Yokoyama S, Yamashita S, Ishibashi S, Sone H, Oikawa S, Shirai K, Ohta T, Bujo H, Kobayashi J, Arai H, Harada-Shiba M, Eto M, Hayashi T, Gotoda T, Suzuki H, Yamada N.	Background to Discuss Guidelines for Control of Plasma HDL-Cholesterol in Japan. J	Atheroscler Thromb	19	207-212	2012
Ina K, Hayashi T, Araki A, Kawashima S, Sone H, Watanabe H, Ohrui T, Yokote K, Takemoto M, Kubota K, Noda M, Noto H, Ding QF, Zhang J, Yu ZY, Yoon BK, Nomura H, Kuzuya M; Japan CDM Group.	Importance of high-density lipoprotein cholesterol levels in elderly diabetic individuals with type IIb dyslipidemia: A 2-year survey of	Geriatr Gerontol Int		1-5	2013
Sun L, Ishida T, Miyashita K, Kinoshita N, Mori K, Yasuda T, Toh R, Nakajima K, Imamura S, Hirata K.	*Plasma activity of endothelial lipase impacts high-density lipoprotein metabolism and coronary risk factors in humans.*	*J Atheroscler Thromb. In press.*	20	1-9	2013
Ishida T, Miyashita K, Shimizu M, Kinoshita N, Mori K, Sun L, Yasuda T, Imamura S, Nakajima K, Stanhope KL, Havel PJ, Hirata K.	*ELISA system for human Endothelial lipase.*	*Clin Chem*	*58*	*1656-64*	*2012*
Gotoda T, Shirai K, Ohta T, Kobayashi J, Yokoyama S, Oikawa S, Bujo H, Ishibashi S, Arai H, Yamashita S, Harada-Shiba M, Eto M, Hayashi T, Sone H, Suzuki H, Yamada N.	Diagnosis and management of type I and type v hyperlipoproteinemia.	J. Atheroscler. Thromb.	19(1)	1-12	2012
Takase S, Osuga JI, Fujita H, Hara K, Sekiya M, Igarashi M, Takanashi M, Takeuchi Y, Izumida Y, Ohta K, Kumagai M, Nishi M, Kubota M, Masuda Y, Taira Y, Okazaki S, Iizuka Y, Yahagi N, Ohashi K, Yoshida H, Yanai H, Tada N, Gotoda T, Ishibashi S, Kadowaki T, Okazaki H.	Apolipoprotein C-ll deficiency with no rare variant in the APOC2 gene.	J. Atheroscler. Thromb.	20(5)	481-493	2013

Yamamoto T, Obika S, Nakatani M, Yasuhara H, Wada F, Shibata E, Shibata MA, Harada-Shiba M	Locked nucleic acid antisense in-hibitor targeting apolipoprotein C-III efficiently and preferentially removed triglyceride from large VLDL particles from murine plas-ma	European Journal of Pharmacol-ogy	723	353-359	2014
Teramoto T, Sasaki J, Harada-Shiba M, Arai H, Ishibasi S, Birou S, Bu-jo H, Daida H, Dohi S, Egusa G, Hiro T, Hirobe K, Iida M, Kihara S, Kinoshita M, Maruyama C, Nohara A, Ohta T, Oikawa S, Okada T, Okamura T, Watatsuki A, Yamashi-ta S, Yokode M, Yokote K.	Familial hypercholesterolemia, Ex-ecutive summary of the Japan Ath-eroscleros Society(JAS) Guide-lines for diagnosis and prevention of atherosclerotic cardiovascular diseases in Japan-2012 version.	J Atheroscler Thromb	21(1)	6-10	2013
Terasaki F, Morita H, Harada-Shiba M, Ohta N, Otsuka K, Nogi S, Miyamura M, Suzuki S, Ito T, Shimomura H, Katsumata T, Miyamoto Y, Ishizaka N.	Familial Hypercholesterolemia with Multiple Large Tendinous Xan-thomas and Advanced coronary Artery Atherosclerosis	Intern Med	52	577-581	2013
Sugisawa T, Okamura T, Makino H, Watanabe M, Kishimoto I, Miya-moto Y, Iwamoto N, Yamamoto A, Yokoyama S, Harada-Shiba M	Defining patients at extremely high risk for coronary artery disease in heterozygous familial hypercholes-terolemia	J Atheroscler Thromb	19(4)	369-375	2012

Original Article

Relationships between Plasma Fatty Acid Composition and Coronary Artery Disease

Hiroshige Itakura[1], Mitsuhiro Yokoyama[2], Masunori Matsuzaki[3], Yasushi Saito[4], Hideki Origasa[5], Yuichi Ishikawa[6], Shinichi Oikawa[7], Jun Sasaki[8], Hitoshi Hishida[9], Toru Kita[10], Akira Kitabatake[11], Noriaki Nakaya[12], Toshiie Sakata[13], Kazuyuki Shimada[14], Kunio Shirato[15], and Yuji Matsuzawa[16], for the JELIS Investigators, Japan

[1]Shinagawa East One Medical Clinic, Tokyo, Japan
[2]Hyogo Prefectural Awaji Hospital, Sumoto, Japan
[3]Division of Cardiology, Department of Medicine and Clinical Science, Yamaguchi University Graduate School of Medicine, Ube, Japan
[4]Chiba University, Chiba, Japan
[5]Division of Biostatistics and Clinical Epidemiology, University of Toyama, Toyama, Japan
[6]Kakogawa Municipal Hospital, Kakogawa, Japan
[7]Division of Endocrinology and Metabolism, Department of Medicine, Nippon Medical School, Tokyo, Japan
[8]International University of Health and Welfare Graduate School of Public Health Medicine, Fukuoka, Japan
[9]Division of Cardiology, Department of Internal Medicine, Fujita Health University School of Medicine, Toyoake, Japan
[10]Kobe City Medical Center General Hospital, Kobe, Japan
[11]Hiraoka Hospital, Osaka, Japan
[12]Nakaya Clinic, Tokyo, Japan
[13]Hisatsune Hospital, Fukuoka, Japan
[14]Division of Cardiovascular Medicine, Department of Medicine, Jichi Medical School, Tochigi, Japan
[15]Saito Hospital, Miyagi, Japan
[16]Sumitomo Hospital, Osaka, Japan

Aim: **The Japan EPA Lipid Intervention Study (JELIS) was the first prospective randomized clinical trial to demonstrate prevention of coronary events by pure eicosapentaenoic acid (EPA). The aim of this study was to examine the relationships between various plasma fatty acid concentrations and the risk of coronary events in JELIS participants.**

Methods: **In 15,534 participants, we calculated the hazard ratio for major coronary events (sudden cardiac death, fatal or nonfatal myocardial infarction, unstable angina pectoris, and angioplasty/stenting or coronary artery bypass grafting) relative to the on-treatment average level of plasma fatty acids with the Cox proportional hazard model.**

Results: **As a result of EPA intervention, the plasma EPA concentration increased, but the docosahexaenoic acid (DHA) concentration did not. The other fatty acids measured decreased slightly. The higher plasma level of EPA (hazard ratio = 0.83, $p = 0.049$, in all participants and hazard ratio = 0.71, $p = 0.018$, in the EPA intervention group), but not of DHA, was inversely associated with the risk of major coronary events. The associations between other fatty acids and the risk of major coronary events were not significant. In all JELIS participants, the risk of major coronary events was significantly decreased (20%) in the group with high (150 μg/mL or more) on-treatment plasma EPA concentration compared with that in the low (less than 87 μg/mL) group.**

Conclusion: **The risk of coronary artery disease is influenced by variations in plasma fatty acid composition. Among n-3 polyunsaturated fatty acids, EPA and DHA exhibited differences in the correlation with the risk of major coronary events.**

J Atheroscler Thromb, 2011; 18:99-107.

Key words; Coronary heart disease, Eicosapentaenoic acid, Docosahexaenoic acid, Lipoprotein

Address for correspondence: Hiroshige Itakura, Shinagawa East One Medical Clinic, 2-16-1 Konan, Minatoku 108-0075, Tokyo, Japan
E-mail: shinagawa-east-one@jouzenkai.or.jp
Received: May 14, 2010
Accepted for publication: September 2, 2010

Introduction

The incidence of coronary artery disease (CAD) is modified by dietary fatty acid composition[1], as is the case for other major CAD risk factors, such as

hypercholesterolemia, hypertension, type-2 diabetes[2], and probably thrombosis. Some fatty acids influence the incidence of CAD through either triglyceride accumulation, inflammation, vasodilation, or platelet aggregation[3], via mediators such as prostaglandins. As shown in the development of metabolic syndrome or insulin resistance[4], excessive accumulation of triglycerides is also a risk factor for CAD. Saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) are the principal ingredients of triglycerides, although details of the relationships between CAD and individual fatty acids remain uncertain; however, some cohort and interventional studies have reported that n-3 polyunsaturated fatty acids (PUFA) have a preventive effect on CAD. The Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico (GISSI) - Prevenzione trial, a randomized control trial using n-3 PUFAs (eicosapentaenoic acid [EPA, C20:5n-3]: docosahexaenoic acid [DHA, C22:6n-3] = 1.2:1) with a purity of ≥85%, reported that n-3 PUFA reduced coronary death in postinfarction patients[5, 6]. Harris and von Schacky found that the concentration of n-3 PUFAs (EPA and DHA) in the erythrocyte membrane (Omega-3 Index) was inversely related to the risk of death from coronary heart disease, and proposed the use of this index as a marker to prevent CAD[7]. Although several studies have examined the relationship between PUFA and the incidence of CAD, no clear consensus yet exists regarding the effects of various fatty acid compositions, including that of PUFA on the development of CAD[8-11]. We also performed the Japan EPA Lipid Intervention Study (JELIS)[12] to investigate whether long-term use of pure EPA decreases the risk of CAD in Japanese hypercholesterolemic patients treated with 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor (pravastatin or simvastatin). At a mean follow-up of 4.6 years, we detected the primary endpoint in 262 (2.8%) patients in the EPA group and 324 (3.5%) controls - a 19% relative reduction ($p = 0.011$)[13]. In addition, we obtained plasma fatty acid concentration data from the participants. In the present supplemental analysis of JELIS, we explored which plasma fatty acids affected the risk of CAD.

Methods

Patients

The enrollment period in JELIS was from November 1996 to November 1999. The planned duration of follow-up of the patients was 5 years, with actual monitoring for a mean of 4.6 (SD1.1) years. The institutional review boards of each facility approved the study, and all patients provided written informed consent. Eligibility criteria were a total cholesterol level of 250 mg/dL or greater, which corresponds to a low-density lipoprotein (LDL) cholesterol level of 170 mg/dL or greater at baseline. The design and inclusion and exclusion criteria were described elsewhere in detail[12]. All patients received 10 mg pravastatin or 5 mg simvastatin once daily as first-line treatment and were counseled to follow the NCEP (National Cholesterol Education Program step) I diet[14].

Study Design

The study population was randomly assigned to receive EPA (EPA group) or not (control group) after a 4- to 8-week washout of antihyperlipidemic drugs. In the EPA group, we administered a daily dose of 1800 mg EPA as 6 capsules, each containing 300 mg of pure (>98%) EPA ethyl ester. The primary endpoint of JELIS was the cumulative incidence of major coronary events (MCE), including sudden cardiac death, fatal or nonfatal myocardial infarction, unstable angina pectoris with documented myocardial ischemia, and angioplasty/stenting or coronary artery bypass grafting. Clinical endpoints were reviewed by expert cardiologists belonging to the Event Evaluation Committee and without knowledge of treatment allocation. Local physicians monitored compliance with dietary instructions and use of medications at each clinic visit.

Measurement of Plasma Fatty Acid

Plasma total fatty acid concentrations were measured annually by a central laboratory (BML Inc., Saitama, Japan) for all patients who gave informed consent for blood sampling to test them. Plasma fatty acid composition was determined by capillary gas chromatography. Briefly, plasma lipids were extracted by Folch's procedure. Then fatty acids (with tricosanoic acid, C23:0, used as the internal standard) were methylated with boron trifluoride and methanol, and methylated fatty acids were analyzed using the SHIMAZU GC-17A gas chromatograph (Shimazu Corporation, Kyoto, Japan) and a BPX70 capillary column (0.25 mm ID × 30 m; SGE International Ltd., Melbourne, Australia).

Statistical Analysis

Differences between the 2 groups with or without EPA treatments were assessed with the χ^2 test for categorical variables and with the Mann-Whitney rank-sum test for continuous data in baseline characteristics. The Mann-Whitney rank-sum test was also used to compare fatty acid values of the quantity of

Table 1. Baseline characteristics of the subjects

	Control group n=8,076	EPA group n=8,321	p value
General characteristics			
Sex (male/female)	2,519/5,557	2,631/5,690	0.555
Age, years	61±9	61±8	0.108
BMI, kg/m^2	24.1±3.3	24.0±3.2	0.619
Systolic blood pressure, mmHg	134.9±20.9	134.9±21.4	0.658
Diastolic blood pressure, mmHg	79.2±12.6	78.9±12.6	0.206
Clinical history			
Coronary artery disease, n (%)	1,550 (19.2)	1,582 (19.0)	0.769
Diabetes, n (%)	1,324 (16.4)	1,357 (16.3)	0.882
Hypertension, n (%)	2,868 (35.5)	2,977 (35.8)	0.724
Smoker, n (%)	1,470 (18.2)	1,648 (19.8)	0.009
Medications, (%)			
Antiplatelet agent, n (%)	1,160 (14.4)	1,121 (13.5)	0.099
Anticoagulant agent, n (%)	246 (3.0)	250 (3.0)	0.876
Nitrate, n (%)	791 (9.8)	753 (9.0)	0.102
Calcium blocker, n (%)	2,501 (31.0)	2,511 (30.2)	0.271
Beta-blocker, n (%)	691 (8.6)	703 (8.4)	0.805

Footnotes: Values are the mean ± S.D. unless otherwise noted.

changes between 2 groups. To evaluate whether on-treatment plasma fatty acid concentrations determined the risk of coronary events in JELIS participants, we calculated the adjusted hazard ratio (HR) of MCE. The HR and its 95% confidence interval (CI) were computed with the Cox proportional hazard model adjusted for age, sex, smoking, history of coronary artery disease, history of diabetes, history of hypertension, use of drugs for coronary artery disease (nitrates, antiplatelet agents, anticoagulant agents), and on-treatment major plasma fatty acid concentrations (C16:0 palmitic acid, C18:0 stearic acid, C18:1 oleic acid, C18:2 linoleic acid, C20:4 arachidonic acid (AA), C20:5 EPA, C22:6 DHA). All probability values of 5% or less (two-sided) were considered significant. Analyses were performed using SAS statistical software (version 9.1; SAS Institute Inc., Cary, NC).

Results

Baseline Characteristics of the Subjects

Table 1 shows the characteristics of the subjects at baseline. Among 18,645 of JELIS participants, 16,397 gave informed consent to annual blood sampling to test plasma fatty acids at registration, and 15,534 during treatment. The rate of smokers was significantly higher (p=0.009) in the EPA group. Except for the rate of smokers, the 2 treatment groups were well-matched at baseline.

Changes in Plasma EPA Concentrations and Other Fatty Acid Profiles with EPA Treatment

Table 2 lists the mean values of serum lipid and plasma fatty acids at baseline (control group; n=8,076, EPA group; n=8,321) and the quantity of change from the baseline. Serum lipid values at baseline did not differ between the two groups. On-treatment LDL cholesterol levels were similar in both groups. Triglyceride level in the EPA group was decreased more than in the control group. Plasma SFA, MUFA, and n-6 PUFA levels in the EPA group were decreased more than in the control group. Among n-3 PUFAs, quantities of changes in plasma docosapentaenoic acid (DPA) and EPA concentrations in the EPA group were significantly higher than those in the control group; however, DHA concentrations were not increased at all by EPA treatment.

Fig. 1 shows histograms of baseline and on-treatment plasma concentrations of EPA and EPA/AA ratios. The mean levels of plasma EPA concentrations at baseline were 93 μg/mL in the control group and 97 μg/mL in the EPA group. On-treatment mean plasma EPA concentrations were 95 μg/mL in the control group and 170 μg/mL in the EPA group (**Fig. 1A**). The mean levels of plasma EPA/AA ratios at baseline were 0.59 in the control group and 0.62 in the EPA group. On-treatment mean plasma EPA/AA ratios were 0.60 in the control group and 1.21 in the EPA group (**Fig. 1B**).

Table 2. Mean serum LDL-cholesterol, HDL-cholesterol, triglyceride, and plasma fatty acid levels at baseline and quantity of changes

	Baseline		Quantity of change		
	Control n=8,076	EPA n=8,321	Control	EPA	p value
LDL-cholesterol (mg/dL)	182±29	182±29	−46±36	−45±38	0.156
HDL-cholesterol (mg/dL)	58±17	59±18	1±16	0.3±15	0.001
Triglyceride (mg/dL)	190±154	188±143	−31±138	−37±124	<0.001
Saturated fatty acid					
Myristic acid (C14:0, μg/mL)	34±25	34±26	−1±30	−3±26	<0.001
Palmitic acid (C16:0, μg/mL)	748±313	749±329	3±339	−27±303	<0.001
Stearic acid (C18:0, μg/mL)	228±74	228±77	1±87	−3±76	0.008
Monounsaturated fatty acid					
Palmitoleic acid (C16:1, μg/mL)	93±58	93±60	−6±54	−13±54	<0.001
Oleic acid (C18:1, μg/mL)	691±370	687±360	−8±394	−49±345	<0.001
n-6 polyunsaturated fatty acid					
Linoleic acid (C18:2, μg/mL)	835±239	834±246	10±268	−38±260	<0.001
γ-Linolenic acid (C18:3, μg/mL)	15±9	15±8	−2±8	−4±8	<0.001
Dihomo-γ-Linolenic acid (C20:3, μg/mL)	35±13	35±13	0.2±12	−6±12	<0.001
Arachidonic acid (C20:4, μg/mL)	162±41	162±42	8±38	−9±38	<0.001
n-3 polyunsaturated fatty acid					
α-Linolenic acid (C18:3, μg/mL)	20±12	20±13	6±17	5±16	<0.001
Eicosapentaenoic acid (C20:5, μg/mL)	93±51	97±55	2±55	69±83	<0.001
Docosapentaenoic acid (C22:5, μg/mL)	25±11	25±11	4±13	17±19	<0.001
Docosahexaenoic acid (C22:6, μg/mL)	169±61	170±61	−2±58	−14±56	<0.001

Footnotes: Values are the mean±S.D.

Relationships between Fatty Acid Concentrations and Risk of MCE

The associations of on-treatment plasma fatty acid values with the risk of MCE are shown in **Fig. 2**. We divided the patients into 2 groups according to mean on-treatment major plasma fatty acid levels, and calculated the hazard ratio of MCE in the higher group relative to the lower group as a standard. In the group of all 15,534 participants (control group; n=7,722, EPA group; n=7,812), a high EPA concentration (above 133 μg/mL as mean) was significantly associated with a lower risk of MCE (HR 0.83, 95% CI: 0.68 to 0.99; p=0.049). Stearic acid concentration (above 225 μg/mL as mean) was also significantly associated with a lower risk of MCE (HR 0.71, 95% CI: 0.54 to 0.93; p=0.014). Linoleic acid exhibited a positive correlation with the risk of MCE, although the relationships were not significant. None of the other fatty acid concentrations exhibited a relationship with the risk of MCE (**Fig. 2A**). In the control group of 7,722 subjects, even a high EPA concentration (above 95 μg/mL as the mean) was not associated with the risk of MCE. Only a high linoleic acid concentration was significantly associated with a higher risk of MCE (HR 1.33, 95% CI: 1.02 to 1.74; p=0.039). Other fatty acids did not exhibit a significant relationship with the risk of MCE (**Fig. 2B**). In the EPA group of 7,812 subjects, a high EPA concentration (above 170 μg/mL as the mean) was significantly associated with a lower risk of MCE (HR 0.71, 95% CI: 0.54 to 0.94; p=0.018). Other fatty acid concentrations did not exhibit a relationship with the risk of MCE (**Fig. 2C**).

We examined in detail the relationship between plasma EPA concentration and the risk of MCE to provide a target level of EPA for the prevention of MCE. As shown in **Fig. 3**, the risk of MCE in all 15,534 participants was significantly lower in the group with the highest plasma EPA concentration (≥150 μg/mL) than in the group with the lowest concentration (<87 μg/mL, median plasma EPA concentration in the control group during the follow-up period) (adjusted HR 0.80, p=0.042). In addition, the risk of MCE in the group with a plasma EPA concentration above 100 μg/mL was lower, although not significantly so, than in the group with a concentration of less than 100 μg/mL. The risk of MCE in groups with a concentration above 150 μg/mL and

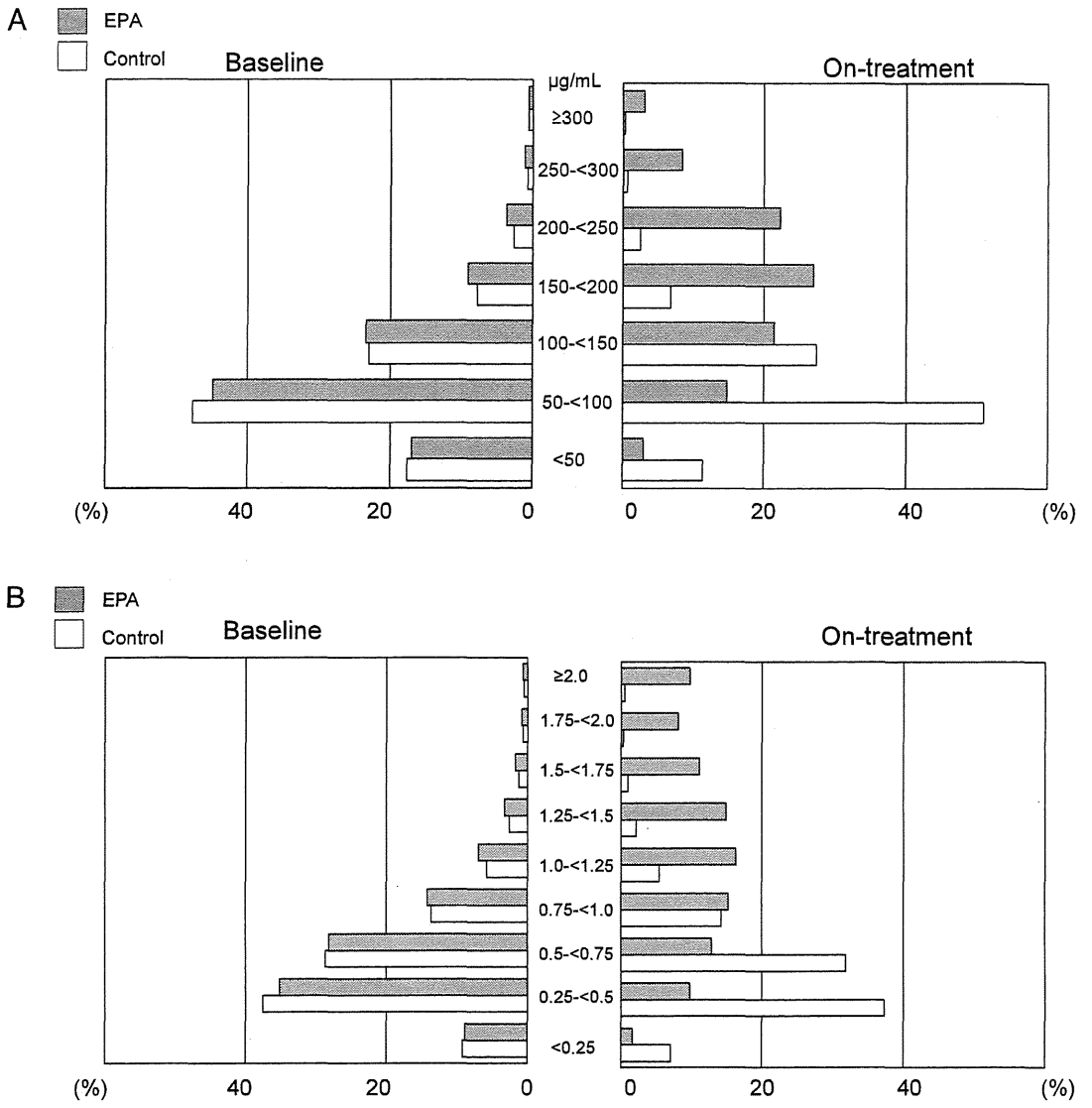

Fig. 1. Distribution of plasma EPA and EPA/AA ratio concentrations.

Footnotes: A) EPA concentrations; B) EPA/AA ratios
Columns show frequencies of patients.

200 μg/mL was significantly lower than in groups with a concentration less than 150 μg/mL (HR=0.82, p=0.032) and 200 μg/mL (HR=0.78, p=0.043), respectively (**Table 3**). Ten percent of patients in the control group and 61% of those in the EPA group attained 150 μg/mL or higher plasma EPA concentration. In addition, the risk of MCE in groups with EPA/AA ratio above 0.75 and 1 was significantly lower than in groups with the ratio less than 0.75 (HR=0.83, p=0.031) and 1 (HR=0.80, p= 0.021), respectively (**Table 3**).

Discussion

Fatty acids are classified as saturated fatty acids, monounsaturated fatty acids, and the n-6 and n-3 polyunsaturated fatty acids. All these fatty acids are known to have different effects. SFA and MUFA are the principal ingredients of triglycerides, although details of the relationships between CAD and individual fatty acids remain uncertain; however, some cohort and interventional studies have reported that n-3PUFA has a preventive effect on CAD. In contrast, oversupply of n-6 PUFA increases the risk of CAD, because of the inflammatory and thrombogenetic

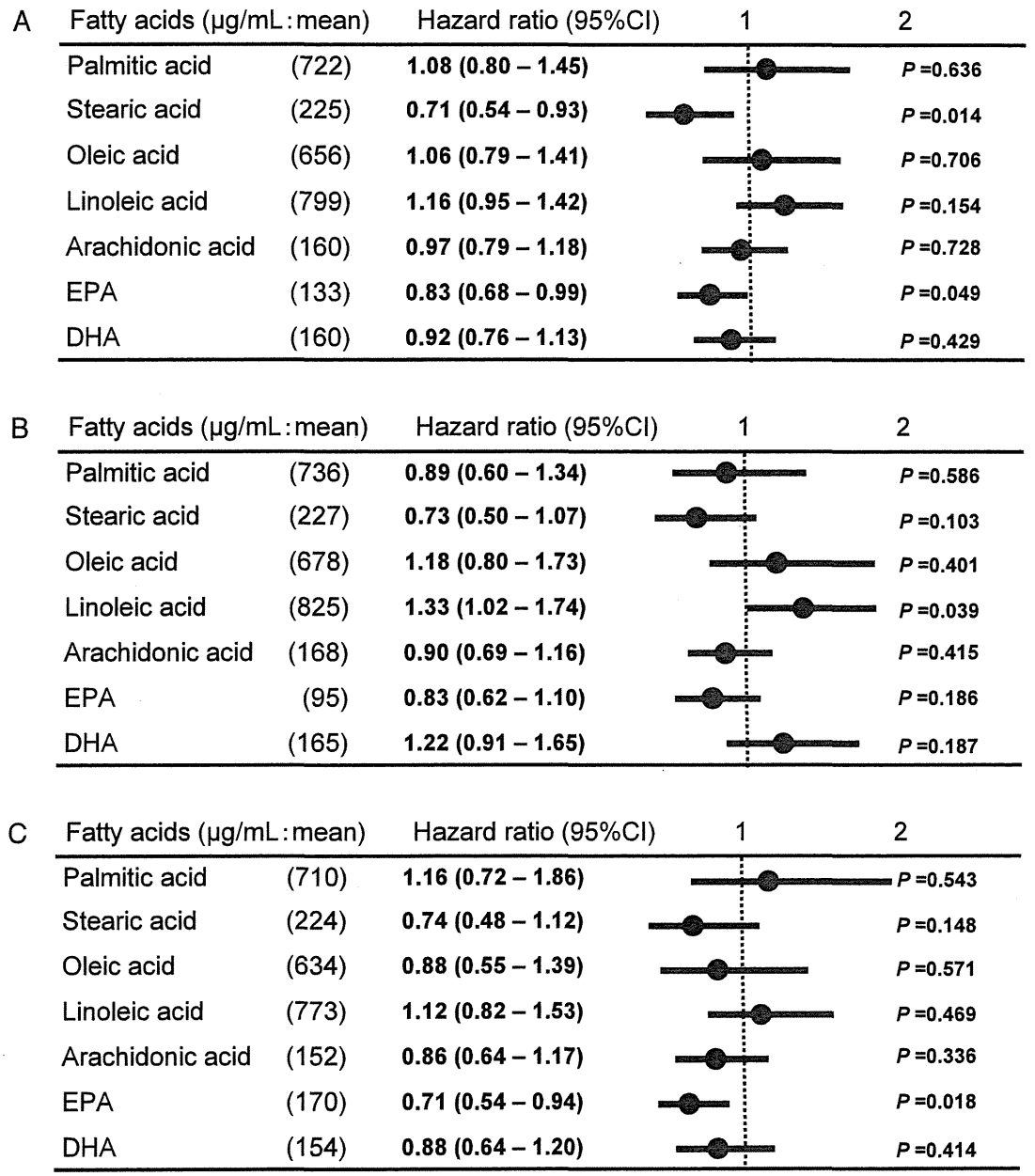

Fig. 2. Relationships between fatty acid levels and major coronary events.

Footnotes: A) All participants; B) Control group; C)EPA group
Risks of major coronary events in the higher groups were calculated relative to the standard in the lower group.
The unit for plasma fatty acids is μg/mL.

effects of metabolites of AA[15].

We had already found that EPA reduced MCE by 19% in a previous study[13], and the result of this sub-analysis of all participants confirmed the findings of JELIS with regard to the composition of fatty acids. In the control group, neither EPA nor DHA concentration exhibited a significant relationship with the risk of coronary events. NIPPON DATA80, which observed 8,879 Japanese men and women from 1980 to 1999, reported that the frequency of fish consumption was associated, although not to a significant extent, with decreased risk of death due to coronary artery disease. The authors speculated that the reason for this was that the majority of Japanese subjects in their study ate fish in amounts above the threshold level shown to be beneficial in previous studies[16]. The

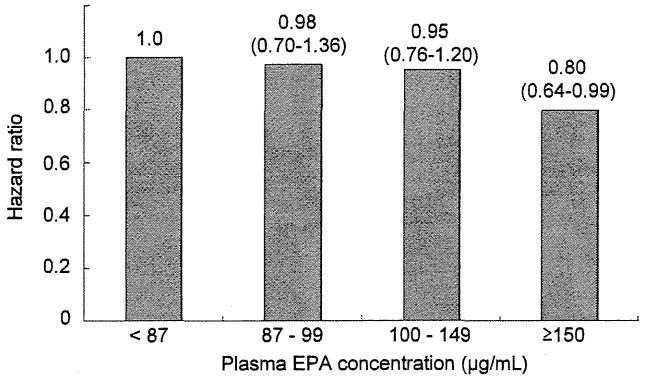

Fig. 3. Relationship between on-treatment EPA concentration and adjusted risk of major coronary events.

Japan Public Health Center-Based (JPHC) Study Cohort I examined the association between a high intake of fish and n-3 PUFAs and the risk of CAD in 41,578 Japanese men and women aged 40 to 59 years who were free of a prior diagnosis of cardiovascular diseases. The study group reported a strong inverse association between dietary intake of n-3 PUFA and the risk of definite myocardial infarction and nonfatal coronary events[17]. In the EPA group in the present study, a higher plasma EPA concentration was associated with a significant reduction of 29% ($p=0.018$) in the risk of MCE. Our findings suggested that larger samples or stronger intervention with EPA agents may be needed to detect significant benefits of EPA in Japanese clinical trials or surveillance. In this analysis, we found that increasing plasma EPA concentration reduced the risk of coronary events. On the other hand, DHA might not play a role in the prevention of coronary events by EPA since the plasma DHA concentration was not increased. We therefore could not determine whether the increase in DHA concentration did not decrease the risk of MCE.

We found that the risk of MCE in all participants was significantly lower in the group with a high plasma EPA concentration (150 μg/mL or more) than in the group with a low concentration. Furthermore, we found that the risk of MCE was significantly lower in the group with a high plasma EPA/AA ratio (0.75 or more) than in the group with a low ratio. It was noted that AA accelerates platelet aggregation and inflammatory reactions, and EPA works as an antagonistic regulator of AA. Recently, a significant positive correlation between EPA/AA ratios and insulin sensitivity as well as a negative correlation between those ratios and insulin resistance were observed in a subject with metabolic syndrome[18]. In fact, we expect that the EPA/AA ratio, which shows the balance of each PUFA concentration, may be used as a precise biomarker for arteriosclerotic disease; however, our results cannot provide definitive target levels since they were obtained through a comparison of control patients with a relatively low EPA concentration in the Japanese population. The desirable EPA value for a much greater decrease in the incidence of coronary artery disease may differ depending on eating habits and risks. Large-scale clinical studies and analyses from more perspectives should be conducted to specify the desirable EPA level for lowering the risk of coronary events. Additionally, among saturated fatty acids, stearic acid concentration, but not palmitic acid concentration was associated with a lower risk of MCE. Many clinical studies have shown the heterogeneity of the effect of saturated fatty acids on risk factors. Lauric, myristic, and palmitic acids are known to be hypercholesterolemic; however, stearic acid is not hypercholesterolemic, and so its intake might beneficially affect CVD risk reduction. The P/S (polyunsaturated/saturated) ratio has long been used as an index in dietary counseling to prevent CAD, but its composition might need to be reconsidered; however, it is unclear whether stearic acid itself protects against cardiovascular disease.

Table 3. Hazard ratios of major coronary events by cut-off point of on-treatment plasma EPA concentration and EPA/AA ratio

Plasma EPA concentration (μg/mL)	Hazard ratio	95% CI	*p* value
Low (<100) vs High (≧100)	0.87	0.72-1.03	0.110
Low (<150) vs High (≧150)	0.82	0.68-0.98	0.032
Low (<200) vs High (≧200)	0.78	0.62-0.99	0.043
Plasma EPA/AA ratio	**Hazard ratio**	**95% CI**	***p* value**
Low (<0.50) vs High (≧0.50)	0.94	0.77-1.14	0.519
Low (<0.75) vs High (≧0.75)	0.83	0.69-0.98	0.031
Low (<1) vs High (≧1)	0.80	0.67-0.97	0.021

JELIS reported that the differences in changes in HDL cholesterol and LDL cholesterol between the two treatment groups were very small[13]. The question thus arises how EPA exhibited its beneficial effects. We speculate that anti-inflammatory effects of EPA might be principally responsible for reducing atherosclerotic lesions and prevent cardiovascular events[15, 19-25]. Concerning the prevention of CAD by EPA as well as its anti-inflammatory effect, it should be noted that EPA exhibits an antiarrhythmic effect[26], inhibits platelet aggregation[27, 28], exhibits vasodilatory activity[29], increases the circulating adiponectin level[30], has a triglyceride-lowering effect[31], and induces plaque stabilization[24, 32]. These effects may function synergistically to prevent CAD. Further study of the relationship between atherosclerosis and these fatty acids is warranted.

In conclusion, plasma fatty acid concentrations were found to be correlated with the risk of coronary events, and administration of pure EPA was found to affect this relationship. Plasma EPA concentration and the EPA/AA ratio may be used as biomarkers of CAD.

Acknowledgements

We thank all trial participants and the large numbers of physicians, nurses, and hospital staff members who made long-term commitments to the study, as well as the patients who participated in the trial.

Sources of Funding

Mochida Pharmaceutical Co. Ltd, Tokyo, Japan bore expenses for the determination of plasma fatty acid concentration. The marketed capsules containing 300 mg EPA ethyl ester were supplied by Mochida Pharmaceutical Co. Ltd. H Itakura received travel funds from Mochida Pharmaceutical Co. Ltd to participate in a scientific meeting. None of the other committee members or investigators received remuneration for performing the study.

References

1) Wolfram G: Dietary fatty acids and coronary heart disease. Eur J Med Res, 2003; 8: 321-324
2) Yamamoto A, Richie G, Nakamura H, Hosoda S, Nobuyoshi M, Matsuzaki M, Tan CE, Keech A, Mabuchi H, Horibe H, Tenba H: Risk factors for coronary heart disease in the Japanese--comparison of the background of patients with acute coronary syndrome in the ASPAC study with data obtained from the general population. Asia-Pacific Collaboration on CHD Risk Factor Intervention study. J Atheroscler Thromb, 2002; 9: 191-199
3) Harris WS: The omega-6/omega-3 ratio and cardiovascular disease risk: uses and abuses. Curr Atheroscler Rep, 2006; 8: 453-459
4) Arai H, Yamamoto A, Matsuzawa Y, Saito Y, Yamada N, Oikawa S, Mabuchi H, Teramoto T, Sasaki J, Nakaya N, Itakura H, Ishikawa Y, Ouchi Y, Horibe H, Shirahashi N, Kita T: Prevalence of metabolic syndrome in the general Japanese population in 2000. J Atheroscler Thromb, 2006; 13: 202-208
5) Gruppo Italiano per lo Studio della Sopravvivenza nell' Infarto miocardico: Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Lancet, 1999; 354: 447-455
6) Marchioli R, Barzi F, Bomba E, Chieffo C, Di Gregorio D, Di Mascio R, Franzosi MG, Geraci E, Levantesi G, Maggioni AP, Mantini L, Marfisi RM, Mastrogiuseppe G, Mininni N, Nicolosi GL, Santini M, Schweiger C, Tavazzi L, Tognoni G, Tucci C, Valagussa F: Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. Circulation, 2002; 105: 1897-1903
7) Harris WS, Von Schacky C: The Omega-3 Index: a new risk factor for death from coronary heart disease? Prev Med, 2004; 39: 212-220
8) Leskinen MH, Solakivi T, Kunnas T, Alho H, Nikkari ST: Serum fatty acids in postinfarction middle-aged men. Scand J Clin Lab Invest, 2005; 65: 485-490
9) Yli-Jama P, Meyer HE, Ringstad J, Pedersen JI: Serum free fatty acid pattern and risk of myocardial infarction: a case-control study. J Intern Med, 2002; 251: 19-28
10) Kark JD, Manor O, Goldman S, Berry EM: Stability of red blood cell membrane fatty acid composition after acute myocardial infarction. J Clin Epidemiol, 1995; 48: 889-895
11) Luostarinen R, Boberg M, Saldeen T: Fatty acid composition in total phospholipids of human coronary arteries in sudden cardiac death. Atherosclerosis, 1993; 99: 187-193
12) Yokoyama M, Origasa H: Effects of eicosapentaenoic acid on cardiovascular events in Japanese patients with hypercholesterolemia: rationale, design, and baseline characteristics of the Japan EPA Lipid Intervention Study (JELIS). Am Heart J, 2003; 146: 613-620
13) Yokoyama M, Origasa H, Matsuzaki M, Matsuzawa Y, Saito Y, Ishikawa Y, Oikawa S, Sasaki J, Hishida H, Itakura H, Kita T, Kitabatake A, Nakaya N, Sakata T, Shimada K, Shirato K: Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. Lancet, 2007; 369: 1090-1098
14) Oliver MF: National cholesterol policies. Eur Heart J, 1993; 14: 581-583
15) James MJ, Gibson RA, Cleland LG: Dietary polyunsaturated fatty acids and inflammatory mediator production. Am J Clin Nutr, 2000; 71: 343S-348S
16) Nakamura Y, Ueshima H, Okamura T, Kadowaki T, Hayakawa T, Kita Y, Tamaki S, Okayama A: Association

between fish consumption and all-cause and cause-specific mortality in Japan: NIPPON DATA80, 1980-99. Am J Med, 2005; 118: 239-245
17) Iso H, Kobayashi M, Ishihara J, Sasaki S, Okada K, Kita Y, Kokubo Y, Tsugane S; JPHC Study Group: Intake of fish and n3 fatty acids and risk of coronary heart disease among Japanese: the Japan Public Health Center-Based (JPHC) Study Cohort I. Circulation, 2006; 17: 195-202
18) Yanagisawa N, Shimada K, Miyazaki T, Kume A, Kitamura Y, Ichikawa R, Ohmura H, Kiyanagi T, Hiki M, Fukao K, Sumiyoshi K, Hirose K, Matsumori R, Takizawa H, Fujii K, Mokuno H, Inoue N, Daida H: Polyunsaturated fatty acid levels of serum and red blood cells in apparently healthy Japanese subjects living in an urban area. J Atheroscler Thromb, 2010; 17: 285-294
19) Zhao Y, Joshi-Barve S, Barve S, Chen LH: Eicosapentaenoic acid prevents LPS-induced TNF-alpha expression by preventing NF-kappaB activation. J Am Coll Nutr, 2004; 23: 71-78
20) Serhan CN: Resolution phase of inflammation: novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. Annu Rev Immunol, 2007; 25: 101-137
21) Schwab JM, Chiang N, Arita M, Serhan CN: Resolvin E1 and protectin D1 activate inflammation-resolution programmes. Nature, 2007; 447: 869-874
22) Arita M, Bianchini F, Aliberti J, Sher A, Chiang N, Hong S, Yang R, Petasis NA, Serhan CN: Stereochemical assignment, antiinflammatory properties, and receptor for the omega-3 lipid mediator resolvin E1. J Exp Med, 2005; 201: 713-722
23) Dona M, Fredman G, Schwab JM, Chiang N, Arita M, Goodarzi A, Cheng G, von Andrian UH, Serhan CN: Resolvin E1, an EPA-derived mediator in whole blood, selectively counterregulates leukocytes and platelets. Blood, 2008; 112: 848-855
24) Matsumoto M, Sata M, Fukuda D, Tanaka K, Soma M, Hirata Y, Nagai R: Orally administered eicosapentaenoic acid reduces and stabilizes atherosclerotic lesions in ApoE-deficient mice. Atherosclerosis, 2008; 197: 524-533
25) Jia Y, Turek JJ: Altered NF-kappaB gene expression and collagen formation induced by polyunsaturated fatty acids. J Nutr Biochem, 2005; 16: 500-506
26) Leaf A, Albert CM, Josephson M, Steinhaus D, Kluger J, Kang JX, Cox B, Zhang H, Schoenfeld D: Prevention of fatal arrhythmias in high-risk subjects by fish oil n-3 fatty acid intake. Circulation, 2005; 112: 2762-2768
27) Terano T, Hirai A, Hamazaki T, Kobayashi S, Fujita T, Tamura Y, Kumagai A: Effect of oral administration of highly purified eicosapentaenoic acid on platelet function, blood viscosity and red cell deformability in healthy human subjects. Atherosclerosis, 1983; 46: 321-331
28) Hirai A, Terano T, Makuta H, Ozawa A, Fujita T, Tamura Y, Yoshida S: Effect of oral administration of highly purified eicosapentaenoic acid and docosahexaenoic acid on platelet function and serum lipids in hyperlipidemic patients. Adv Prostaglandin Thromboxane Leukot Res, 1989; 19: 627-630
29) Terano T, Shiina T, Tamura Y: Eicosapentaenoic acid suppressed the proliferation of vascular smooth muscle cells through modulation of various steps of growth signals. Lipids, 1996; 31 Suppl: S301-304
30) Nomura S, Shouzu A, Omoto S, Inami N, Ueba T, Urase F, Maeda Y: Effects of eicosapentaenoic acid on endothelial cell-derived microparticles, angiopoietins and adiponectin in patients with type 2 diabetes. J Atheroscler Thromb, 2009; 16: 83-90
31) Nozaki S, Matsuzawa Y, Hirano K, Sakai N, Kubo M, Tarui S: Effects of purified eicosapentaenoic acid ethyl ester on plasma lipoproteins in primary hypercholesterolemia. Int J Vitam Nutr Res, 1992; 62: 256-260
32) Thies F, Garry JM, Yaqoob P, Rerkasem K, Williams J, Shearman CP, Gallagher PJ, Calder PC, Grimble RF: Association of n-3 polyunsaturated fatty acids with stability of atherosclerotic plaques: a randomised controlled trial. Lancet, 2003; 361: 477-485

Serum Level of Triglycerides Is a Potent Risk Factor Comparable to LDL Cholesterol for Coronary Heart Disease in Japanese Patients with Type 2 Diabetes: Subanalysis of the Japan Diabetes Complications Study (JDCS)

Hirohito Sone, Sachiko Tanaka, Shiro Tanaka, Satoshi Iimuro, Koji Oida, Yoshimitsu Yamasaki, Shinichi Oikawa, Shun Ishibashi, Shigehiro Katayama, Yasuo Ohashi, Yasuo Akanuma, and Nobuhiro Yamada, for the Japan Diabetes Complications Study Group*

Context: Risk factors for cardiovascular complications in Japanese patients with diabetes have not been fully elucidated.

Objective: Our objective was to determine incidence of and risk factors for coronary heart disease (CHD) and stroke in Japanese diabetic patients.

Design and Settings: We conducted a prospective study at 59 hospitals throughout Japan.

Patients: Patients included 940 men and 831 women with type 2 diabetes (mean age, 58.2 yr) without a history of cardiovascular complications who were followed for a median of 7.86 yr.

Intervention: This was an observational study.

Main Outcome Measures: Incidence of CHD and stroke was evaluated.

Results: Incidences of CHD and stroke per 1000 person-years were 9.59 and 7.45, respectively, whereas those of myocardial and brain infarctions were 3.84 and 6.29, respectively. Multivariate Cox analysis revealed that the serum log-transformed triglyceride level was a potent and independent predictor of CHD [hazard ratio (HR) = 1.54; 95% confidence interval (CI) = 1.22–1.94 per 1 SD increase), comparable to low-density lipoprotein (LDL) cholesterol (HR = 1.49; 95% CI = 1.25–1.78 per 1 SD increase). Triglycerides and LDL cholesterol linearly and continuously increased CHD risk, and subjects in the top third for both had markedly high risks of CHD, and their effects were possibly additive. However, serum triglycerides worked independently of blood pressure levels. Systolic blood pressure was the only significant predictor for stroke except for age (HR = 1.31; 95% CI = 1.04–1.65, per 1 SD increase).

Conclusions: In Japanese patients with type 2 diabetes, the serum triglyceride level was a leading predictor of CHD, comparable to LDL cholesterol. Because the serum triglyceride level is not a leading predictor of CHD in diabetic subjects in Western countries, ethnic group-specific strategies for prevention of diabetic macroangiopathy may be indicated. **(*J Clin Endocrinol Metab* 96: 3448–3456, 2011)**

As in other regions of the world, type 2 diabetes also confers a substantially enhanced risk of cardiovascular disease (CVD) in East Asia where the diabetic population has been explosively increasing (1). Compared with type 2 diabetic patients in Western countries, those in East Asian countries, including Japan, are suggested to have different features regarding cardiovascular complications. Diabetic patients in East Asia have a much lower

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2011-0622 Received March 9, 2011. Accepted July 27, 2011.
First Published Online August 24, 2011

* Author affiliations are shown at the bottom of the next page.

Abbreviations: CHD, Coronary heart disease; CVD, cardiovascular disease; ECG, electrocardiogram; GFR, glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; UKPDS, United Kingdom Prospective Diabetes Study.

incidence of coronary heart disease (CHD) than those in Western countries (2), and CVD is not necessarily a leading cause of mortality among diabetic patients in Japan (3). However, the incidence of stroke in East Asia, which includes Japan, is higher than that of myocardial infarction, which is opposite to what has been observed among Western diabetic subjects (2). In addition, as we previously reported, many other traits regarding cardiovascular complications differ considerably between Western and Japanese diabetic populations, such as the influence of metabolic syndrome (4, 5) or alcohol drinking (6), the relationship between predictors for macro- and microvascular complications (7), or the degree of obesity (8, 9), which is closely associated with insulin resistance and atherosclerosis.

Risk factors for cardiovascular complications in diabetic subjects have been predominantly reported in White, Black, and Hispanic subjects in Western countries, but those issues in East Asians, including Japanese, with diabetes have not been fully elucidated. In particular, although the serum triglyceride level seems to have a significant influence on cardiovascular complications in diabetic subjects in East Asia (10–12), it has not been confirmed by large-scale prospective studies. Clarifying those issues could contribute to ethnic group-specific diabetes care and prevention of cardiovascular disease for those of Asian origin, who account for more than 60% of the world's diabetes population (1). Therefore, we investigated the association between risk factors and the incidence of CHD and stroke in a nationwide clinical trial of Japanese patients with type 2 diabetes mellitus.

Patients and Methods

Recruitment of patients

The present analysis was conducted as part of the Japan Diabetes Complications Study, a multicenter prospective study on the incidence of and risk factors for macro- and microvascular complications among Japanese patients with type 2 diabetes (4). For this analysis of macrovascular complications, 940 men (mean age 57.8 ± 7.1 yr) and 831 women (mean age 58.7 ± 6.8 yr) who registered from January 1995 to March 1996 from outpatient clinics in 59 university and general hospitals nationwide that specialize in diabetes care were selected after consideration of the exclusion criteria prespecified in the study protocol. Excluded were patients with impaired glucose tolerance, a history of angina pectoris, myocardial infarction, stroke, peripheral artery disease, familial hypercholesterolemia, type III hyperlipidemia (diagnosed by broad β-band on electrophoresis) or nephrotic syndrome (urine protein $\geq$3.5 g/d and serum total protein $\leq$6.0 mg/dl), and serum creatinine levels greater than 1.3 mg/dl (120 μmol/liter).

Diabetes mellitus and impaired glucose tolerance were diagnosed according to the Report of the Committee of the Japan Diabetes Society on the Classification and Diagnostic Criteria of Diabetes Mellitus, which is almost identical in terms of cutoff values for glucose levels to those of the World Health Organization (WHO). The protocol for the study, which is in accordance with the Declaration of Helsinki and the Ethical Guidelines for Clinical/Epidemiological Studies of the Japanese Ministry of Health Labor and Welfare, received ethical approval from the institutional review boards of all of the participating institutes. Written informed consent was obtained from all patients enrolled.

Clinical and laboratory measurements

Patients were assessed yearly after the baseline evaluation. Mean values of at least two measurements each year were obtained for glycated hemoglobin (HbA1c), fasting plasma glucose, and fasting serum lipids. HbA1c assays were performed according to procedures outlined by the Laboratory Test Committee of the Japan Diabetes Society with the standard samples supplied by the society, which is known to be converted by the formula HbA1c (Japan Diabetes Society) (percent) = HbA1c (National Glycohemoglobin Standardization Program) (percent) − 0.4%, with 5.8% as the upper limit of normal. All other laboratory tests were done at each participating institute. Serum low-density lipoprotein (LDL) cholesterol was calculated using Friedewald's equation, except where triglycerides exceeded 400 mg/dl, in which case the LDL cholesterol data were treated as missing. This was applicable to 20 subjects. The estimated glomerular filtration rate (GFR) was calculated according to the following equation generated by The Japanese Society of Nephrology: GFR (milliliter per minute per 1.73 m^2) = 194 × serum creatinine$^{-1.094}$ × age$^{-0.287}$ × 0.739 (if female) (13). All other measurements, including those for body weight, blood pressure, and waist/hip circumference, were done at least once yearly. Waist and hip circumferences were measured at the levels of the umbilicus and trochanters, respectively. A 12-lead electrocardiogram (ECG) and chest x-ray were performed annually. A baseline dietary survey, comprised of food records and a food frequency questionnaire that included alcohol consumption, was undertaken. Information regarding cigarette smoking was collected using a self-administered questionnaire.

Outcome measures

The outcomes considered in the analysis were a fatal or first nonfatal manifestation of CHD or stroke, all of which were diagnosed yearly by predefined criteria (4). CHD consists of angina pectoris and myocardial infarction, and their diagnoses were according to criteria defined by the WHO/MONICA (Multinational Monitoring of Trends and Determinants in Cardiovascular Disease) project (14, 15), and angina pectoris was defined as

Department of Internal Medicine (H.S.), University of Tsukuba Institute of Clinical Medicine, Ibaraki, Japan 310-0015; EBM Research Center (Sh.T.) and Translational Research Center (Sa.T.), Kyoto University School of Medicine, Kyoto, Japan 606-8507; Department of Biostatistics and Epidemiology (S.Ii., Y.O.), University of Tokyo School of Medicine, Tokyo, Japan 113-0033; Fukui Chuo Clinic (K.O.), Fukui, Japan 910-0023; Center for Advanced Science and Innovation (Y.Y.), Osaka University, Osaka, Japan 565-0871; Department of Medicine (S.O.), Nippon Medical School, Tokyo, Japan 113-8602; Department of Endocrinology and Metabolism (S.Is.), Jichi Medical College, Tochigi, Japan 329-0498; The Fourth Department of Medicine (S.K.), Saitama Medical School, Saitama, Japan 350-0495; The Institute for Adult Diseases Asahi Life Foundation (Y.A.), Tokyo, Japan 160-0023; and University of Tsukuba (N.Y.),305-0006

typical effort-dependent chest pain or oppression relieved at rest or by use of nitroglycerine as validated by an exercise-positive ECG and/or angiography. A patient with a first percutaneous coronary intervention or coronary artery bypass graft was also counted as having a CHD event.

Diagnosis of stroke was according to guidelines defined by the Ministry of Health, Labor, and Welfare of Japan (16) and WHO criteria (17). Stroke events were defined as a constellation of focal or global neurological deficits or disturbance of cerebral function that was sudden or rapid in onset and for which there was no apparent cause other than a vascular accident such as epilepsy or brain tumors on the basis of a detailed history, neurological examination, and ancillary diagnostic procedures such as computed tomography, magnetic resonance imaging, cerebral angiography, and lumbar puncture. Stroke events were classified as cerebral infarction (including embolus), intracranial hemorrhage (including subarachnoid hemorrhage), transient ischemic attack, or stroke of undetermined type in accordance with WHO criteria (17). No cases of asymptomatic lesions detected by brain imaging (*i.e.* silent infarction) were included. Only first-ever CHD or stroke events during the study period were counted in the analysis and in a patient having both CHD and stroke events; each event was counted separately. Information regarding primary outcome and other clinical parameters for each subject was collected through an annual report from each physician. Adjudication of endpoints was made by central committees comprised of experts in each complication who were masked as to risk factor status based on additional data such as computed tomography or magnetic resonance imaging of the brain or sequential changes in ECG.

Statistical analysis

All statistical analyses and data management were conducted at a central data center. Patient characteristics were described as mean ± SD, median, interquartile range, or percentage. After dividing subjects into three groups, *i.e.* CHD, stroke, or no CVD (subject with neither CHD nor stroke), we compared the no-CVD group with the CHD or stroke groups separately by Dunnett's *t* test and Fisher's exact test for numerical and categorical variables, respectively. Univariate and multivariate Cox regression analyses were used to estimate the adjusted hazard ratios (HR) and 95% confidence intervals (CI) for risk factors. A histogram was used to check normality of distributions of these variables. The distributions of triglycerides and lipoprotein(a) were skewed, and we conducted Cox analysis using the log-transformed values after confirming their normality instead of using the raw data. To directly compare the impact of risk factors that have different units or means, we also calculated the HR per 1 SD increment for most variables. To explore potential nonlinear relationships, we used multivariate-adjusted generalized additive models with a spline function of three degrees of freedom. All *P* values are two sided, and the significance level is 0.05. All statistical analyses were conducted using SAS packages version 9.2 (SAS Institute Inc., Cary, NC).

Results

Table 1 summarizes the baseline characteristics according to the occurrence of CHD and stroke events. During the median follow-up period of 7.86 yr, the total person-years studied were 11,743 (6106 for men and 5637 for women), and the crude incidence per 1000 patient-years of all CHD was 9.59 (11.18 in men and 7.85 in women), and among those with CHD, that of myocardial infarction was 3.84 (4.95 in men and 2.62 in women). The crude incidence per 1000 patient-years of all strokes was 7.45 (8.70 in men and 6.07 in women); among these, the incidence of brain infarction was 6.29 (7.44 in men and 5.03 in women). In terms of therapeutic measures, the proportion of patients who were being administered antihypertensive agents was significantly higher among those in whom CHD or stroke occurred, and the proportion of patients receiving biguanides was more than double in patients with stroke in comparison with those who did not experience these events.

HR with a 95% CI of each factor for CHD and stroke events estimated by the Cox regression analysis are shown in Table 2. In multivariate analysis for CHD, serum levels of both LDL cholesterol and log-transformed triglycerides were the most significant and the strongest predictors, having almost identical HR of 1.5 with an increment of 1 SD of each variable. These results were fundamentally the same even when the HR were calculated with an increment of 1 mmol/liter of these lipid variables [LDL cholesterol, 1.64 (95% CI = 0.33–2.02), $P < 0.001$; triglycerides, 1.63 (95% CI = 1.29–2.07), $P < 0.001$]. HbA1c and systolic blood pressure had borderline significance, with *P* values <0.1. With regard to stroke, except for age, systolic blood pressure was the only other significant predictor. Spline curves of the HR with 95% CI between CHD events and LDL cholesterol or triglyceride levels (Fig. 1) demonstrated linear positive relationships for both lipid variables.

Because the serum triglyceride level was found to be a characteristically strong predictor for CHD in our cohort, we investigated the combined roles of triglycerides with other major risk factors for CHD (Fig. 2). In comparison with subjects in the bottom third of both triglyceride values and either body mass index, HbA1c, systolic blood pressure, or LDL cholesterol as the reference (reference HR = 1), the risk for CHD in subjects with values in the top third for both triglycerides and either of these factors was approximately three times higher, which is statistically significant. Subjects in the top third for triglycerides were at a significantly high risk of CHD regardless of the concurrent systolic blood pressure levels. In contrast, subjects in the top third for both triglycerides and LDL cholesterol had a markedly high risk of CHD, and their effects were possibly additive, although the *P* value for interaction was 0.66. HbA1c also appears to work additively

TABLE 1. Patient characteristics at baseline according to occurrence of CHD and stroke events (mean ± SD)

	No CVD	CHD	*P* value (*vs.* no CVD)	Stroke	*P* value (*vs.* no CVD)
No. of patients (no. of women)	1577 (757)	109 (41)	0.036[a]	85 (33)	0.099[a]
Age (yr)	58.2 ± 7.0	59.8 ± 6.5	0.037	60.7 ± 6.1	0.003
Diabetes duration (yr)	10.8 ± 7.2	11.7 ± 6.8	0.38	11.0 ± 6.9	0.97
BMI (kg/m^2)	23.0 ± 3.0	23.4 ± 2.8	0.35	23.6 ± 3.1	0.087
Waist circumference (cm)	79.1 ± 9.2	81.6 ± 8.2	0.017	81.9 ± 8.7	0.017
Systolic blood pressure (mm Hg)	131 ± 16	135 ± 16	0.028	138 ± 16	<0.0001
Diastolic blood pressure (mm Hg)	76 ± 10	79 ± 9	0.068	79 ± 9	0.079
Fasting plasma glucose (mmol/liter))	8.8 ± 2.4	9.2 ± 2.5	0.20	9.2 ± 2.7	0.39
HbA1c (%)	7.9 ± 1.3	8.1 ± 1.4	0.077	8.1 ± 1.4	0.17
Serum LDL cholesterol (mmol/liter)	3.1 ± 0.8	3.5 ± 0.8	<0.0001	3.2 ± 1.0	0.84
Serum HDL cholesterol (mmol/liter)	1.4 ± 0.5	1.3 ± 0.4	0.031	1.4 ± 0.4	0.30
Serum triglycerides (mmol/liter)[b]	1.1 (0.8)	1.4 (0.8)	0.016	1.3 (0.7)	0.25
Serum lipoprotein(a) (μmol/liter)[b]	0.81 (1.1)	0.95 (1.1)	0.56	1.0 (1.0)	0.049
Estimated GFR (10 ml/min · 1.73 m^2)	87.4 ± 28.9	82.0 ± 29.5	0.12	91.2 ± 33.8	0.42
Therapeutic measures					
Diabetes					
Diet only (%)	18.6	14.7	0.31	14.1	0.30
Insulin (%)	21.7	25.7	0.33	23.5	0.68
Sulfonylureas (%)	57.2	62.4	0.29	58.8	0.76
α-Glucosidase inhibitors (%)	20.3	22.0	0.67	21.2	0.85
Biguanides (%)	5.1	1.6	0.21	13.5	0.026
Insulin sensitizer (%)	2.0	3.7	0.26	1.2	0.59
Antihypertensive agents (%)	24.7	34.9	0.018	35.7	0.023
Agents for dyslipidemia (%)	23.8	29.4	0.19	25.0	0.79
Diet					
Energy intake (kJ/d)[b]	7079 (2248)	6801 (1903)	0.67	7259 (2726)	0.97
Fat intake (g/d)[b]	51 (21)	52 (18)	0.59	53.6 (28)	0.50
Exercise (kJ/d)[b]	530 (1112)	577 (1020)	0.91	442 (922)	0.73
Current smoker (%)	26.7	37.1	0.021	32.1	0.29
Alcohol intake: never, ≤3 drinks, >3 drinks (%)[c]	62.8/31.0/6.2	61.1/34.3/4.6	0.66	53.6/35.7/10.7	0.13

[a] *P* values for differences in gender proportion.

[b] Median (interquartile range).

[c] One drink is equivalent to 12.6 g ethanol based on the U.S. Department of Agriculture definition.

with triglycerides for CHD risk, but their interaction was not statistically significant.

Discussion

The current analysis of data from the nationwide study of Japanese subjects with type 2 diabetes demonstrated an approximately 1.6-fold increased CHD risk for an increment of 1 mmol/liter in LDL cholesterol, which is almost identical to what is observed in the United Kingdom Prospective Diabetes Study (UKPDS) (18). However, a striking difference between results of this study and those of the UKPDS (18) was that in our cohort, the serum triglyceride level was an additional strong predictor for CHD, showing almost the same 1.5-fold increased risk for the same 1 SD increment as with LDL cholesterol. In the current study, the inclines of the spline curves for LDL cholesterol and triglyceride levels are quite similar, indicating that both lipid variables equivalently affected CHD events in our cohort. The curve for triglycerides demonstrated that when a triglyceride level of 1.1 mmol/liter (100 mg/dl) is defined as a reference, its concentration as low as approximately 1.5 mmol/liter (134 mg/dl) could represent a significant CHD risk, which is quite close to the therapeutic target suggested in the current guidelines, which is 1.68 mmol/liter (150 mg/dl).

Although it is true that the serum triglyceride level is an established independent cardiovascular risk factor in general populations in Western (19) as well as in Asian (20) countries, it is well recognized that its potency as a CHD predictor is not as strong as that of LDL cholesterol (21). Actually, serum triglycerides were not among the significant CHD predictors in the UKPDS patients (18). On the other hand, among East Asians with diabetes, serum triglyceride values have been suggested to have stronger associations with cardiovascular morbidity (10, 12) and mortality (11) than those of LDL cholesterol, although these studies were either cross-sectional (10, 12) or relatively small scale and short term (11). Those results (10–12), together with results of the current relatively large-

TABLE 2. HR with 95% CI of each factor for CHD and stroke risk analyzed by Cox models

	Adjusted by age and sex						Multivariate adjusted[a]					
	CHD			Stroke			CHD			Stroke		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Gender (female; reference is men)	0.68	0.47–1.00	0.05	0.61	0.39–0.94	0.03	0.57	0.35–0.92	0.02	0.67	0.38–1.18	0.17
Age (per 5 yr)	1.16	1.01–1.34	0.04	1.24	1.05–1.46	0.01	1.16	1.00–1.35	0.06	1.30	1.09–1.57	<0.01
Diabetes duration (per 10 yr)	1.10	0.86–1.42	0.45	0.84	0.62–1.15	0.27	1.26	0.96–1.64	0.09	0.88	0.63–1.23	0.44
Energy intake (per 418 kJ/d)	1.01	0.96–1.07	0.65	0.99	0.93–1.06	0.75	1.01	0.96–1.07	0.68	0.98	0.92–1.05	0.58
Exercise (per 418 kJ/d)	0.96	0.89–1.04	0.36	0.99	0.92–1.08	0.87	0.98	0.91–1.06	0.58	1.01	0.93–1.10	0.78
Current smoker (yes; reference is no)	1.39	0.90–2.15	0.14	1.21	0.74–1.98	0.46	1.41	0.91–2.17	0.12	1.18	0.70–1.97	0.53
Alcohol drinking status[b]												
≤3 drinks (reference)	1.00			1.00			1.00			1.00		
>3 drinks	0.78	0.33–1.85	0.57	1.59	0.78–3.26	0.20	0.59	0.24–1.44	0.24	1.50	0.66–3.39	0.34
Nondrinker	1.13	0.73–1.75	0.60	0.87	0.52–1.45	0.59	0.91	0.58–1.42	0.66	1.12	0.65–1.91	0.68
Body mass index												
Per 1 kg/m^2	1.04	0.98–1.11	0.22	1.06	0.98–1.13	0.13	1.00	0.93–1.08	1.00	1.00	0.92–1.08	0.95
Per 1 SD	1.13	0.93–1.36		1.18	0.95–1.46		1.00	0.80–1.25		0.99	0.78–1.27	
Waist circumference												
Per 10 cm	1.24	0.99–1.55	0.06	1.31	1.02–1.67	0.04	1.18	0.81–1.73	0.39	1.21	0.79–1.84	0.39
Per 1 SD	1.22	0.99–1.49		1.28	1.02–1.60		1.17	0.82–1.65		1.19	0.81–1.75	
Systolic blood pressure												
Per 10 mm Hg	1.12	1.00–1.26	0.05	1.20	1.06–1.37	0.01	1.11	0.98–1.26	0.09	1.18	1.03–1.36	0.02
Per 1 SD	1.21	1.00–1.45		1.36	1.09–1.68		1.19	0.97–1.45		1.31	1.04–1.65	
Diastolic blood pressure												
Per 10 mm Hg	1.17	0.97–1.40	0.10	1.20	0.97–1.48	0.10	1.02	0.80–1.30	0.87	0.93	0.71–1.21	0.57
Per 1 SD	1.17	0.97–1.40		1.19	0.97–1.47		1.02	0.80–1.30		0.93	0.71–1.21	
Estimated GFR												
Per 10 ml/min · 1.73 m^2	0.95	0.88–1.02	0.15	1.06	1.00–1.13	0.07	0.95	0.89–1.03	0.19	1.05	0.99–1.13	0.12
Per 1 SD	0.85	0.69–1.06		1.19	0.99–1.43		0.87	0.70–1.08		1.17	0.96–1.41	
HbA1c												
Per 1%	1.21	1.06–1.38	0.00	1.17	1.01–1.35	0.03	1.15	1.00–1.33	0.05	1.11	0.94–1.31	0.24
Per 1 SD	1.28	1.08–1.51		1.23	1.02–1.48		1.20	1.00–1.45		1.14	0.92–1.42	
Fasting plasma glucose												
Per 1 mmol/liter	1.08	1.01–1.16	0.04	1.07	0.99–1.17	0.11	0.99	0.91–1.09	0.90	1.02	0.91–1.13	0.75
Per 1 SD	1.21	1.01–1.44		1.19	0.96–1.46		0.99	0.79–1.23		1.04	0.80–1.35	
LDL cholesterol												
Per 1 mmol/liter	1.71	1.39–2.10	<0.01	1.05	0.80–1.37	0.74	1.61	1.30–1.98	<0.01	1.00	0.76–1.32	1.00
Per 1 SD	1.56	1.32–1.86		1.04	0.83–1.30		1.49	1.25–1.77		1.00	0.79–1.26	
HDL cholesterol												
Per 1 mmol/liter	0.57	0.35–0.93	0.02	0.68	0.40–1.16	0.15	0.99	0.56–1.74	0.97	0.86	0.46–1.61	0.64
Per 1 SD	0.78	0.63–0.97		0.85	0.67–1.07		1.00	0.78–1.27		0.94	0.72–1.23	
Log–triglycerides (per 1 SD)	1.39	1.16–1.65	<0.01	1.20	0.97–1.47	0.09	1.54	1.22–1.94	<0.01	1.13	0.86–1.46	0.38
Log–Lp(a) (per 1 SD)	1.22	0.99–1.49	0.06	1.14	0.91–1.44	0.25	1.15	0.93–1.43	0.20	1.17	0.92–1.49	0.19

[a] Adjusted by gender, age, diabetes duration, body mass index, systolic blood pressure, HbA1c, LDL cholesterol, HDL cholesterol, triglycerides, smoking status, and alcohol intake.

[b] One drink is equivalent to 12.6 g ethanol based on the U.S. Department of Agriculture definition.

scale, long-term prospective study, strongly indicate that the serum triglyceride level is one of the leading predictors of CHD and is comparable to LDL cholesterol in East Asian subjects with type 2 diabetes. In this sense, although lowering levels of LDL cholesterol is given priority over that of triglycerides in most current guidelines for diabetes (22), more attention toward triglycerides should be given in East Asians with diabetes. In contrast to the favorable results of statin trials, fibrates, which mainly lower triglyceride levels, failed to significantly reduce cardiovascular events in diabetic subjects (23). However, this study mostly involved White subjects; therefore, the effects of lowering triglycerides in East Asians with diabetes are still to be determined.

Details of the etiology and pathological mechanisms for the stronger association of serum triglyceride levels with CHD in diabetic subjects in East Asia than in Western countries cannot be fully elucidated from results of the epidemiological studies discussed above. Although triglycerides *per se* do not seem to be directly involved in the atherogenic process, its conjunction with small dense LDL or remnant particles based on insulin-resistant status, which are important accelerators of atherosclerosis in diabetes, is well known (21). However, because we did not

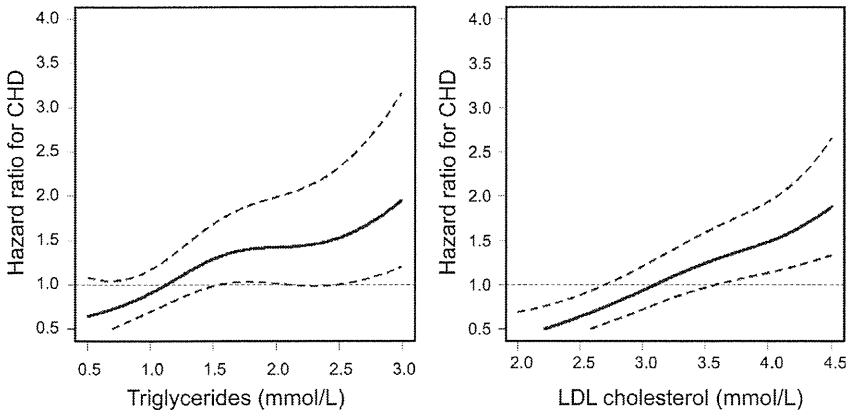

FIG. 1. Spline curves of HR with 95% CI showing relationships between major risk factors and CHD events estimated by generalized additive models.

measure the small dense LDL, remnant particles, or fasting insulin levels to determine the degree of insulin resistance, we cannot address their role among our study subjects. It was reported in Japanese subjects with type 2 diabetes that the serum triglyceride level is associated with insulin resistance, the visceral fat area (24) and C-reactive protein (25). A prospective study showed that the triglyceride value was a strong predictor of recurrent coronary events in patients with relatively low LDL cholesterol (26). Because more than one fourth of our subjects were taking agents for dyslipidemia, many of which were statins that lower LDL cholesterol, the significance of triglycerides as a predictor of CHD could be exaggerated.

It should be noted that the high-density lipoprotein (HDL) cholesterol level, which has a negative correlation with the triglyceride level, was not a significant predictor for CHD in our cohort after multivariate adjustment, although in the UKPDS (18), low HDL cholesterol values were the second strongest predictor for CHD after LDL cholesterol, and the triglyceride value was not an independent predictor after multivariate adjustment. It is already known (27, 28), and was actually observed in our cohort, that the serum level of HDL cholesterol is naturally higher in East Asians than in Western populations. Therefore, it could be possible that the influence of HDL cholesterol was not apparent, and instead, that of triglycerides was enhanced in Japanese. Another possibility was that when two explanatory variables with significant correlations, as in the case of HDL cholesterol and triglycerides, were in a regression model, the stronger and more precisely measured factor had significance, and the other seemed to be absorbed. Our findings are consistent with other studies of a Japanese general population (29, 30) that showed triglycerides as an independent risk factor not substantially influenced by total or HDL cholesterol.

Moderate alcohol intake is known to increase both triglyceride and HDL cholesterol levels and reduces CHD. Therefore, the expected effects of ethanol would not likely account for adverse association of elevated triglyceride levels with elevated CHD risk, and ethanol intake would also not likely account for the lack of expected inverse association between HDL cholesterol levels and CHD in this study. It could be possible that the low intake of ethanol in the study population might explain the lack of the expected ethanol effects.

Our results also imply that the triglyceride level seemed to have affected CHD risk possibly in an additive fashion to the LDL cholesterol level (although not statistically significant) despite the fact that it worked independently of blood pressure. Even among subjects in the top tertile of either triglyceride or LDL cholesterol values, only those in whom the rest of these lipid variables were in the middle or the top tertile were at significantly higher risk compared with those who were in the lowest tertiles of both of these variables. Although it is not known whether this phenomenon can be seen in diabetic subjects of other ethnicities, its mechanism requires investigation.

The significant elevation of baseline proportions of those receiving therapy with antihypertensive agents or biguanides among subjects with cardiovascular events should be carefully interpreted. It is not rare that in observational studies the use of drug therapy is associated with worse cardiovascular outcome because of so-called indication bias or treatment selection bias (31, 32). Therefore, the possible reason for this contradictory result is that patients who originally had a higher cardiovascular risk tended to be prescribed these agents, which had been proved to have antiatherogenic effects in clinical trials (33, 34), because our patients were cared by diabetes specialists.

A markedly lower incidence of CHD compared with Western diabetic populations was confirmed in our Japanese subjects. The incidence of myocardial infarction in our cohort was approximately one fourth of that reported in the United Kingdom (35, 36), whereas the incidence of stroke was quite similar (35, 37). In addition, the current finding that stroke is more frequent than myocardial infarction in Japanese subjects with diabetes, which is the reverse of that in other ethnic groups (2), is a reflection of the same relationship that has been observed in the Japanese general population (38). In terms of risk factors for stroke, as was seen in Western diabetic subjects (37, 39), blood pressure was confirmed to be a leading predictor of stroke. This strong correlation, which is universally seen beyond ethnic groups, might have concealed the relation-

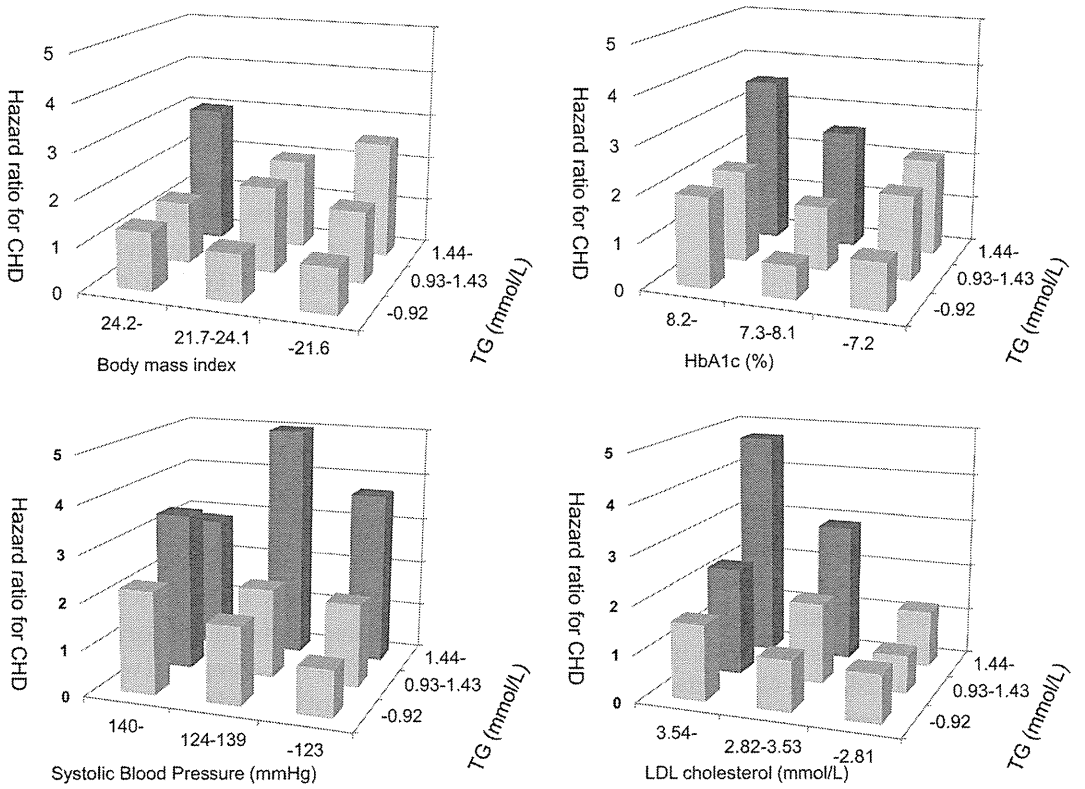

FIG. 2. Combined roles of triglycerides (TG) with other major risk factors for CHD. Each variable was stratified according to tertiles. Columns of categories with a significantly elevated HR compared with the reference category, which is the combination of the lowest tertiles of both parameters for each combination, are shown by *dark shading*.

ship between stroke and lipid variables including triglycerides, which was prominent in CHD.

The strengths of this study lie in its long-term prospective design of a nation-representative cohort consisting of patients from a large number of institutions throughout Japan. However, the number of patients was not necessarily large enough to draw firm conclusions, and our patients comprised only Japanese recruited from clinics specializing in diabetes care. Therefore, our results may not be representative of all East Asians. A limitation was the lack of standardization in measurement methods for laboratory testing, although in Japan, laboratory tests are well standardized on a nationwide level. Another limitation was that we could not detect asymptomatic angina pectoris, which could possibly occur in diabetic subjects, although asymptomatic myocardial infarctions were detected by an annual ECG.

In conclusion, an elevated triglyceride level was a strong predictor for CHD in Japanese subjects with type 2 diabetes, which implies that more therapeutic attention toward serum triglycerides should be given in Japanese with diabetes. These results could be crucial in ethnic group-specific diabetes care and could also highlight the clinical background in the development of cardiovascular complications in patients with diabetes.

Appendix

The Japan Diabetes Complications Study (JDCS) Group (including current and former members with their affiliations at the time of their participation in this study) includes primary investigators Hirohito Sone and Nobuhio Yamada (University of Tsukuba) and Chief in Assessment Committee Yasuo Akanuma (Institute for Adult Diseases Asahi Life Foundation).

Other investigators are as follows: Keita Ato, Masaaki Eto, and Hiroshi Ito (Asahikawa Medical College); Naotake Hashimoto, Azuma Kanatsuka, Yasushi Saito, Kenichi Sakurai, Kazuo Takahashi, Kazuo Yagi, and Kotaro Yokote, (Chiba University); Tadami Takekoshi and Takanobu Wakasugi (Fukui Prefectural Hospital); Shigetake Toyooka (Fukui Red Cross Hospital); Yukihiro Bando (Fukui Saiseikai Hospital); Tsugihiko Nakai, Koji Oida, and Jinya Suzuki (Fukui University); Yasuaki Fukumoto and Seiichi Sumi (Garatia Hostiptal); Tomokazu Awaya, Genshi Egusa, Rumi Fujikawa, Masamichi Okubo, and Kiminori Yamane (Hiroshima University); Takao Koike and Narihito Yoshioka (Hokkaido University); Yasuo Akanuma, Motonobu Anai, Ritsuko Honda, Shoji Kawatsu, and Masatoshi Kikuchi (Institute for Adult Diseases Asahi Life Foundation); Shun Ishibashi (Jichi Medical School); Masanobu Kawakami, Kazuyuki Namai, Hiroyuki Tamemoto, and Hideo Toyoshima (Jichi Medical School Saitama Medical Center); Masami Nemoto and Takashi Sasaki (Jikei University); Ryuzo Kawamori and Yasushi Tanaka (Juntendo University); Toshihiko Ishida (Kagawa University); Toshihide Kawai and Izumi Takei (Keio University); Yoshikuni Fujita, Keiji Tanaka, and Yoshihiro Yajima (Kitazato University); Noboru Furukawa,

Hideki Kishikawa, Tetsushi Toyonaga, and Kaku Tsuruzoe (Kumamoto University); Yoichi Imamura, Shingo Komichi, Zenji Makita, Kyohei Nonaka, and Kentaro Yamada (Kurume University); Naoto Nakamura and Koji Nakano (Kyoto Prefectural University of Medicine); Toyoshi Iguchi and Hajime Nawata (Kyushu University); Yasuhisa Matsushima (Matsudo City Hospital); Hideo Takahashi (Minami Akatsuka Clinic); Hiroyuki Toyoshima (Minoh City Hospital); Shoichi Akazawa, Eiji Kawasaki, and Shigenobu Nagataki (Nagasaki University); Toshio Hayashi, Nigishi Hotta, and Jiro Nakamura (Nagoya University); Kentaro Doi, Yu Harano, Hisashi Makino, and Yasunao Yoshimasa (National Cardiovascular Center); Yoichi Hayashi (Nihon University); Shinnichi Oikawa (Nippon Medical School); Ryuzou Abe (Ohta Memorial Hospital); Hiroaki Seino, Susumu Suzuki, and Daishiro Yamada (Ohta-Nishinouchi Hospital); Mitsuru Hoshi, Eiichi Imano, and Takao Watarai (Osaka Koseinenkin Hospital); Masatoshi Imaizumi, Hideki Taki, and Ryuhei Todo (Osaka National Hospital); Keisuke Kosugi, Yasuhisa Shimizu, and Yutaka Umayahara (Osaka Police Hospital); Munehide Matsuhisa, Junichiro Miyagawa, Mitsuyoshi Nanba, Kohei Okita, Kaoru Takemura, and Yoshimitsu Yamasaki (Osaka University); Yoshihito Atsumi, Kazuhiro Hosokawa, and Kempei Matsuoka (Saiseikai Central Hospital); Junko Nakano and Hirotaka Umezu (Saiseikai Fukushima General Hospital); Akihiko Hoshino, Toshihiko Nishiyama, and Tetsushi Nogami (Saisekai Kumamoto Hospital); Hideo Nunome (Saiseikai Mito Hospital); Shigehiro Katayama, Susumu Kurihara, and Atsuhito Togashi (Saitama Medical College); Kenichi Yamada (Sakura National Hospital); Shinichi Araki, Atsunori Kashiwagi, and Yoshihiko Nishio (Shiga University of Medical Science); Yukio Yoshimura (Shikoku University); Tatsuhide Inoue (Shizuoka General Hospital); Masafumi Kitaoka (Showa General Hospital); Toshio Kitada, Akio Shirai, and Ryoichiro Watanabe (Takeda General Hospital); Takaichi Miyagawa (Tama Minami Clinic); Osamu Mokuta, Ryo Okazaki, and Yoshikazu Sakamoto (Teikyo Universiy Ichihara Hospital); Yasushi Ishigaki and Kazuma Takahashi (Tohoku University); Yoh Miyashita and Koji Shirai (Toho University Sakura Hospital); Akira Tanaka (Tokyo Medical and Dental University); Yoshiaki Fujita (Tokyo Metropolitan Institute of Gerontology); Hideki Ito (Tokyo Metropolitan Geriatric Hospital); Yasuhiko Iwamoto, Reiko Kawahara, Yasue Omori, and Asako Sato (Tokyo Women's Medical University); Yasumichi Mori, Toshio Murase, Mitsuhiko Noda, and Masato Odawara (Toranomon Hospital); Masashi Kobayashi and Masaharu Urakaze (Toyama University); Rei Aida, Hitomi Fujii, Satoshi Iimuro, Takashi Kadowaki, Yasuo Ohashi, Junichi Osuga, Yasuyoshi Ouchi, Akane Takahashi, Sachiko Tanaka, and Shiro Tanaka (University of Tokyo); Hirohito Sone, Nobuhiro Yamada, and Kamejiro Yamashita (University of Tsukuba); Ryo Kawasaki and Hidetoshi Yamashita (Yamagata University); Hisahiko Sekihara and Yasuo Terauchi (Yokohama City University); Tetsuo Nishikawa (Yokohama Rosai Hospital); and Hiroto Furuta and Kishio Nanjo (Wakayama Medical University).

Acknowledgments

We thank the many diabetologists and patients at 59 participating institutes all over Japan. Thanks are extended to Ms. Satomi Fukuya and Ms. Yasuko Maruyama for their excellent secretarial assistance.

Address all correspondence and requests for reprints to: Hirohito Sone, M.D., Ph.D., FACP, Professor, Department of Internal Medicine, University of Tsukuba Institute of Clinical Medicine, 3-2-7 Miyamachi, Mito, Ibaraki, Japan 310-0015. E-mail: jdcstudy@md.tsukuba.ac.jp.

This work was supported by the Ministry of Health, Labor and Welfare, Japan. The sponsor had no role in the design and conduct of the study.

Disclosure Summary: The authors declare that there is no duality of interest associated with this manuscript.

All authors researched data, contributed to the discussion, and wrote and edited the manuscript. H.S. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. **Chan JC, Malik V, Jia W, Kadowaki T, Yajnik CS, Yoon KH, Hu FB** 2009 Diabetes in Asia: epidemiology, risk factors, and pathophysiology. JAMA 301:2129–2140
2. **Lee ET, Keen H, Bennett PH, Fuller JH, Lu M** 2001 Follow-up of the WHO Multinational Study of Vascular Disease in Diabetes: general description and morbidity. Diabetologia 44(Suppl 2):S3–13
3. **Hotta N, Nakamura J, Iwamoto Y, Ohno Y, Kasuga M, Kikkawa R, Toyota T** 2010 Causes of death in Japanese diabetics: a questionnaire survey of 18,385 diabetics over a 10-year period. J Diabet Invest 1:66–76
4. **Sone H, Mizuno S, Fujii H, Yoshimura Y, Yamasaki Y, Ishibashi S, Katayama S, Saito Y, Ito H, Ohashi Y, Akanuma Y, Yamada N** 2005 Is the diagnosis of metabolic syndrome useful for predicting cardiovascular disease in Asian diabetic patients? Analysis from the Japan Diabetes Complications Study. Diabetes Care 28:1463–1471
5. **Sone H, Tanaka S, Ishibashi S, Yamasaki Y, Oikawa S, Ito H, Saito Y, Ohashi Y, Akanuma Y, Yamada N** 2006 The new worldwide definition of metabolic syndrome is not a better diagnostic predictor of cardiovascular disease in Japanese diabetic patients than the existing definitions: additional analysis from the Japan Diabetes Complications Study. Diabetes Care 29:145–147
6. **Sone H, Yamada N, Mizuno S, Aida R, Ohashi Y** 2004 Alcohol use and diabetes mellitus. Ann Intern Med 141:408–409; author reply 409
7. **Sone H, Mizuno S, Yamada N** 2005 Vascular risk factors and diabetic neuropathy. N Engl J Med 352:1925–1927
8. **Sone H, Ito H, Ohashi Y, Akanuma Y, Yamada N** 2003 Obesity and type 2 diabetes in Japanese patients. Lancet 361:85
9. **Sone H, Yoshimura Y, Ito H, Ohashi Y, Yamada N** 2004 Energy intake and obesity in Japanese patients with type 2 diabetes. Lancet 363:248–249
10. **Ko GT, Chan JC, Chow CC, Yeung VT, Chan WB, So WY, Ma RC, Ozaki R, Cockram CS** 2003 Triglyceride, albuminuria and blood pressure are the major associations of non-fatal cardiovascular disease in Chinese type 2 diabetes. Acta Diabetol 40:80–84
11. **Chan WB, Tong PC, Chow CC, So WY, Ng MC, Ma RC, Osaki R, Cockram CS, Chan JC** 2005 Triglyceride predicts cardiovascular mortality and its relationship with glycaemia and obesity in Chinese type 2 diabetic patients. Diabetes Metab Res Rev 21:183–188
12. **Tseng CH, Tseng CP, Chong CK, Cheng JC, Tai TY** 2006 Independent association between triglycerides and coronary artery disease in Taiwanese type 2 diabetic patients. Int J Cardiol 111:80–85
13. **Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, Yamagata K, Tomino Y, Yokoyama H, Hishida A** 2009 Revised

equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis 53:982–992
14. **Beaglehole R, Stewart AW, Butler M** 1987 Comparability of old and new World Health Organization criteria for definite myocardial infarction. Int J Epidemiol 16:373–376
15. **Tuomilehto J, Kuulasmaa K** 1989 WHO MONICA Project: assessing CHD mortality and morbidity. Int J Epidemiol 18:S38–S45
16. **The Committee of Ministry of Health, Labor and Welfare on the Diagnostic Criteria of Stroke.** 1984 Report of The Committee of Ministry of Health, Labor and Welfare on the Diagnostic Criteria of Stroke. Tokyo: Ministry of Health, Labor and Welfare, Japan
17. **Aho K, Harmsen P, Hatano S, Marquardsen J, Smirnov VE, Strasser T** 1980 Cerebrovascular disease in the community: results of a WHO collaborative study. Bull World Health Organ 58:113–130
18. **Turner RC, Millns H, Neil HA, Stratton IM, Manley SE, Matthews DR, Holman RR** 1998 Risk factors for coronary artery disease in non-insulin dependent diabetes mellitus: United Kingdom Prospective Diabetes Study (UKPDS: 23). BMJ 316:823–828
19. **Sarwar N, Danesh J, Eiriksdottir G, Sigurdsson G, Wareham N, Bingham S, Boekholdt SM, Khaw KT, Gudnason V** 2007 Triglycerides and the risk of coronary heart disease: 10,158 incident cases among 262,525 participants in 29 Western prospective studies. Circulation 115:450–458
20. **Patel A, Barzi F, Jamrozik K, Lam TH, Ueshima H, Whitlock G, Woodward M** 2004 Serum triglycerides as a risk factor for cardiovascular diseases in the Asia-Pacific region. Circulation 110:2678–2686
21. **Mooradian AD** 2009 Dyslipidemia in type 2 diabetes mellitus. Nat Clin Pract Endocrinol Metab 5:150–159
22. **Bitzur R** 2011 Diabetes and cardiovascular disease: when it comes to lipids, statins are all you need. Diabetes Care 34(Suppl 2):S380–S382
23. **Keech A, Simes RJ, Barter P, Best J, Scott R, Taskinen MR, Forder P, Pillai A, Davis T, Glasziou P, Drury P, Kesäniemi YA, Sullivan D, Hunt D, Colman P, d'Emden M, Whiting M, Ehnholm C, Laakso M** 2005 Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomised controlled trial. Lancet 366:1849–1861
24. **Taniguchi A, Nakai Y, Sakai M, Yoshii S, Hamanaka D, Hatae Y, Kawata M, Yamanouchi K, Okumura T, Doi K, Tokuyama K, Nagasaka S, Fukushima M** 2002 Relationship of regional adiposity to insulin resistance and serum triglyceride levels in nonobese Japanese type 2 diabetic patients. Metabolism 51:544–548
25. **Yanagawa T, Taniguchi A, Fukushima M, Nakai Y, Nagasaka S, Ohgushi M, Matsumoto K, Kuroe A, Ohya M, Seino Y** 2007 Leptin, triglycerides, and interleukin 6 are independently associated with C-reactive protein in Japanese type 2 diabetic patients. Diabetes Res Clin Pract 75:2–6
26. **Sacks FM, Tonkin AM, Craven T, Pfeffer MA, Shepherd J, Keech A, Furberg CD, Braunwald E** 2002 Coronary heart disease in patients with low LDL-cholesterol: benefit of pravastatin in diabetics and enhanced role for HDL-cholesterol and triglycerides as risk factors. Circulation 105:1424–1428
27. **Zhang L, Qiao Q, Tuomilehto J, Hammar N, Alberti KG, Eliasson M, Heine RJ, Stehouwer CD, Ruotolo G** 2008 Blood lipid levels in relation to glucose status in European men and women without a prior history of diabetes: the DECODE Study. Diabetes Res Clin Pract 82:364–377
28. **Zhang L, Qiao Q, Tuomilehto J, Hammar N, Janus ED, Söderberg S, Mohan V, Ramachandran A, Dong YH, Lam TH, Pang ZC** 2009 Blood lipid levels in relation to glucose status in seven populations of Asian origin without a prior history of diabetes: the DECODA study. Diabetes Metab Res Rev 25:549–557
29. **Iso H, Naito Y, Sato S, Kitamura A, Okamura T, Sankai T, Shimamoto T, Iida M, Komachi Y** 2001 Serum triglycerides and risk of coronary heart disease among Japanese men and women. Am J Epidemiol 153:490–499
30. **Yamamoto A, Richie G, Nakamura H, Hosoda S, Nobuyoshi M, Matsuzaki M, Tan CE, Keech A, Mabuchi H, Horibe H, Tenba H** 2002 Risk factors for coronary heart disease in the Japanese: comparison of the background of patients with acute coronary syndrome in the ASPAC study with data obtained from the general population. Asia-Pacific Collaboration on CHD Risk Factor Intervention study. J Atheroscler Thromb 9:191–199
31. **Delgado-Rodríguez M, Llorca J** 2004. Bias. J Epidemiol Community Health 58:635–641
32. **Stukel TA, Fisher ES, Wennberg DE, Alter DA, Gottlieb DJ, Vermeulen MJ** 2007 Analysis of observational studies in the presence of treatment selection bias: effects of invasive cardiac management on AMI survival using propensity score and instrumental variable methods. JAMA 297:278–285
33. **UK Prospective Diabetes Study (UKPDS) Group** 1998 Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). Lancet 352:854–865
34. **UK Prospective Diabetes Study (UKPDS) Group** 1998 Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. BMJ 317:703–713
35. **UK Prospective Diabetes Study (UKPDS) Group** 1998 Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. Lancet 352:837–853
36. **Mulnier HE, Seaman HE, Raleigh VS, Soedamah-Muthu SS, Colhoun HM, Lawrenson RA, de Vries CS** 2008 Risk of myocardial infarction in men and women with type 2 diabetes in the UK: a cohort study using the General Practice Research Database. Diabetologia 51:1639–1645
37. **Mulnier HE, Seaman HE, Raleigh VS, Soedamah-Muthu SS, Colhoun HM, Lawrenson RA, De Vries CS** 2006 Risk of stroke in people with type 2 diabetes in the UK: a study using the General Practice Research Database. Diabetologia 49:2859–2865
38. **Kitamura A, Sato S, Kiyama M, Imano H, Iso H, Okada T, Ohira T, Tanigawa T, Yamagishi K, Nakamura M, Konishi M, Shimamoto T, Iida M, Komachi Y** 2008 Trends in the incidence of coronary heart disease and stroke and their risk factors in Japan, 1964 to 2003: the Akita-Osaka study. J Am Coll Cardiol 52:71–79
39. **Davis TM, Millns H, Stratton IM, Holman RR, Turner RC** 1999 Risk factors for stroke in type 2 diabetes mellitus: United Kingdom Prospective Diabetes Study (UKPDS) 29. Arch Intern Med 159:1097–1103