Table 2 Relative Risk of Coronary Events According to Serum Lipid Concentrations During Treatment[a)]

	Men					Women				
	N	*Event*	*RR*	*95%CI*	*p value*	*N*	*Event*	*RR*	*95%CI*	*p value*
TC (mg/dl)										
<200	3,442	24	1.00	(Referent)		5,833	22	1.00	(Referent)	
200–219	3,643	23	0.99	(0.56–1.77)	0.984	8,194	14	0.52	(0.27–1.02)	0.057
220–239	3,029	25	1.46	(0.83–2.56)	0.192	7,070	18	0.88	(0.47–1.64)	0.687
240–259	1,431	15	2.01	(1.05–3.88)	0.036	3,668	22	2.19	(1.21–3.98)	0.010
260–	1,030	18	3.48	(1.86–6.52)	<0.001	2,248	17	2.82	(1.48–5.36)	0.002
LDL-C (mg/dl)										
<120	4,680	27	1.00	(Referent)		8,050	22	1.00	(Referent)	
120–139	3,542	23	1.24	(0.71–2.16)	0.456	8,418	17	0.83	(0.44–1.57)	0.566
140–159	2,406	21	1.84	(1.03–3.26)	0.038	6,185	19	1.42	(0.77–2.64)	0.263
160–179	1,057	12	2.60	(1.31–5.17)	0.006	2,673	17	3.29	(1.74–6.23)	<0.001
180–	648	17	6.58	(3.53–12.25)	<0.001	1,564	17	5.78	(3.03–11.00)	<0.001
TG (mg/dl)										
<100	1,521	11	1.00	(Referent)		6,337	18	1.00	(Referent)	
100–149	3,663	22	0.84	(0.41–1.74)	0.634	10,444	32	0.98	(0.55–1.76)	0.946
150–199	3,127	33	1.51	(0.76–3.02)	0.243	5,861	17	0.87	(0.44–1.71)	0.684
200–249	1,768	18	1.46	(0.68–3.15)	0.330	2,429	9	1.12	(0.50–2.53)	0.783
250–	2,494	21	1.24	(0.58–2.65)	0.572	1,921	17	2.62	(1.32–5.21)	0.006
HDL-C (mg/dl)										
<40	2,198	36	1.00	(Referent)		1,758	10	1.00	(Referent)	
40–44	2,133	23	0.64	(0.38–1.09)	0.099	2,794	17	1.12	(0.51–2.45)	0.776
45–49	2,207	17	0.44	(0.25–0.80)	0.006	4,101	24	1.09	(0.52–2.28)	0.819
50–54	1,956	13	0.39	(0.21–0.74)	0.004	4,440	13	0.57	(0.25–1.30)	0.179
55–59	1,402	8	0.33	(0.15–0.72)	0.005	4,053	13	0.66	(0.29–1.51)	0.324
60–	2,679	8	0.17	(0.08–0.36)	<0.001	9,867	16	0.33	(0.15–0.73)	0.006
LDL-C/HDL-C										
<2.0	2,851	11	1.00	(Referent)		7,426	11	1.00	(Referent)	
2.0–2.4	2,719	11	1.10	(0.48–2.55)	0.817	6,909	19	1.95	(0.92–4.10)	0.080
2.5–2.9	2,598	17	1.91	(0.89–4.10)	0.095	5,884	14	1.68	(0.76–3.72)	0.199
3.0–3.4	1,889	20	3.21	(1.53–6.74)	0.002	3,545	21	4.57	(2.19–9.54)	<0.001
3.5–4.0	1,082	13	3.87	(1.72–8.72)	0.001	1,728	12	5.04	(2.21–11.49)	<0.001
4.0–	1,194	28	8.06	(3.95–16.44)	<0.001	1,398	15	8.56	(3.88–18.88)	<0.001

RR, relative risk; CI, confidence interval. Other abbreviations see in Table 1.

a) Coronary events included acute myocardial infarction and sudden cardiac death. Adjustment for age, hypertension, diabetes mellitus, body mass index, ECG abnormality, family history of CHD, cigarette smoking, and alcohol use.

Table 3 Relative Risk of Coronary Events and Baseline Characteristics[a)]

	Men					Women					*Heterogeneity p value[b)]*
	N	*Event*	*RR*	*95%CI*	*p value*	*N*	*Event*	*RR*	*95%CI*	*p value*	
Age (years)											
<55	6,281	49	1.00	(Referent)		6,137	8	1.00	(Referent)		0.008
55–59	2,182	14	0.74	(0.41–1.34)	0.320	6,488	15	1.82	(0.77–4.29)	0.174	
60–64	2,164	17	0.87	(0.50–1.53)	0.627	7,112	29	3.02	(1.38–6.62)	0.006	
≥65	1,948	25	1.42	(0.86–2.34)	0.168	7,276	41	4.11	(1.92–8.82)	<0.001	
Obesity[c)]	4,621	40	0.99	(0.66–1.48)	0.956	8,700	32	0.91	(0.59–1.40)	0.663	0.676
Hypertension[d)]	5,705	68	2.15	(1.42–3.26)	<0.001	12,511	62	2.05	(1.32–3.18)	0.001	0.864
Diabetes mellitus[e)]	2,513	29	1.58	(1.03–2.43)	0.037	3,747	31	3.07	(1.99–4.74)	<0.001	0.019
ECG abnormality[f)]	1,681	26	1.86	(1.18–2.91)	0.007	3,473	23	1.67	(1.04–2.70)	0.035	0.972
Family history of CHD[g)]	637	10	2.00	(1.04–3.84)	0.038	1,289	13	3.34	(1.85–6.04)	<0.001	0.317
Cigarette smoking[g)]	5,506	52	1.46	(0.98–2.17)	0.063	1,105	9	2.94	(1.43–6.02)	0.003	0.148
Alcohol use[g)]	9,224	70	0.63	(0.41–0.96)	0.031	2,337	6	0.61	(0.26–1.45)	0.266	0.933

Abbreviations see in Tables 1,2.

a) Coronary events included acute myocardial infarction and sudden cardiac death. Adjustment for age, hypertension, diabetes mellitus, body mass index, ECG abnormality, family history of CHD, cigarette smoking, and alcohol use. b) Heterogeneity between men and women, based on the likelihood ratio test. c) Body mass index ≥25 kg/m². d) Systolic blood pressure ≥160 mmHg and/or diastolic blood pressure ≥95 mmHg or medication for hypertension. e) Fasting plasma glucose ≥140 mg/dl or medication. f) Study physician's diagnosis. g) Self-reported information.

tive association was more evident in men. The relative risk for coronary events was substantially increased in patients with LDL-C/HDL-C ≥3.0 in both men and women.

The increase in the risk of coronary events for each 10 mg/dl elevation of LDL-C concentration during the treatment period was 18% (95% CI 12–24%) in men and 21% (95% CI 15–27%) in women, and the decrease in CHD risk associated with each 10 mg/dl elevation of HDL-C concentration was 39% in men and 33% in women. The relationships of coronary events with baseline LDL-C and HDL-C concentrations were also examined, but were much weaker than those observed during the treatment period. With each 10 mg/dl elevation of LDL-C concentration at baseline, the increase in the relative risk was 7% for men

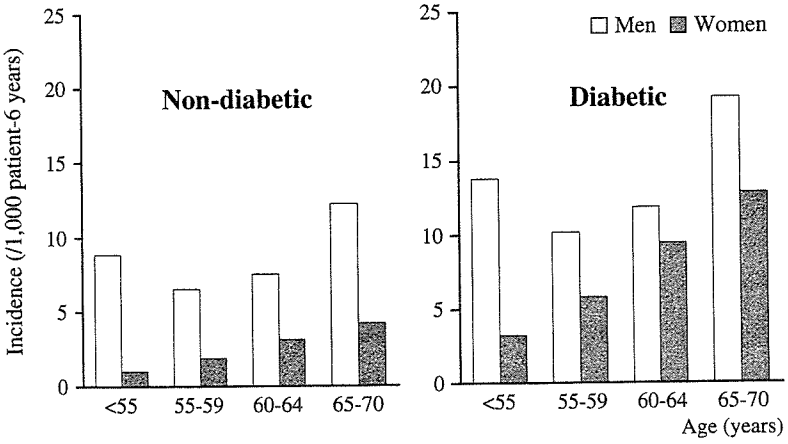

Fig 1. Estimated rates of coronary events according to age in men and women with and without diabetes mellitus (DM). Incidence rates were calculated from coronary heart disease (CHD) relative risks and the proportion of patients in each age category, for men and women separately, using Cox proportional hazards model, in which adjustment was made for age, hypertension, DM, body mass index, ECG abnormality, family history of CHD, cigarette smoking, and alcohol use.

and 9% for women and the decrease in risk with each 10 mg/dl elevation of HDL-C at baseline was 20% in both men and women.

Patient Baseline Characteristics and Risk of Coronary Events

The effect of age on the risk of coronary events was seen in women, but not in men (Table 3). Hypertension, DM, ECG abnormalities and a family history of CHD were also risk factors for coronary events in both men and women, but increased risks associated with DM and a family history of CHD were more marked for women than for men; the relative risk with DM was 1.58 in men and 3.07 in women, and the corresponding values for a family history of CHD were 2.00 in men and 3.34 in women. Obesity was unrelated to coronary events in either men or women. Although alcohol ingestion was protective in both men and women to the same extent, cigarette smoking was more strongly related to an increased risk of coronary events in women.

Discussion

This report addresses the gender differences in the relationship of serum lipid concentrations and other risk factors to CHD risk in Japanese patients under long-term treatment for hypercholesterolemia. Although serum TC and LDL-C concentrations were very similarly related to CHD risk in men and women, there was a difference between men and women in the relationship to serum TG and HDL-C concentrations. An inverse relationship of HDL-C to CHD risk was seen in men and women, but the HDL-C concentration showing a decreased risk of CHD differed by sex. The risk was significantly decreased at HDL-C ≥45 mg/dl in men and at HDL-C ≥60 mg/dl in women. The findings agree with observations published in the United States and Europe[2,3] and further indicate that the criterion of "low HDL-C" must be differential for men and women. An increased risk was observed only in women with an extremely high concentration of TG (≥250 mg/dl). Interpretation of this finding is difficult, and we do not have a clear idea about the implication of the present finding on serum TG.

In the present study, men did not show a clear increase in the risk of coronary events with increasing age, whereas there was a progressive increase in the risk with advancing age in women. The latter finding could be a reflection of the increase in serum TC and LDL-C concentrations with increasing age after menopause. The lack of an increasing trend in the association between age and coronary events in men is an unexpected finding, and may have been due to unknown characteristics of the male participants in the present study.

Whereas DM was related to increased CHD risk in both men and women, the increased risk was much greater in women, as indicated by a statistically significant interaction (p=0.019). These results did not change when further adjusted for TC or LDL-C. However, the risk difference between men and women for DM was not unique to the J-LIT patients. In a meta-analysis of 10 prospective studies, Lee et al showed that the effect of DM on the CHD risk was greater in women than in men![14] They showed that the relative risk of coronary death for DM patients vs non-DM patients was 2.58 (95% CI 2.05–3.26) in women and 1.85 (95% CI 1.47–2.33) in men (interaction p=0.045)![14] It was further noted in a later study that DM diminished the female advantage for lower CHD incidence![15] That DM is a stronger CHD risk factor in women may be related to the lower concentrations of HDL-C. Walden suggested that lower HDL-C concentrations in diabetic women as compared with men might be relevant to a stronger association between DM and CHD in women![16] In the present study, mean HDL-C concentrations in female diabetic patients were lower than those of non-diabetic patients (55.5 vs 57.5 mg/dl, p<0.001), but there was no difference in the HDL-C concentrations between the 2 groups in men (50.8 vs 51.3 mg/dl, p=0.09). The relative risk for DM was unchanged with adjustment for HDL-C. When the predicted rates of CHD incidence according to age were examined in men and women with and without DM (Fig 1), the increase in CHD incidence with aging was augmented in the presence of DM. Notably, DM diminished the women's advantage of having a lower CHD incidence in older patients.

Both cigarette smoking and family history of CHD were related to a greater increase in the risk of coronary events in women than in men. These differential increases in men and women may have been caused by random variation, as indicated by the lack of statistical significance for the interaction. As regards the effect of cigarette smoking, some studies suggest that smoking is a stronger risk factor in women than in men[2,17] but others have failed to find such a finding![18]

Finally, the present study results indicated that hypertension was an important risk factor in men and women equally, and that alcohol ingestion was protective in both sexes. These findings are in agreement with observations reported elsewhere![19–21]

In conclusion, the incidence of coronary events was 60% lower in women than in men among the J-LIT participants. Although the relationship of serum TC and LDL-C concentrations to coronary events was similar in men and women, the HDL-C concentration associated with a decreased risk of coronary events was slightly higher in women. DM was a stronger risk factor in women, and traded off the women's advantage of having a lower risk of coronary events, especially in aged patients.

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Original Article

Effect of Probucol on Elderly Hypercholesterolemic Patients in the FAST Study

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Abstract The present study involved a detailed post hoc comparison of the efficacy and safety of lipid-lowering therapy in elderly hypercholesterolemic patients from the Fukuoka Atherosclerosis Trial (FAST). The FAST cohort of 246 hypercholesterolemic patients included 76 patients who were (75 years old. Patients were randomized to receive probucol (500 mg/day) or pravastatin (10 mg/day) therapy, or to a control group (diet alone), and then were followed for 2 years. In patients ≥75 years old, either probucol or pravastatin achieved a significant reduction of carotid intima-media thickness (IMT). In patients <75 years old, lipid-lowering therapy also achieved a significant reduction of IMT. In patients ≥75 years old receiving probucol, the relative risk (95% confidence interval) of all-cause mortality was 0.15 (0.02 to 1.28) and that for major coronary events was 0.12 (0.02 to 1.04). In conclusion, probucol reduced the incidence of cardiovascular disease in elderly hypercholesterolemic patients as well as younger patients.

Key words: elderly hypercholesterolemic patients, probucol, pravastatin, intima-media thickness, carotid atherosclerosis, cardiovascular disease

INTRODUCTION

Atherosclerosis is a common disease in the elderly, and atherosclerotic lesions may cause myocardial infarction or stroke. Measurement of the carotid artery intima-media thickness (IMT) by high-resolution B-mode ultrasonography allows noninvasive detection of early carotid atherosclerosis, and the IMT is also a reliable end-point for trials assessing the effect of interventions on disease progression. Furthermore, ultrasonography can directly quantify early atherosclerotic changes and the response to risk factor modification[1], allowing the use of a smaller patient population to determine the benefits of treatment or accurately assess the presence of early atherosclerosis. The Fukuoka Atherosclerosis Trial (FAST) was the first study to demonstrate the benefit of probucol therapy for patients with hypercholesterolemia and to also reveal an effect of probucol on the incidence of cardiovascular events[2].

A direct relationship between the serum low-density lipoprotein (LDL)-cholesterol level and the risk of coronary heart disease (CHD) has been most clearly demonstrated in studies on middle-aged men. Although a similar relationship has also been observed

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in middle-aged women and in some older populations (≥65 years), the relationship is reported to be weaker in the elderly and it has been less convincingly established for elderly women compared with elderly men[3]. This may be partly because women and elderly patients have been poorly represented in prior clinical trials of cholesterol-lowering therapy. Consequently, the value of screening the lipid profile and performing cholesterol-lowering therapy in these populations is still unclear[4].

The aim of the present study was to perform a detailed post hoc assessment that compared the effect of lipid-lowering therapy on carotid atherosclerosis in younger and older patient subsets (≥75 and <75 years old) from the FAST population.

METHODS

Patient Selection and Study Protocol

The study design and the baseline characteristics of the patients have been described elsewhere[2]. Briefly, between February 1996 and February 2000, men and women aged 30-89 years with hypercholesterolemia who met the following criteria were eligible to participate in the present study. Exclusion criteria included a serum triglyceride level >350 mg/dl, uncontrolled heart failure, recent myocardial infarction (≤6 months), severe or unstable angina pectoris, hypothyroidism/hyperthyroidism or other endocrine diseases, secondary hyperlipidemia, uncontrolled diabetes mellitus, uncontrolled hypertension, heavy alcohol intake, obese patients on weight reduction programs, diseases that might interfere with drug absorption, any severe illness, and treatment with certain drugs (including corticosteroids, androgens, other lipid-lowering agents, or antiacids containing aluminum salts). Hospital visits for monitoring were scheduled after 2 weeks of therapy and then every 4 weeks thereafter. At each visit, a brief physical examination was performed, and the number of tablets was counted to assess compliance. In both groups, lipids, lipoproteins, and other laboratory parameters (to confirm safety) were also measured at each visit. Written informed consent was obtained from each patient, and this trial was approved by the Ethics Committee of Kyushu University Hospital.

The procedure for measurement of carotid IMT and its reproducibility have been described elsewhere[2]. In brief, ultrasonography was done with the patient in the supine position using an Aloka SSD-2000 (Aloka, Tokyo, Japan) with a 7.5 MHz transducer. The IMT of the far wall of both the right and left common carotid arteries was measured at 2, 2.5, and 3 cm proximal to the carotid bifurcation. The IMT was defined as the distance between two echogenic lines separated by a hypoechoic or anechoic space, with the outer line corresponding to the medial-adventitial border and the inner line representing the luminal-intimal border. The mean IMT was calculated as the average value of the measurements obtained at 6 sites (3 per vessel) in the bilateral carotid arteries. Stenosis was defined as plaque (IMT ≥1.10 mm) occupying more than half of the luminal circumference of the artery on a transverse scan.

Laboratory Tests

Blood samples were collected between 8 and 9 am after a 12-hour fast. Serum cholesterol and triglyceride levels were measured by enzymatic methods. Using the calcium heparin method, high-density lipoprotein (HDL) cholesterol was measured in

the supernatant obtained after precipitation of apolipoprotein B-containing lipoproteins by and LDL cholesterol was calculated using Friedewald's formula. Measurements were done on the day of blood collection, or else the blood was stored at -4 (C for no longer than 3 days until assay.

Statistical Analysis

All data were recorded on standard forms and were entered into a database. Results are expressed as percentages or as the mean (SD. The mean values of numerical variables were compared by the Mann-Whitney U test, while categorical variables were compared by the chi-square test, as appropriate.

The endpoint was the effect of each treatment on the incidence of major atherosclerotic events. The relative risk and 95% confidence interval were calculated with the Cox regression model. In all analyses, $P < 0.05$ was considered to indicate statistical significance. All data were analyzed on an intention-to-treat basis.

RESULTS

Baseline Characteristics

The baseline characteristics of the subjects have been summarized elsewhere[2]. Briefly, the mean age of the patients was 66.1 years and 31.3% were men. The average systolic blood pressure and diastolic blood pressure were 130.8 and 77.1 mm Hg, respectively. Of the 246 patients, 59.3% were recent or former smokers, 42.5% had a history of hypertension, and 22.9% had diabetes mellitus. Baseline serum total cholesterol and LDL-cholesterol levels were 253.0 mg/dL and 166.1 mg/dL, respectively, while the HDL-cholesterol level was 57.0 mg/dL and the serum triglyceride level was

Table 1 Baseline characteristics (including lipids) for the two subgroups of interest (patients ≥75 years old and patients <75 years old)

		Probucol			Pravastatin			Diet alone		
		Age≥75 (n=27)	Age<75 (n=55)	chi-square test	Age≥75 (n=27)	Age<75 (n=55)	chi-square test	Age≥75 (n=22)	Age<75 (n=59)	chi-square test
		No.(%)	No.(%)		No.(%)	No.(%)		No.(%)	No.(%)	
Sex	M	4(14.8)	21(32.0)	0.0568	1(3.6)	21(32.0)	0.0018	6(27.3)	24(40.7)	0.3939
	F	23(85.2)	34(61.8)		27(96.4)	34(61.8)		16(72.7)	35(59.3)	
Smoking	+	12(44.4)	36(65.5)	0.1149	13(46.4)	31(56.4)	0.5321	15(68.2)	39(66.1)	1.0000
	−	15(55.6)	19(34.5)		15(53.6)	24(43.6)		7(31.8)	20(33.9)	
CVD	+	17(63.0)	18(32.7)	0.0181	19(67.9)	15(27.3)	0.0009	14(63.6)	20(33.9)	0.0308
	−	10(37.0)	37(67.3)		9(32.1)	40(72.7)		8(36.4)	39(66.1)	
IHD	+	5(18.5)	4(7.3)	0.2480	8(28.6)	7(12.7)	0.1410	7(31.8)	4(6.8)	0.0104
	−	22(81.5)	51(92.7)		20(71.4)	48(87.3)		15(68.2)	55(93.2)	
HT	+	12(44.4)	25(45.5)	1.0000	16(57.1)	23(41.8)	0.2757	9(40.9)	17(28.8)	0.4415
	−	15(55.6)	30(54.5)		12(42.9)	32(58.2)		13(59.1)	42(71.2)	
DM	+	3(11.1)	12(21.8)	0.3818	5(17.9)	13(23.6)	0.7472	4(18.2)	19(32.2)	0.3331
	−	24(88.9)	43(78.2)		23(82.1)	42(76.4)		18(81.8)	40(67.8)	

	Probucol					p-value Mann Whitney's U-test	Pravastatin					p-value Mann Whitney's U-test	Diet alone					p-value Mann Whitney's U-test
	Age≥75(n=27)			Age<75(n=55)			Age≥75(n=27)			Age<75(n=55)			Age≥75(n=22)			Age<75 (n=59)		
	No.	Mean	S.D.	No.	Mean	S.D.	No.	Mean	S.D.	No.	Mean	S.D.	No.	Mean	S.D.	No.	Mean	S.D.
sBP	27	73.3±23.1		55	133.3±22.6	0.8434	28	130.2±24.4		55	128.7±22.0	0.5369	22	141.5±24.5		59	127.0±17.0	0.0077
dBP	27	21.6±11.5		55	80.0±13.1	0.0526	28	71.5±10.2		55	77.7±11.3	0.0187	22	78.7±8.2		59	77.4±10.2	0.2531
BMI	27	21.6± 3.3		55	23.9± 4.5	0.0109	28	21.8± 5.7		55	24.4±3.4	0.0118	22	22.3±2.1		59	23.1± 2.9	0.1723
IMT	27	1.5± 0.8		55	1.3± 0.5	0.7556	28	1.4± 0.9		55	1.2±0.4	0.6748	22	1.3±0.5		59	1.3± 0.5	0.8943
TC	27	257.9±25.5		55	249.7±25.5	0.0962	28	258.0±23.5		55	248.2±25.1	0.0349	22	256.0±23.3		59	254.9±30.3	0.3895
LDL-C	27	171.3±31.1		55	163.5±24.7	0.2734	28	161.5±29.2		55	160.2±33.0	0.4325	22	175.2±27.1		59	170.2±36.3	0.4509
HDL-C	27	61.1±19.9		55	56.2±18.7	0.2864	28	62.2±14.3		55	53.9±15.6	0.0218	22	55.1±10.0		59	57.0±16.5	0.8400
TG	27	127.9±56.0		55	150.2±72.3	0.2119	28	171.8±75.0		55	167.1±89.8	0.4880	22	128.5±44.8		59	138.5±63.7	0.6479

CVD: cerevral vascular disease
IHD: ischemic heart disease
HT: hypertension
DM: diabetes mellitus
sBP: systolic blood pressure
dBP: diastolic blood pressure
BMI: body mass index
IMT: intima-media thickness
TC: total cholesterol
LDL: LDL-cholesterol
HDL: HDL-cholesterol
TG: triglyceride

149.2 mg/dL. The mean IMT was 1.308 mm. There were no statistically significant differences in any of these baseline characteristics among the three treatment groups (probucol, pravastatin, and diet alone).

Baseline characteristics (including lipids) for the two subgroups of interest (patients ≥75 years old and patients <75 years old) are shown in the Table 1. In general, the three treatment groups were well matched for age and sex at baseline. The elderly subgroup (≥75 years old) included a higher proportion of women, and more patients had cerebrovascular disease compared with the younger subgroup (<75 years old) ($p<0.01$ for probucol, $p<0.01$ for pravastatin, and $p<0.01$ for diet alone; chi-square test). The potential importance of chance differences in baseline characteristics between any of the four subpopulations receiving either of the two active treatments was evaluated by assessing the relationship of all listed baseline variables to total mortality or major coronary events.

Drug Treatment and Serum Lipids

The percent changes of serum lipids after 2 years of treatment are displayed in Fig. 1. Mean between-group differences (intention-

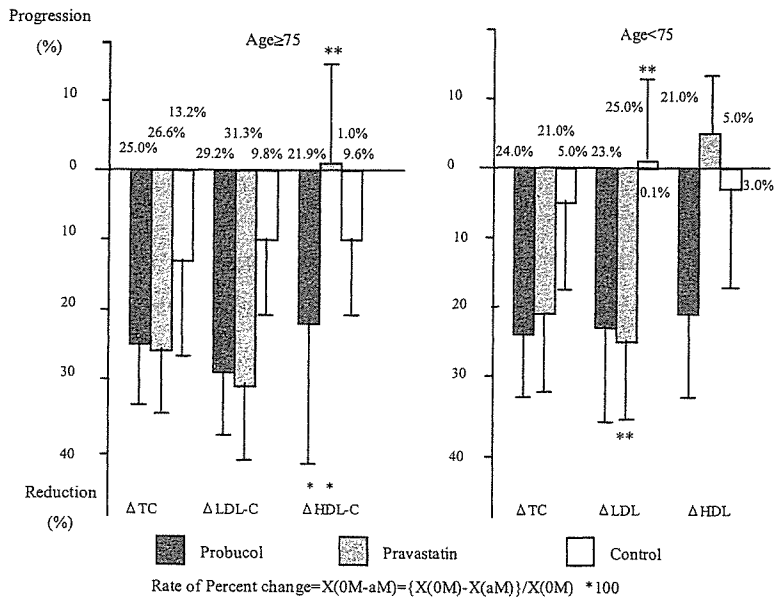

Fig. 1 Percent changes of serum lipids after 2 years. Among patients ≥75 years old, there was a significant decrease of serum total cholesterol in each of the three subgroups (by 25.0%, 26.6%, and 13.2% compared with baseline, respectively). There was also a significant decrease of serum LDL-cholesterol by 29.2%, 31.3%, and 9.8%, respectively, while the HDL-cholesterol levels of the probucol and control groups were significantly lower after 2 years. Patients <75 years old from the probucol and pravastatin groups showed a significant decrease of serum total and LDL-cholesterol levels (by 23.6% and 21.0% or 23.3% and 25.5% compared with baseline, respectively). In the probucol group, HDL-cholesterol was significantly reduced after 2 years (21.9%, $p<0.01$).

to-treat) of the percent change from baseline over the full duration of the trial are shown for total cholesterol, LDL cholesterol, and HDL cholesterol.

Patients ≥ 75 Years Old

After 2 years of treatment, there was a decrease of serum total cholesterol in each of the three groups, which showed a significant reduction of 25.0%, 26.6%, and 13.2% compared with baseline, respectively. After 2 years, there was also a significant decrease of serum LDL-cholesterol in the three groups, with the reduction being 29.2%, 31.3%, 9.8%, respectively. The serum HDL-cholesterol level of the pravastatin group was increased by 1.0% after 2 years, but this change was not significant. On the other hand, the HDL-cholesterol level showed a significant decrease in the probucol and control groups by 21.9% and 9.6%, respectively (Mann-Whitney U test). Triglyceride levels showed no significant changes throughout the study.

Patients < 75 Years Old

After 2 years of treatment, there was a significant decrease of serum total cholesterol and LDL-cholesterol levels in the probucol and pravastatin groups, with a reduction of 23.6% and 21.0% versus 23.3% and 25.5% compared with baseline, respectively. In the control group, total cholesterol and LDL-cholesterol levels were also lower at the end of the study, but the changes were not significant. The HDL-cholesterol level of the probucol group was significantly reduced after 2 years (21.9%, $p<0.01$). In the pravastatin group and the control group, however, HDL-cholesterol showed no significant changes throughout the study. Triglyceride levels also showed no significant changes throughout the study in any of the groups.

Intima-Media Thickness

The percent change of carotid IMT after 2 years is shown in Fig. 2. The decrease of IMT in patients ≥75 years old from the

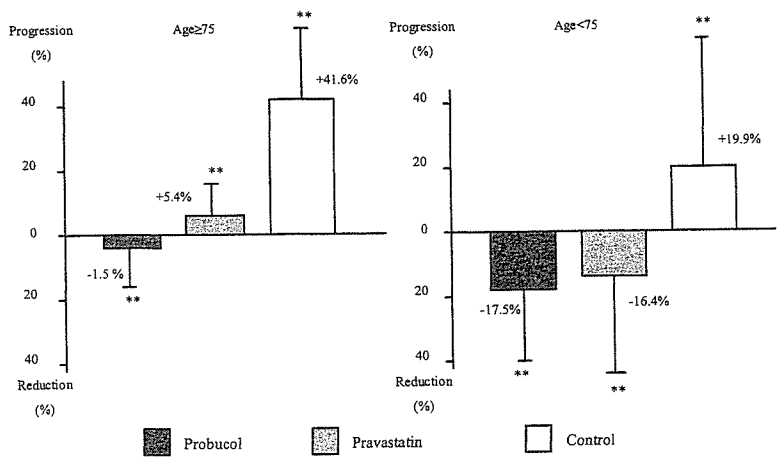

Fig. 2 Percent changes of carotid IMT after 2 years. Among patients ≥75 years old from the probucol and pravastatin groups, IMT showed a significantly greater decrease compared with that in patients <75 years old (both $p<0.01$). In the control group, IMT increased significantly by 19.9% ($p<0.05$). The change of IMT was significantly different in the treated groups compared with the control group (both $p<0.001$).

probucol and pravastatin groups was significant compared with that in patients <75 years old (both $p<0.01$; Mann-Whitney U test). In the control group, however, IMT showed a significant increase of 19.9% after 2 years ($p<0.05$; Mann-Whitney U test). The changes of IMT in the probucol and pravastatin groups were significantly different compared with that in the control group (both $p<0.001$; Mann-Whitney U test), while there was no significant difference in the change of IMT between the probucol and pravastatin groups at 24 weeks after the completion of treatment. There was no significant increase of IMT in the probucol group after 2 years of treatment.

Total and CHD Mortality

Among the 82 patients in the probucol group, two suffered a major cardiovascular event (2 deaths from coronary heart disease). Major events occurred in 4 of the 83 patients from the pravastatin group (3 deaths from coronary heart disease and 1 nonfatal myocardial infarction) and 11 of the 81 patients from the control group (8 deaths from coronary heart disease and 3 nonfatal myocardial infarctions). Of the 16 deaths that occurred during this study, two were in the probucol group, 5 were in the pravastatin group, and 9 were in the control group. Among these 16 patients, 13 deaths were from cardiovascular causes, while the others were due to gastrointestinal bleeding and infection. Total mortality and CHD mortality in the patients ≥75 years old are shown in Table 2.

Total cardiovascular events were significantly reduced in patients ≥75 years old from the probucol group compared with the control group (relative risk: 0.12; $p<0.05$). The reduction of relative risk was slightly greater than that observed for patients <75 years old (relative risk: 0.20; p=N.S.), but there were overlapping 95% confidence intervals. The relative risk of total death was similarly reduced by probucol in both age groups (86% reduction for patients ≥75 years old), and this decrease was statistically significant. Although the relative risk of total death was also reduced by pravastatin in both age groups (43% reduction for patients ≥75 years old), the change was not significant. The total cardiovascular event rate and total death rate over the duration of the study were more than three times higher in control group patients ≥75 years old (27.3% and 22.7%, respectively) compared with patients <75 years old (8.5% and 6.8%, respectively). Conse-

Table 2 Effect of probucol and pravastatin on clinical events in hypercholesteromoic patients

	Patients, n (%)													
	Probucol n=82		Pravastatin n=83		Control n=81		Probucol				Pravastatin			
							Hazards ratio	95%C.I.		p	Hazards ratio	95%C.I.		p
Age≥75	n=72		n=28		n=22									
All cardiovascular events	1	(3.7)	4	(14.3)	6	(27.3)	0.1247	0.0150	1.0358	0.0184	0.476	0.1343	1.6875	0.2439
Fatal MI	1	(3.7)	3	(10.7)	5	(22.7)	0.1509	0.0176	1.2923	0.0416	0.4317	0.1031	1.8072	0.2403
Non-fatal MI	0	(0.0)	1	(3.6)	1	(4.5)					0.6899	0.0431	11.0317	0.7936
PTCA/CABG	0	(0.0)	1	(3.6)	0	(0.0)								
All cerebrovascular events	0	(0.0)	0	(0.0)	0	(0.0)								
All other events	0	(0.0)	1	(3.6)	0	(0.0)								
All deaths	1	(3.7)	4	(14.3)	5	(22.7)	0.1498	0.0175	1.2828	0.0407	0.5713	0.1533	2.1287	0.4023
Age<75	n=55		n=55		n=59									
All cardiovascular events	1	(1.8)	0	(0.0)	5	(8.5)	0.2080	0.0243	1.7804	0.0968				
Fatal MI	1	(1.8)	0	(0.0)	3	(5.1)	0.3430	0.0357	3.2978	0.3197				
Non-fatal MI	0	(0.0)	0	(0.0)	2	(3.4)								
PTCA/CABG	0	(0.0)	0	(0.0)	0	(0.0)								
All cerebrovascular events	0	(0.0)	0	(0.0)	0	(0.0)								
All other events	0	(0.0)	1	(1.8)	1	(1.7)					1.3237	0.0823	21.2987	0.8434
All deaths	1	(1.8)	1	(1.8)	4	(6.8)	0.2557	0.0286	2.2879	0.1726	0.2990	0.0334	2.6794	0.2322

quently, the absolute risk reduction for patients ≥75 years old was more than three times that for patients <75 years old in the case of both total cardiovascular events and total deaths.

DISCUSSION

FAST was the first clinical trial to clearly demonstrate the benefit of probucol for elderly hypercholesterolemic patients and to also demonstrate an effect of probucol on the incidence of cardiovascular events. FAST showed that probucol therapy could achieve a significant reduction in the risk of major coronary events in patients ≥75 or < 75 years old, as well as significant improvement of all the tertiary CHD and atherosclerosis-related study end-points that were positive in the entire FAST cohort. The magnitude of the observed risk reduction in these subgroups was very similar to that reported for the entire study cohort and for other clinically relevant subgroups that have been analyzed. Although FAST was not specifically designed to assess changes of mortality in elderly subjects, high event rates combined with the substantial percentage of patients in this subgroup allowed us to detect a significant reduction of both all-cause mortality and CHD mortality. Safety and tolerability showed no important differences between the two age groups and were largely consistent with the findings for the entire study cohort[2].

In the subjects ≥75 versus <75 years old, LDL cholesterol showed similar changes (26% vs. 22%). This finding is consistent with other data suggesting that the cholesterol-lowering effect of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitors is enhanced as patients become older[5]. Baseline total cholesterol, LDL-cholesterol, and HDL-cholesterol levels showed no significant relationship with the response to treatment (reduction in relative risk) in any of the subpopulations examined (data not shown), as was also the case for the entire study cohort[2]. The reduction of LDL cholesterol was more significant in the pravastatin group than in the probucol or control groups. Although the control group showed a significant reduction of LDL cholesterol with diet alone, an increase of carotid IMT still occurred, unlike the outcome in the active treatment groups. After 2 years of therapy, there was a significant decrease of serum LDL-cholesterol in all three groups compared with baseline. It was interesting that probucol had an antiatherogenic effect and caused a reduction of CHD events in patients ≥75 years old.

Lipid peroxidation of LDL has been demonstrated to be an important risk factor for the development of atherosclerosis[6][7]. There are several possible reasons, including the increased susceptibility of LDL to oxidation with aging[8], which can be partly explained by modification of its fatty acid composition and a decrease of the antioxidant (vitamin E) content[8]. Recently, Napoli et al. reported that resistance of LDL to peroxidative modification was lower in elderly men than in young men[9]. Furthermore, age was correlated with the extent of lipid peroxidation, supporting the hypothesis that LDL contributes to the increment of plasma lipid peroxides with aging[10][11]. Since oxidation of LDL is considered to be a key event in atherogenesis, it could be an additional reason why atherosclerosis is related to aging.

FAST showed that probucol therapy could delay the increase of IMT independently of its LDL or HDL cholesterol-lowering effect, and a reduction of IMT occurred

earlier with probucol than with pravastatin[2]. In the present study, patients ≥75 years old showed a significantly smaller change of IMT after probucol therapy compared with patients < 75 years old irrespective of the cholesterol-lowering effect. However, it was clearly demonstrated that probucol could reduce the risk of all-cause mortality and major coronary events in CHD patients ≥75 years old. The above findings suggest that there may be another mechanism involved in the effect of probucol. Other investigators have shown that suppression of atherogenesis by probucol is independent of its cholesterol-lowering action and is presumably due to an antioxidant effect on lipids[12)13)]. Because mortality and CHD events increase with age[14)], the absolute reduction of the death rate and event rate was substantially greater for patients ≥75 years old compared with those <75 years old. The relationship between serum cholesterol and the development of CHD has been observed in various epidemiological studies, but is reported to be weaker in elderly persons compared with middle-aged subjects[15)~19)], so the above findings may be unexpected. However, limited data are available about the predictive value of cholesterol in elderly patients with established CHD. Taken together with the results of the present study, the above findings may indicate the importance of ancillary effects of probucol other than cholesterol lowering for reducing the incidence of cardiovascular events. In fact, our data suggest that probucol may have multiple actions, but further studies are needed to investigate the relative contribution of each effect of this drug.

A difference between the effect of probucol and pravastatin on the IMT was not demonstrated by the present study, perhaps because the sample size was small. A large-scale investigation would be necessary to determine whether probucol and pravastatin therapy have a different influence on the IMT. Lack of a placebo control group was another limitation of our study. However, the use of quantitative B-mode ultrasound allowed us to obtain unbiased data. Although FAST was not specifically designed to assess the influence of lipid-lowering therapy on mortality in the elderly, high event rates combined with the substantial percentage of elderly patients in the study population provided the power to demonstrate a significant reduction of both all-cause mortality and CHD mortality among elderly patients receiving probucol. Safety and tolerability showed no important differences related to age or sex, and were generally consistent with the results for the entire study cohort[2)].

In conclusion, the present findings suggest that hypercholesterolemia in the elderly is a morbid state requiring treatment and that probucol is a useful drug for reducing the incidence of cardiovascular disease in hypercholesterolemic elderly persons.

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（和文抄録）

高齢者の高コレステロール血症に対するProbucolの効果

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【目的】高齢者（75歳以上）の高コレステロール血症患者に対して，積極的な脂質低下療法が頚動脈硬化の進展抑制および主要冠動脈イベントリスク低下が認められるか否かについて検討した．

【方法】FASTの対象患者（246例）のうち，75歳以上（76例）と75歳未満（168例）について，脂質低下療法（Probucol Pravastain）および食事療法により，その有効性について頚動脈エコーを用いて評価した．総頚動脈の内膜中膜複合体厚（IMT）を測定し，左右6点のIMTの平均値をIMT値とした．1次エンドポイントは2年間のIMT値の変化率とし，2次エンドポイントは主要冠動脈イベントとした．

【結果】Probucol群及びPravastain群では，年齢に関係なく，高齢者においても動脈硬化の進展抑制を認めた．Probucol群における高齢者のControl群に対する各臨床イベントの相対リスク（95%信頼期間）は総死亡が0.15（0.02-1.28），総冠動脈イベント0.12（0.02-1.04）と有意な進展を認めた．一方，Pravastain群との間では，各臨床イベントの相対リスクに有意差は認められなかった．Probucol群とPravastain群との間では，各臨床イベントの相対リスクに有意差は認められなかった．

【結論】75歳以上の高齢者に対してもProbucolは，頚動脈硬化の進展抑制効果が認められ，さらに主要冠動脈イベントの相対リスクの低下作用を認められる可能性が示唆された．

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Association between fast-migrating low-density lipoprotein subfraction as characterized by capillary isotachophoresis and intima-media thickness of carotid artery

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Abstract

Background: A mildly modified LDL subfraction that is characterized by an increased negative charge exists in plasma. This electronegative LDL separated by ion-exchange chromatography has been shown to be inflammatory and its proportion is increased in patients with hyperlipidemia and diabetes mellitus. The present study examined the association between the level of fast (f)-migrating LDL subfraction characterized by capillary isotachophoresis (cITP) and carotid-artery intima-media thickness (CA-IMT).

Methods and results: This study included 469 subjects who underwent a physical examination. CA-IMT was determined by high-resolution B-model ultrasonoraphy. Levels of charge-based LDL subfractions were measured by cITP on a Beckman P/ACE MDQ system. An increased serum LDL-C level and cITP fLDL level were associated with increased CA-IMT after adjusting for age. The extent of the associations between cITP fLDL and CA-IMT and between LDL-C and CA-IMT were similar as assessed by a receiver-operating characteristic curve analysis. LDL-C, triglyceride, and remnant-like particle cholesterol levels were independently correlated with cITP fLDL, and the LDL-C level had the strongest correlation with cITP fLDL. The association between the cITP fLDL level and CA-IMT was significant in the high LDL-C stratum but not in the low stratum, indicating that it is modified by the LDL-C level. The high-LDL-C-high-fLDL group had the highest relative risk for a high CA-IMT among the groups with each combination of LDL-C and cITP fLDL level.

Conclusion: The cITP fLDL level was associated with CA-IMT and its combination with the LDL-C level is a stronger indicator for a high CA-IMT.

Keywords: Low density lipoprotein (LDL) cholesterol; Intima-media thickness; Carotid atherosclerosis; Capillary isotachophoresis; Fast-migrating LDL subfraction

1. Introduction

Serum level of low-density lipoprotein cholesterol (LDL-C) is an established risk factor of coronary artery disease (CAD), and a reduction in LDL-C levels has been shown to be associated with reduced death rates caused by CAD. LDL is composed of heterogeneous particles that differ in size, composition, and electric charge. Qualitatively modified forms of LDL have been shown to exist in human plasma, including small dense LDL, oxidative modified LDL, glycated LDL, and diasylated LDL [1–5], and they are

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all atherogenic. These forms of modified LDL all have an increased negative charge. The electronegative subfraction of LDL [LDL(−)] has been separated from plasma by anion-exchange chromatography techniques [6,7]. LDL(−) in plasma can also be generated by other processes including enrichment with nonesterified fatty acids or enzymatic modification by phospholipase A2 or cholesteryl esterase/trypsin [7].

Although the origins of LDL(−) are complex and not fully understood, LDL(−) has been shown to have proinflammatory activity on endothelial cells [8] and its proportion is increased in patients with hypertriglyceridemia [9], familial hypercholesterolemia (FH) [9], and diabetes mellitus (DM) [10], patients on hemodialysis [11], and patients with anigographically documented CAD [12].

However, there is still little information available on whether or not the LDL(−) subfraction level is a marker for atherosclerosis and whether or not its association with atherosclerosis is independent of the LDL-C level, partly because of the lack of a routine analytical technique for this modified LDL subfraction.

The current ion-exchange chromatography method for measuring the LDL(−) subfraction gives the proportion of LDL(−) protein content in total LDL separated by ultracentrifugation [6]. Therefore, it is disadvantageous for routine analysis in that it is time-consuming and requires a relatively large amount of samples, and the absolute level of LDL(−) in plasma cannot be determined.

Capillary isotachophoresis (cITP) is another technique that separates and quantifies LDL subfractions according to electric charge. It was originally developed by the research group of Schmitz [13,14]. Fast-migrating LDL (fLDL) carries more negative charge than slow-migrating LDL (sLDL) [13,14]. Since lipoproteins are pre-stained with a fluorescent lipophilic dye, LDL subfractions can be measured directly in plasma and with high sensitivity (only several microliters of sample are necessary). Separation and on-line detection can both be performed within just a few minutes. Therefore, analytical cITP technique may be useful for the routine analysis of lipoprotein profiles. We previously showed that the absolute levels of lipoprotein subfractions can be determined as a peak area relative to an internal marker and the levels of cITP fLDL and sLDL were proportional to the protein content of LDL [15–17].

Measurement of the thickness of the intima and media of carotid arteries by high-resolution B-mode carotid ultrasonography has been used as a non-invasive method for detecting early carotid atherosclerosis [18,19]. Carotid-artery intima-media thickness (CA-IMT) is associated with the prevalence of cardiovascular disease and with cardiovascular risk factors [20].

We investigated the hypothesis that the cITP fLDL subfraction level is associated with CA-IMT. We also hypothesized that the cITP fLDL level contributes to the ability of LDL-C to predict the risk of CA-IMT after controlling for conventional cardiovascular risk factors.

2. Methods

2.1. Subjects

This study included 469 male subjects (aged between 21 and 88 years) who participated in a health examination. This study was approved by the Ethics Committees of Kyushu University Hospital, and samples were collected only after the participants had given their informed consent.

The prevalence of hypertension, diabetes mellitus, and smoker in the study subjects was 39.7% ($n=186$), 13.9% ($n=65$), and 32.6% ($n=153$), respectively. Twelve subjects (2.6%) had a history of stroke, and 12 (2.6%) had a history of coronary heart disease. Hypertension was defined as systolic blood pressure $\geq$140 mmHg, diastolic pressure $\geq$90 mmHg, or treatment with antihypertensive medications. Diabetes mellitus was defined as a self-reported history of diabetes, a fasting plasma glucose concentration $\geq$126 mg/dl, or the use of anti-diabetic drugs. Smokers were defined as those who had smoked past or who were present smokers. Subjects who refused ultrasound examination or who had a fasting blood glucose concentration $\geq$400 mg/dl or triglyceride (TG) level $\geq$400 mg/dl were excluded from the study.

Blood was drawn between 9 and 12 a.m. after an overnight fast and stored at −80 °C until analysis. Storage of samples at −80 °C for up to 5 months does not apparently affect measurements for cITP LDL subfractions [17].

2.2. Ultrasonographic measurement

Common carotid-artery lesions were assessed by high-resolution B-mode ultrasonography with a 7.5 MHz mechanical sector transducer on an Aloka SSD-2000 (Aloka Co. Ltd., Tokyo, Japan), as described previously [21,22]. All assessment of carotid arteries was performed by three specially trained technicians who were unaware of the clinical history or risk factor profile. IMT was measured at points 20, 25, 30 mm proximal to the flow divider on the far wall of the right and left common carotid arteries at the end of the diastolic phase. Using this information, mean CA-IMT was determined for each individual.

2.3. Measurement of serum lipids and lipoproteins

Serum levels of total cholesterol (TC), TG, high-density lipoprotein cholesterol (HDL-C) were measured by enzymatic methods. Serum LDL-C levels were calculated indirectly using the Friedewald formula.

Remnant-like particle cholesterol (RLP-C) levels were measured by an RLP-Cholesterol Immunoseparation Assay using a commercially available kit (JIMRO-II, Japan Immunoresearch Laboratories Co., Ltd., Gunma, Japan) [23,24]. Briefly, the RLP immunoseparation gel was washed before use three times with RLP buffer by low-speed centrifugation and suspended by repeatedly inverting the container. After 150 μl of the suspended gel was aliquoted into Hitachi

microsample cups, 5 μl serum samples were added and the mixture was stirred using a steel bead for 2 h at room temperature on an RLP Mixer J-100 (Otsuka Electrics Co., Ltd, Tokyo, Japan). After the gel had settled for 15 min, the cholesterol level in the supernatant was measured with cholesterol reagents included in the assay kit using an auto-analyzer (Hitachi 7600-020S).

2.4. *Quantification of lipoprotein subfractions by cITP*

Capillary isotachophoresis of serum lipoproteins was performed on a Beckman P/ACE MDQ system (Beckman-Coulter Inc., Tokyo, Japan) according to the method of Bottcher et al. [13] with some modifications, as previously described [15–17,25,26]. Briefly, 6 μl of serum was diluted with 14 μl of leading buffer consisting of 10 mM HCl and 18 mM ammediol (2-amino-2-methyl-1,3-propanediol) (pH 8.8), prestained with 10 μl 0.1 mg/ml NBD C6-ceramide (Molecular Probe Inc., OR, USA) for 5 min at room temperature, and mixed with 50 μl of the mixture containing leading buffer with 0.35% hydroxypropylmethylcellulose (HPMC), spacers, and 5-carboxy-fluorescein as an internal marker. The spacers were *N*-(2-acetamido)-2-aminoethanesulfonic acid (ACES), D-glucuronic acid, 1-octanesulfonic acid sodium salt, 3-(*N*-tris[hydroxymethyl]methylamino)-2-hydroxypropanesulfonic acid (TAPSO), *N*-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid (TAPS), L-serine, L-glutamine, L-methionine, and glycine. The terminating buffer contained 24 mM β-alanine and 13 mM ammediol, and was adjusted to pH 10.5 with saturated barium hydroxide solution. A dimethylpolysiloxane-modified fused silica capillary (AT^{TM}-1) was purchased from Alltech Japan Inc. (Tokyo, Japan). The sample was injected at 20 psi for 18 s into a 30-cm long capillary (i.d. 180 μm), and separation was performed at a constant 30 μA for 1 min and 10 kV for 7 min. The separated zones were monitored with argon-laser-induced fluorescence detection (excitation, 488 nm; emission, 520 nm). Each peak was identified and the peak area in relative fluorescence units was analyzed using 32 Karat Software version 5.0 (Beckman-Coulter Inc., Tokyo, Japan). Levels of cITP lipoprotein subfractions were expressed as the peak area relative to the internal marker.

2.5. *Statistical analysis*

All of the statistical analysis was performed using the SAS (Statistical Analysis System) Software Package (Version 9.1, SAS Institute, CA, USA) at the Fukuoka University. The distribution of variables were examined by the Shapiro–Wilk test [27]. The 33.3th and 66.7th percentiles were used to produce tertiles of CA-IMT. Linear trends of risk factors across tertiles of CA-IMT after adjusting for age were examined by an analysis of covariance (ANCOVA) using a general linear model. Correlation between variables was examined by Spearman correlation. Log-transformed values of TG and RLP-C were used in the data analysis. Low and high LDL-C strata were defined as $<$ and $\geq$ the median value of LDL-C (118 mg/dl), respectively, and low and high CA-IMT were defined as $<$ and $\geq$ the median value of CA-IMT (0.77 mm). The strength of the associations between the cITP fLDL and LDL-C levels was compared using a receiver operating characteristic (ROC) curve analysis. An ROC-curve (plot of sensitivity versus 1-specificity) analysis is a powerful tool for assessing the ability of a continuous variable to discriminate between two groups of subjects, and does not depend on the cutoff value selected. The area under the ROC curve represents the probability for a randomly chosen low CA-IMT subject to exhibit a value lower than the level observed among randomly chosen high CA-IMT subjects. A value of 0.5 means that the distributions of the values in the two groups are similar; conversely, a value of 1.0 means that the distributions of the values in the two groups do not overlap. We determined the area under the ROC curve by the trapezoidal rule and evaluated its significance by the Wald chi-square test, as described previously [28]. Stepwise multiple regression analysis was used to examine the independent variables that are related to cITP fLDL. The significance of the association between the combination of LDL-C and cITP fLDL and CA-IMT after controlling for age and other related variables was examined by a multivariate logistic regression analysis using dummy variables. The odds ratio and 95% confidence interval (CI) were given for each combination of LDL-C and cITP fLDL. All p values are two-tailed. The significance level was considered to be 5% unless indicated otherwise.

3. Results

Table 1 shows the mean levels of conventional risk factors of CAD, serum levels of lipids and lipoproteins, and RLP-C levels according to tertiles of CA-IMT. Increased age was associated with increased CA-IMT (tertile III versus tertile II versus tertile I: 64.4 ± 0.9 year versus 59.7 ± 0.9 year versus 48.9 ± 1.0 year, $p < 0.05$, by an analysis of variance). The prevalence of DM and serum levels of TC and LDL-C were positively and significantly associated with CA-IMT after adjusting for age, as assessed by an analysis of covariance (Table 1). Body mass index (BMI), prevalence of HT and smoking, and serum levels of TG, HDL-C, and RLP-C were not significantly associated with CA-IMT after adjusting for age (Table 1).

Fig. 1 shows the typical cITP lipoprotein profiles of subjects with low (0.54 mm) and high CA-IMT (1.17 mm). As shown, capillary isotachophoresis clearly separated lipoproteins into eight fractions within 8 min. Peaks 6 and 7 are the two LDL subfractions with fast and slow electrophoretic mobility. Subject with high CA-IMT had apparently higher levels of both fLDL and sLDL than that with low CA-IMT (Fig. 1).

Table 2 shows that age-adjusted mean levels of intermediate-migrating HDL decreased and cITP fLDL and sLDL increased across tertiles of CA-IMT. This result indi-

Table 1
Age-adjusted mean levels of risk factors according to tertiles of carotid-artery intimal-media thickness (CA-IMT)

	Tertiles of CA-IMT			
	Low (<0.67 mm)	Middle (0.67–0.83 mm)	High (≥0.83 mm)	
No. of subjects	142	159	168	
Age (year)	48.9 ± 1.0	59.7 ± 0.9	64.4 ± 0.9	<0.05
Body mass index (kg/m^2)	22.6 ± 0.3	23.9 ± 0.2	23.5 ± 0.2	n.s.
Hypertension (%)	23	40	53	n.s.
Diabetes mellituss (%)	5	12	22	<0.05
Smoking (%)	37	31	30	n.s.
TC (mg/dl)	191 ± 3	199 ± 2	204 ± 3	<0.05
log(TG)	4.7 ± 0.0	4.8 ± 0.0	4.8 ± 0.0	n.s.
HDL-C (mg/dl)	53 ± 1	54 ± 1	52 ± 1	n.s.
LDL-C (mg/dl)	113 ± 3	118 ± 2	123 ± 2	<0.05
log(RLP-C)	2.7 ± 0.0	2.8 ± 0.0	2.9 ± 0.0	n.s.

TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; RLP-C, remnant-like particle cholesterol. The units of TG and RLP-C were mg/dl.

[a] Assessed by an analysis of covariance or logistic regression analysis after adjusting for age. Continuous variables were adjusted for age by means of linear regression, and categorial variables were adjusted for age by means of logistic regression.

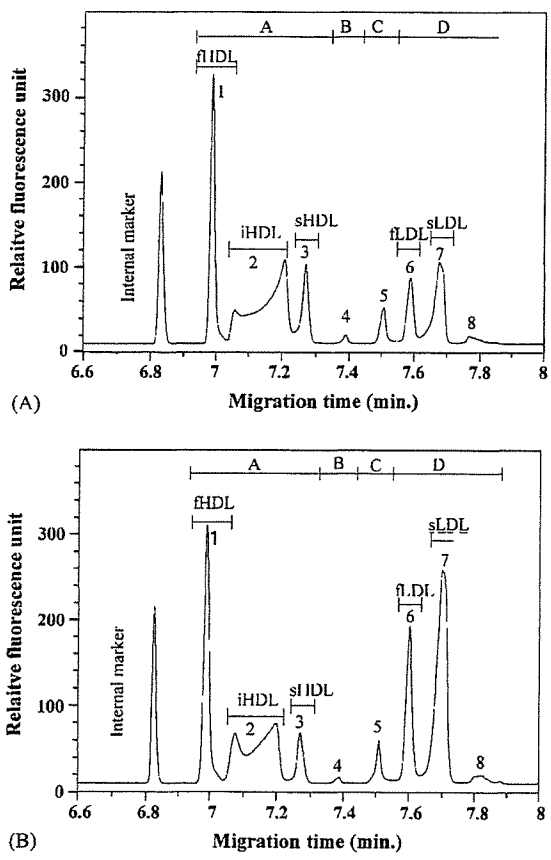


Fig. 1. Lipoprotein profiles as determined by capillary isotachophoresis in serum from subjects with low (A) and high (B) carotid-artery intima-media thickness (CA-IMT: 0.54 and 1.17 mm, respectively). The various lipoprotein subfractions are depicted as follows [13,14]: A, HDL; B, chylomicron/remnants; C, VLDL/IDL; D, LDL. fHDL, fast-migrating HDL; iHDL, intermediate-migrating HDL; sHDL, slow-migrating HDL; fLDL, fast-migrating LDL; sLDL, slow-migrating LDL.

cates that both cITP fLDL and sLDL were positively associated with CA-IMT independent of age. The strength of the associations between cITP fLDL and CA-IMT (two levels) and between LDL-C and CA-IMT were compared by an ROC curve analysis. Fig. 2 shows the plot of sensitivity (true positive) versus 1-specificity (false positive) for the LDL-C level and cITP fLDL level. The area under the ROC curve was similar for cITP fLDL and LDL-C (0.578 and 0.582, respectively).

The cITP fLDL levels were negatively correlated with HDL-C levels ($r=-0.135$, $p<0.01$) and significantly ($p<0.01$) and positively correlated with age, BMI, and serum levels of TC, TG, LDL-C, and RLP-C ($r=0.156$, 0.168, 0.524, 0.208, 0.545, and 0.147, respectively). Stepwise multiple regression analysis selected LDL-C, TG, and RLP-C as independent variables that were related to cITP fLDL (Table 3). The LDL-C level had the strongest correlation with cITP fLDL (Table 3). Fig. 3 shows the correlation between cITP fLDL and LDL-C levels in subjects with low, middle, and high CA-IMT. As shown, cITP fLDL levels were significantly correlated with LDL-C levels in all the three groups of subjects. As also shown in Fig. 3, the regression lines of cITP fLDL versus LDL-C levels in subjects with middle and high CA-IMT (dotted lines) were shifted towards higher cITP fLDL levels as compared with that in subjects with low CA-IMT (solid line). This result indicates that cITP fLDL levels were higher in subjects with middle and high CA-IMT than in subjects with low CA-IMT after controlling for LDL-C levels.

Therefore, LDL-C levels were stratified into low and high strata and the association between cITP LDL and CA-IMT was examined according to LDL-C strata to test its relation to LDL-C levels. As shown in Table 4, the association between cITP fLDL and CA-IMT was significant in the high LDL stratum [odds ratio (95% CI): 2.2 (1.2–3.8)] but not in the low LDL stratum after adjusting for age by a multiple logistic regression analysis. This result indicates that

Table 2
Age-adjusted mean levels of lipoprotein subfractions as measured by capillary isotachophoresis (cITP) according to tertiles of carotid-artery intimal-media thickness (CA-IMT)

	Tertiles of CA-IMT			p^a
	Low (<0.67 mm)	Middle (0.67–0.83 mm)	High (≥0.83 mm)	
cITP fHDL	1.46 ± 0.04	1.47 ± 0.04	1.40 ± 0.04	n.s.
cITP iHDL	2.22 ± 0.04	2.14 ± 0.03	2.10 ± 0.03	<0.05
cITP sHDL	0.41 ± 0.01	0.40 ± 0.01	0.39 ± 0.01	n.s.
cITP VLDL/IDL	0.91 ± 0.04	1.04 ± 0.04	1.01 ± 0.04	n.s.
cITP fLDL	1.09 ± 0.03	1.16 ± 0.02	1.20 ± 0.03	<0.05
cITP sLDL	1.29 ± 0.05	1.36 ± 0.04	1.46 ± 0.04	<0.05

Levels of cITP lipoprotein subfractions are expressed as peak area relative to the internal marker. fHDL, iHDL, and sHDL, fast-intermediate, and slow-migrating high-density lipoprotein; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein; fLDL and sLDL, fast- and slow-migrating low-density lipoprotein.

[a] Assessed by an analysis of covariance. Variables were adjusted for age by means of linear regression.

Table 3
Stepwise multivariable regression analysis of the independent variables related to fast-migrating low-density lipoprotein (fLDL) as determined by capillary isotachophoresis (cITP)

Step	Variable entered	Partial correlation coefficient	F	p
1	LDL-C	0.573	190.1	<0.001
5	log(TG)	0.306	67.3	<0.001
4	log(RLP-C)	0.204	25.6	<0.001

Levels of cITP fLDL are expressed as peak area relative to the internal mark.

the association between cITP fLDL and CA-IMT was modified by the LDL-C level. Fig. 4 shows a three-dimensional plot of the age-adjusted relative risk for a high CA-IMT for each combination of cITP fLDL and LDL-C levels. The high-LDL-C-high-fLDL group had the highest risk for a high CA-IMT among the four groups: the low-LDL-C-low-fLDL group, the low-LDL-C-high-fLDL group, the high-LDL-C-low-fLDL group, and the high-LDL-C-high-fLDL group. Similar results were obtained after additionally adjusting for HT, DM, and smoking (data not shown). These results indicate that the combination of cITP fLDL and LDL-C level was a stronger indicator for a high CA-IMT than either cITP fLDL or LDL-C alone.

4. Discussion

With advances in techniques in lipoprotein analysis, a new LDL subfraction in plasma that is characterized by a greater negative charge than native LDL has attracted considerable attention. The electronegative LDL subfraction separated by ion-exchange chromatography has been shown to contain mildly modified LDL that could be produced from multiple origins [7] and is associated with a pathogenic state that is related to atherosclerosis [9–11]. Therefore, this negatively charged LDL subfraction could be a novel marker for atherosclerosis. However, there is still little evidence to support this point because of the lack of routine analytical techniques for this LDL subfraction. Ion-exchange chromatography is excellent for the separation of LDL(−) and for preparative use [6]. However, since it requires the separation of LDL by ultracentrifugation, the absolute level of LDL(−) in plasma cannot be measured with this technique and routine analysis is also difficult.

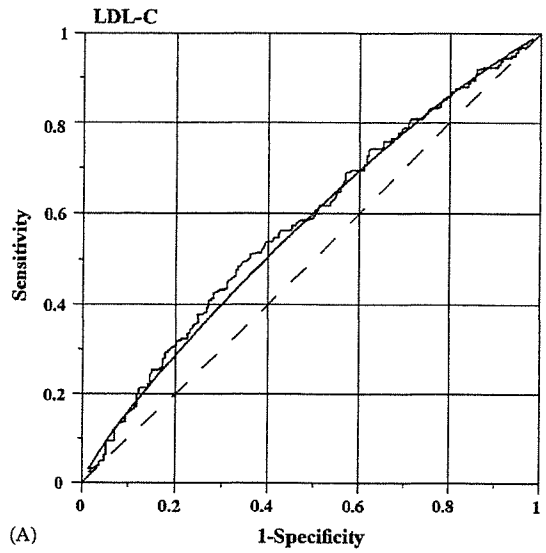
Analytical capillary isotachophoresis is a new technique for routine analysis of LDL subfractions according to their electric charges, which was established by the research group of Schmit et al. [13,14]. Several microliters of serum or plasma can be directly analyzed and separation and detection of cITP fast- and slow-migrating LDL can be performed within minutes. However, little attention has been paid to this technique [15–17,25,26], and therefore the clinical significance of the cITP fLDL subfraction is still unclear. We have previously shown that cITP can be used to quantify charge-based LDL subfractions [17] and express the absolute levels of cITP lipoprotein subfractions as the peak area relative to an internal marker [15–17,25,26].

We are the first to report that cITP fLDL and sLDL levels are associated with carotid-artery IMT. This finding is not unexpected because serum levels of LDL-C are associated with CA-IMT and levels of cITP LDL subfractions were correlated with LDL-C levels (Table 3, Fig. 3). We also found using an ROC curve analysis that the ability of cITP fLDL to predict for a high CA-IMT was similar to that of LDL-C (Fig. 2).

Table 4
Multiple logistic regression analysis of the association between fast-migrating LDL determined by cITP and carotid-artery intima-media thickness after adjusting for age in low and high LDL-C strata

	Regression coefficient ± S.E.	Odds ratio (95% confidence interval)	Wald chi-square	p
Low LDL-C	0.12 ± 0.37	1.1 (0.54–2.3)	0.10	n.s.
High LDL-C	0.78 ± 0.29	2.2 (1.2–3.8)	7.36	<0.01

The median value of LDL-C (118 mg/dl) was used to produce low and high LDL-C strata.



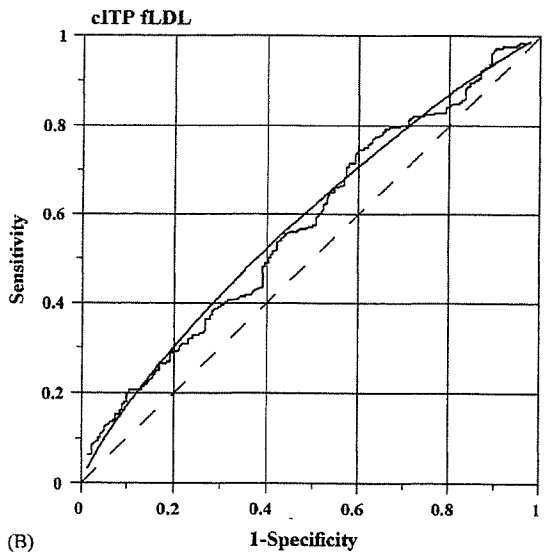


Fig. 2. ROC curves of the true-positive rate (sensitivity) vs. the false-positive rate (1-specificity) for LDL-C (A) and cITP fLDL (B). The smooth curves are model-fitted curves by the method of Swets [31].

Our finding that the cITP fLDL level was significantly related to the serum TG level (Table 3) agrees with that of Sanchez-Quesada et al., who reported that patients with hypertriglyceridemia had an increased proportion of LDL(−) [9]. Therefore, a high TG level could contribute to the increased electronegativity of LDL. We also observed a significant correlation between cITP fLDL and RLP-C levels (Table 3). The RLP-C level has been shown to be associated with CA-IMT independent of LDL-C and TG levels in a group of 50-year-old Caucasian men [29]. In our study subjects who had a wide range of ages, we observed no statistically significant association between the RLP-C level and CA-IMT after adjusting for age. The mechanism by which

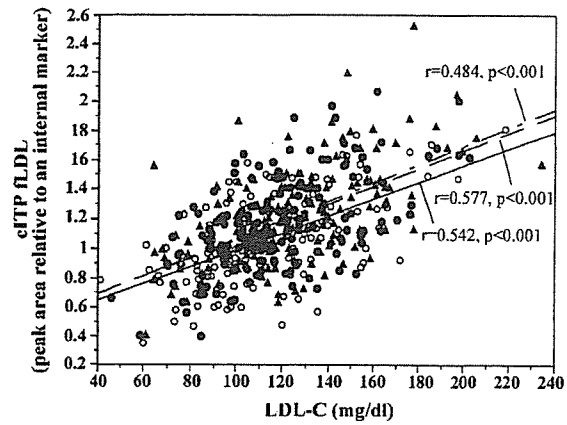


Fig. 3. Correlation between levels of fast-migrating LDL as determined by capillary isotachophoresis (cITP fLDL) and LDL-C levels in subjects with low (○), middle (●) and high (▲) carotid-artery intima-media thickness (CA-IMT).

RLP-C is related to cITP fLDL and whether or not it contributes to the association between cITP fLDL and atherosclerosis need further investigation.

Despite a strong correlation between cITP fLDL and LDL-C levels, we found that the association between the cITP fLDL level and CA-IMT was modified by LDL-C levels (Table 4) and the combination of cITP fLDL and LDL-C levels is a better indicator for a high CA-IMT (Fig. 4). Therefore, increased cITP fLDL could be a potentially useful marker for a high CA-IMT when the LDL-C level is high. Although the result of this cross-sectional study cannot be used to determine whether or not cITP fLDL subfraction is a causal factor for a high CA-IMT, our finding suggests that mildly modified

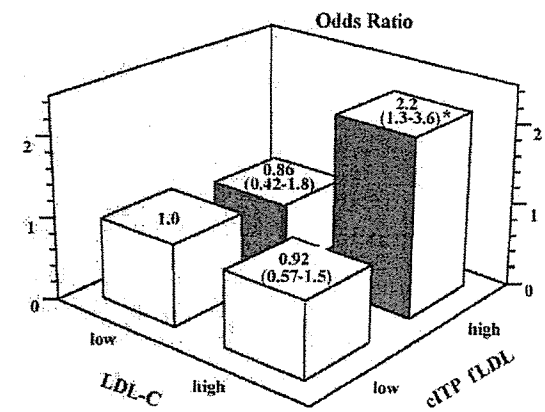


Fig. 4. Age-adjusted odds ratios [95% confidence interval (CI)] for a high carotid-artery intima-media thickness (CA-IMT) in each combination of LDL-C level and cITP fLDL level (low-LDL-C-low-fLDL, low-LDL-C - high fLDL, high-LDL-C-low-fLDL, and high-LDL-C-high-fLDL groups). Two levels of CA-IMT were produced using the median value (given a value of 0 if CA-IMT < 0.77 mm and 1 if CA-IMT ≥ 0.77 mm). The median value of LDL-C (118 mg/dl) and the 66.7th percentile value of cITP fLDL (1.37) were used to make dummy variables for each group. $^{*}p < 0.01$, as assessed by a multiple logistic regression analysis.

LDL in human blood could be important in the pathogenesis of atherosclerosis, especially under a high LDL-C level.

Our finding that cITP fLDL was associated with CA-IMT supports the notion that the electronegative subfraction of LDL is associated with risk factors of CAD, as reported by other authors [8–11], and the prevalence of angiographically documented CAD [12]. However, in the present study, the absolute levels of cITP fLDL were examined in its relation to CA-IMT, while other authors reported an association between the proportion of LDL(−) in total LDL and risk factors for CAD [8–11] or the prevalence of CAD [12]. The absolute plasma level of LDL(−) cannot be determined by anion-exchange chromatography because LDL has to be separated from plasma by ultracentrifugation or other technique before it is used for the separation of LDL(−). Therefore, the proportion of LDL(−) reported in previous studies is not equivalent to the level of cITP fLDL in the present study. However, the more negative-charged LDL subfractions separated by the two different techniques are closely related. We have previously shown that cITP fLDL represents an electronegative fraction of LDL because the cITP sLDL subfraction was converted to the fLDL subfraction when LDL was subjected to in vitro oxidation by $CuSO_4$ [17]. Bittolo-Bon et al., who separated plasma LDL into four subfractions using a different buffer system in capillary isotachophoresis, also reported that the ratio of fast-migrating (LDL1 and LDL2) and slow-migrating (LDL3 and LDL4) LDL subfractions determined by cITP was strongly and positively correlated with the proportion of LDL(−) determined by anion-exchange chromatography [30]. We observed no significant associations between the proportion of cITP fLDL in total cITP calculated from the absolute levels of cITP fLDL and sLDL and the ratio of cITP fLDL to sLDL and CA-IMT (data not shown). Therefore, our findings indicate that the absolute level of cITP fLDL but not the proportion of cITP fLDL in total LDL is important as a marker for a high CA-IMT.

In conclusion, fast LDL as characterized by analytical cITP was associated with carotid-artery intima-media thickness and could be a potentially useful marker for early atherosclerosis in combination with the LDL-C level. Further investigations are needed to clarify whether or not this conclusion can be applied to coronary atheosclerosis.

Acknowledgments

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