid and Dietary Long-chain ω-3 PFA (Sum, EPA+DHA) (% kcal) Higher by 2 Standard Deviations,* Linolenic Acid and Sum, EPA+DHA in Same Regression Model (Model 5P), All Participants, "Nonintervened" Persons, Nonhypertensive Persons

		Linolenic Acid				Sum, EPA+DHA			
		Systolic Blood Pressure		Diastolic Blood Pressure		Systolic Blood Pressure		Diastolic Blood Pressure	
Group	No. of Persons	Difference mm Hg	Z-Score	Difference mm Hg	Z-Score	Difference mm Hg	Z-Score	Difference mm Hg	Z-Score
All persons	4680	−0.60	−1.43	−0.50†	−1.71	−0.03	−0.08	−0.28	−0.96
"Nonintervened" persons	2238	−0.97	−1.52	−0.87‡	−1.95	−0.32	−0.57	−0.45	−1.07
Nonhypertensive persons from total cohort	3671	−0.77	−2.05	−0.61‡	−2.16	−0.23	−0.65	−0.45	−1.66
Nonhypertensive persons from "Nonintervened" cohort	2038	−0.73	−1.39	−0.73	−1.89	−0.52	−1.08	−0.54	−1.46

PFA indicates polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

See footnotes for Tables 1–3.

*Two standard deviation difference is 0.566 %kcal for linolenic acid and 0.318 %kcal for sum, EPA+DHA, similar for the 3 subcohorts; 2 SD differences in gram/24 hours are 1.623 and 0.789.

†*P* value for cross-country heterogeneity ≤0.01; ‡*P* value for cross-country heterogeneity ≤0.05.

Long-Chain ω-3 PFA From Foods and BP

Findings for the relation to BP of Sum EPA+DHA+DPA and Sum EPA+DHA were similar: For the 4 groups, DBP differences with 2 SD higher EPA+DHA (0.318% kcal, = about 0.79 g/d) ranged (model 5 P) from −0.28 mm Hg to −0.54 mm Hg; SBP differences were generally smaller (Table 4).

Corresponding analyses were done on the relation to BP of EPA and DHA considered separately. Results were qualitatively similar to the foregoing: eg, Model 5P, with 2 SD higher EPA, DBP lower by −0.21 to −0.56 mm Hg; for DHA, −0.31 to −0.61 mm Hg. Findings were similar from regressions of SBP, DBP on EPA, DHA, EPA+DHA ingested only from fish/shellfish and their products.

In multiple regression models with these 2 highly correlated variables considered together in the same model, the relation of EPA to SBP and DBP varied across cohorts, ie, was nonsignificantly positive for all 4680 participants and the 2238 "nonintervened" persons, nonsignificantly inverse for the 3671 nonhypertensive persons and the subcohort of 2038 nonhypertensive persons. DHA-BP relations also varied in sign across cohorts and all had low-order Z-scores.

Discussion

Main findings of this population-based study on food ω-3 PFA intake of individuals and their blood pressure are: (1) Consistent independent inverse relations of total ω-3 PFA to systolic and diastolic pressure; (2) estimated effect size small, <1.0 mm Hg with 2 SD higher ω-3 PFA intake (about 1.9 g/d); (3) estimated effect size larger for nonhypertensive persons and for persons not reporting lifestyle modification (eg, special diet, use of nutritional supplements), diagnosed CVD or diabetes, prescribed medication for major chronic disease; (4) similar inverse relations also of linolenic acid to SBP/DBP; (5) for long-chain ω-3 PFA (sum EPA+DHA, EPA separately, DHA separately) qualitatively similar weaker inverse relation to DBP.

To the best of our knowledge these INTERMAP data indicating low-order independent inverse relations of food ω-3 PFA (total, linolenic acid, long-chain) to BP are the first comprehensive population-based findings on this matter. The Finnish Kuopio Study of 722 middle-aged men reported a significant independent cross-sectional relation of dietary ALA to SBP and mean arterial pressure, but not to DBP; no data were given on long-chain ω-3 PFA.[9]

These INTERMAP observational data on food ω-3 PFA and BP are concordant with results from metaanalyses of randomized trials assessing whether ω-3 PFA supplements (mostly fish oil capsules) influence BP; in particular, our data are similar qualitatively and quantitatively in indicating a low-order favorable BP effect, including in nonhypertensive persons,[3–6] and are also compatible with the small nonsignificant differences (−0.4/−0.6 mm Hg) reported recently from the Kanwu Study Group RCT on 162 healthy nonhypertensive adults.[18]

As to BP influences of ALA per se, Wendland et al[8]—based on 3 RCTs involving 348 persons—reported small nonsignificant effect sizes (−0.72/−0.17 mm Hg). Similarly, for long-chain ω-3 PFA per se, the 1993 metaanalysis of RCTs[4] estimated effect sizes overall (ie, for all participants, hypertensive and nonhypertensive) for DHA of −1.50/−0.77 mm Hg/gram, and for EPA of −0.93/−0.53 mm Hg/gram. Given limitations in statistical power, it is consistent with the foregoing that no significant influences of EPA supplements on BP were noted in a recent overview of 4 small RCTs in nonhypertensive people.[7] For DHA supplements (4 g/d), that article reported sizable BP effects on 24-hour and day-time ambulatory BP of 56 overweight hypercholesterolemic adults.[7] High order collinearity of EPA and DHA intakes from foods limits ability to estimate their separate influences on BP. Our separate regression analyses on EPA-BP and DHA-BP relations yielded similar low-order associations for each. Because dietary ALA is a metabolic precursor of EPA and DHA,[8] it is a reasonable expectation that all these dietary ω-3 PFA relate to BP.

Limitations of the INTERMAP findings include: their cross-sectional nature, but they are the only available population-based data on food ω-3 PFA (total, linolenic acid,

long-chain) and BP, and their results are consistent with those from RCTs; underestimation of effect size attributable to limited reliability in measurement of nutrients (regression dilution bias); possible confounding of the overall data on food ω-3 PFA and BP by special diets, dietary supplements, and drug treatment for high BP/CVD/diabetes, but the findings prevailed with multivariate control for those and other possible confounders for the 4680 participants. In addition, the data indicating larger influences of ω-3 PFA on BP for persons not experiencing such interventions are coherent with the inference that the ω-3 PFA-BP relation may be etiologically significant.

Possible mechanisms whereby ω-3 PFA may favorably influence BP are, based on animal experimental data: enhanced endothelial vasodilator function,[19,20] reduced reactivity of resistant vessel vascular smooth muscle,[20,21] increased vascular compliance.[22]

As noted, if feasible intakes of food ω-3 PFA do indeed influence BP favorably for people in the general population, effect size is apparently small, based on INTERMAP and RCT results. This finding, anticipated by INTERMAP, needs to be kept in perspective: First, with multiple nutrients having "small" independent influences, combined effect becomes sizable, ie, improved nutrition is capable of preventing or lowering unfavorable BP levels for most people, as the Dietary Approaches to Stop Hypertension and Optimal Macro-Nutrient Intake Heart feeding trial results indicate.[23–25] Second, long-term effects of habitual eating patterns, from early life into middle-age, may be greater, as data on salt intake and BP indicate.[26,27] Third, estimates indicate that lowering of population average SBP by "small" amounts (eg, 2 mm Hg) can result in reduction of mortality rates of 6% for stroke and 4% for coronary heart disease (CHD).[26] Fourth, enhanced ω-3 PFA intake from foods may contribute to decreased risk of CHD/CVD not only by modestly lowering BP, also by favorably influencing dyslipidemia, by anticoagulant, and antiarrhythmic effects.[28–32] Population-wide feasibility of greater ω-3 PFA intake from foods, vegetable and marine sources is indicated by findings for INTERMAP Japanese—compared with Chinese, U.K., U.S.A.—participants, ie, linolenic acid and long-chain ω-3 PFA both substantially higher, especially the latter. As to specific food sources, ω-3 PFA in 100 g cooked fatty fish (175 kcal) is 2.70g; 100 g canned pink salmon (unsalted) (134 kcal), 1.90g; 20g walnuts (unsalted) (134 kcal), 1.36g; 10g flax seed (45 kcal), 1.83g; 5g canola oil (45 kcal), 0.46g; 5g soy bean oil (45 kcal), 0.34g.

In conclusion, there was a weak inverse relationship to BP of ω-3 polyunsaturated fat intake from foods (total, linolenic acid, long-chain) with control for multiple possible confounders. This finding was stronger for nonhypertensive people and persons not experiencing dietary/medical intervention, ie, was stronger after removing sources of possible bias, a result consistent with the inference that the ω-3 PFA–BP relationship may be etiologically significant, albeit low-order.

Perspectives

Recent research data indicate that multiple improvements in food intake lower BP levels of adults, both prehypertensive and hypertensive. Nutrients possibly accounting for these favorable effects include greater intake of minerals (calcium, magnesium, phosphorus); vegetable protein; polyunsaturated fatty acids including omega-3; and reduced intake of total fat, saturated fatty acids, cholesterol, sugars—over and above known favorable effects on BP of reduced sodium chloride, increased potassium, and prevention/correction of overweight/obesity and excess alcohol intake. The findings of the present study indicating a low-order favorable influence of food ω-3 fatty acid intake on BP of individuals from general population samples are consistent with metaanalytic data of RCTs on this matter. Thus, these results on a major CHD/CVD risk factor lend modest support to current recommendations for increased ingestion of ω-3 fatty acids from marine and vegetable sources.

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Disclosures

None.

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Effect of Combined Cardiovascular Risk Factors on Individual and Population Medical Expenditures

—— A 10-Year Cohort Study of National Health Insurance in a Japanese Population ——

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Background Although obesity is required for some criteria defining metabolic syndrome, clustering of other risk factors also indicates an increased risk of cardiovascular disease. Whether the relationship between cardiovascular risk factor clustering and medical expenditures differs with body mass index (BMI) requires investigation, especially in a population with a low prevalence of obesity such as that in Japan.

Methods and Results A 10-year cohort study of 4,478 Japanese National Health Insurance beneficiaries aged 40–69 years in a community between 1990 and 2001 was carried out in the present study. The clustering of cardiovascular risk factors showed a positive and graded relationship to personal medical expenditures in participants who are overweight (BMI ≥25.0) and normal weight (BMI <25.0). The individual medical expenditures per month were 1.7-fold higher for participants with 2 or 3 risk factors and overweight than for those without these factors (26,782 vs 15,377 Japanese yen). Differences in the geometric means were similarly significant after adjustment for other confounding factors. However, the excess medical expenditures by risk clustering of normal weight categories within the total medical expenditures were higher than those of overweight categories because more participants were of normal weight.

Conclusions Cardiovascular risk factor clustering and being overweight can be a useful predictor of medical expenditures in a Japanese population. (*Circ J* 2007; **71**: 807–813)

Key Words: Medical expenditures; Metabolic components; Overweight; Risk factors

Hypertension, dyslipidemia, diabetes and obesity are cardiovascular risk factors that are difficult to control, but which are widespread in many developed countries[1] These factors are often clustered[2–6] which has resulted in a high incidence of cardiovascular disease accounted for by metabolic syndrome, recognized as visceral fat accumulation[2–7] The individual components of metabolic syndrome impose a major economic burden on the health-care system[8–12] However, few studies have examined the combined effects of multiple cardiovascular risk factors on medical expenditures[13,14]

Furthermore, the National Cholesterol Education Program considers each risk factor to have a similar effect on atherosclerosis[15] On the contrary, the International Diabetes Federation defines waist circumference as a requirement for a diagnosis of metabolic syndrome[16] However, other studies have shown that high-risk individuals with metabolic risk factors often go undetected if obesity is a required criterion[17,18] Thus, whether the relationship between cardiovascular risk factor clustering and medical expenditures differs with body mass index (BMI) should be determined, especially in a population with a low prevalence of obesity such as the Japanese.

The present study examines the influence of cardiovascular risk factor clustering on medical expenditures in individuals who are overweight and of normal weight defined by BMI. Our a priori hypothesis is that clustering of cardiovascular risk factors has a positive, graded association with medical expenditures. Furthermore, we investigated whether overweight participants with risk factor clustering actually have high medical expenditures and if so, the proportion of the excess medical expenditures in the total medical expenditures consumed by these participants.

Methods

Medical Expenditures in Japan

Medical expenditures in Japan are based on a public medical insurance institution[19,20] that comprises 2 systems. Everyone living in Japan is required to enrol in either of the 2 insurance systems, and this is called 'health-insurance for

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