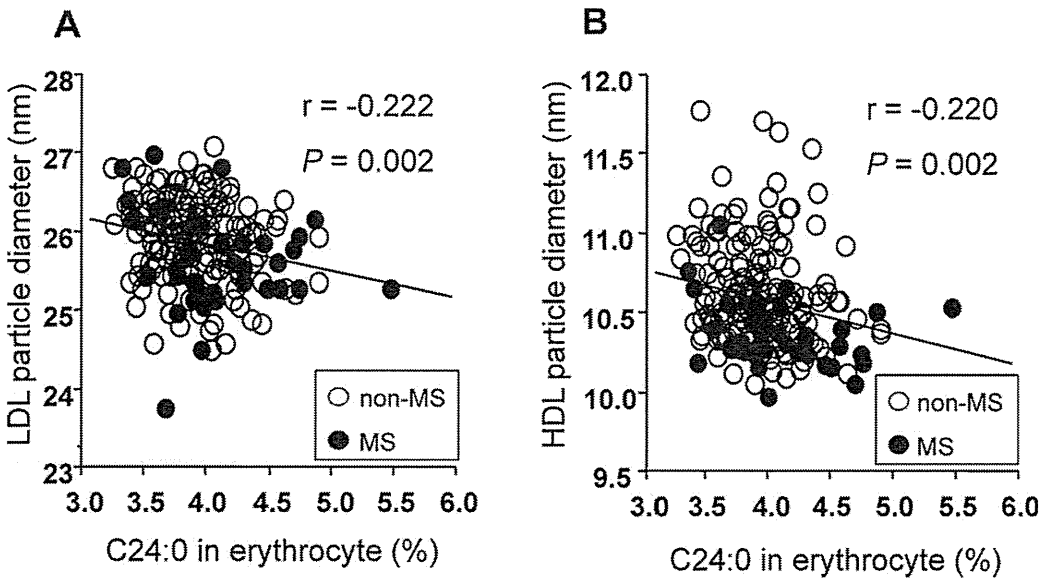

Fig. 1 – Correlation of the level of C24:0 in erythrocyte with low density lipoprotein (LDL) particle diameter (A) and high density lipoprotein diameter (B).

components and precise atherogenic lipoprotein profiles remains unclear.

Several studies have reported associations between dietary fatty acids and atherogenic lipoproteins. Consumption of dietary *trans* fatty acids is associated with an increase in small, dense LDL particles [29]. In animal experiments, HDL particle size was significantly smaller in male Hartley guinea pigs that were fed *trans* fatty acids compared with guinea fed other diets [30]. Dietary unsaturated fats similarly reduce LDL size relative to saturated fats, although the composition of dietary fat is not a major factor affecting LDL size [31]. However, in this study population, only a high level of saturated VLCFA C24:0, but not other fatty acids, in erythrocytes showed a significant correlation with both small LDL and HDL particle size. This indicates that the accumulation of VLCFAs may play a crucial role in the pathogenesis of atherosclerosis.

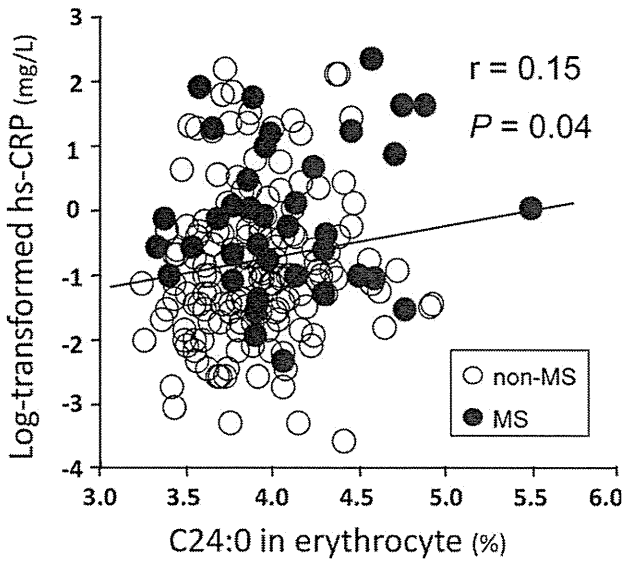

Fig. 2 – Correlation of the level of C24:0 in erythrocyte with log-transformed high sensitive C reactive protein (hs-CRP).

We also found a significant association between increased erythrocyte C24:0 level and high hs-CRP levels, indicating that the accumulation of C24:0 may interact with the inflammatory state in MS. Long chain saturated fatty acids (>C12:0) have relatively high melting points; therefore, increased levels of saturated fatty acids have the potential to reduce cell membrane fluidity. Reduced erythrocyte membrane fluidity may be associated with endothelial dysfunction and increased oxidative stress [32,33]. We recently reported that macrophages with accumulated saturated VLCFAs obtained from mice with peroxisome dysfunction produced several inflammatory cytokines and increased oxidative stress [34]. A recent report showed the alteration of long-chain fatty acid composition in plasma and erythrocytes due to higher levels of chronic oxidative stress are associated with the pathophysiology of depression [35]. These results suggest that the accumulation of saturated VLCFAs in various cells and organs may be involved in inflammation and oxidative stress during the pathogenesis of MS.

This study has several limitations. First, we have no data available on the dietary fatty acid intake of our subjects. The effects of fatty acid intake on the accumulation of saturated VLCFA in erythrocytes will require additional study. Second, we did not assess plasma parameters associated with peroxisomal beta-oxidation or the enzyme activity related to the elongation of fatty acids. Therefore, additional studies are needed to clarify the contribution of erythrocyte VLCFAs to MS.

5. Conclusion

We have confirmed the association between a saturated VLCFA (C24:0) and MS. In addition, we found that a high level

of C24:0, but not other fatty acids, in erythrocytes was significantly correlated with atherogenic lipoprotein profiles and an inflammation marker. In conclusion, measuring the level of C24:0 in erythrocytes may be a useful marker to evaluate MS atherogenicity.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

The authors would like to thank Dr. Hirotaka Takizawa and Dr. Kiyotaka Fujii at Nagasu Kashiwado clinic for their assistances to collect data of study subjects, and Enago (www.enago.jp) for the English language review.

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ORIGINAL ARTICLE

Mortality risk of triglyceride levels in patients with coronary artery disease

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ABSTRACT

Objective The association between triglyceride level and the risk of coronary artery disease (CAD) remains controversial. In particular, the prognostic significance of triglyceride levels in established CAD is unclear. We aimed to assess the relationship between triglyceride levels and long-term (>10 years) prognosis in a cohort of patients after complete coronary revascularisation.

Design Observational cohort study.

Setting Departments of cardiology and cardiovascular surgery in a university hospital.

Patients Consecutive patients who had undergone complete revascularisation between 1984 and 1992. All patients were categorised according to the quintiles of fasting triglyceride levels at baseline.

Main outcome measures The risk of fasting triglyceride levels for all-cause and cardiac mortality was assessed by multivariable Cox proportional hazards regression analyses.

Results Data from 1836 eligible patients were assessed. There were 412 (22.4%) all-cause deaths and 131 (7.2%) cardiac deaths during a median follow-up of 10.5 years. Multivariable analyses including total and high-density lipoprotein cholesterol and other covariates revealed no significant differences in linear trends for all-cause mortality according to the quintiles of triglyceride (p for trend=0.711). However, the HR increased with the triglyceride levels in a significant and dose-dependent manner for cardiac mortality (p for trend=0.031). Multivariable analysis therefore showed a significant relationship between triglyceride levels, when treated as a natural logarithm-transformed continuous variable, and increased cardiac mortality (HR 1.51, p=0.044).

Conclusions Elevated fasting triglyceride level is associated with increased risk of cardiac death after complete coronary revascularisation.

Several epidemiological studies have investigated the relationships between serum triglyceride levels and morbidity and mortality rates of coronary artery disease (CAD).[1–4] However, the evidence for elevated triglyceride levels as an independent risk factor for CAD remains controversial because there is no uniformity in data obtained in large epidemiological studies. There is a concern that adjustment for other abnormal lipid profiles, such as high-density lipoprotein (HDL) cholesterol levels, attenuates the relationship between triglycerides and CAD because there is an inverse correlation between triglyceride levels and HDL cholesterol levels. Nevertheless, meta-analyses have shown that serum triglyceride levels are an independent risk factor for morbidity and mortality rates of CAD in primary prevention.[5–8]

Unlike primary prevention, there are few data on the long-term prognostic significance of triglyceride levels in secondary prevention of CAD. The relationship between triglyceride levels and mortality risk after complete coronary revascularisation has not been established. We aimed to assess the relationship between triglyceride levels and long-term prognosis in a cohort of patients with CAD after complete coronary revascularisation.

METHODS

Subjects

We analysed data from consecutive patients who had undergone coronary revascularisation, including percutaneous coronary intervention (PCI) and coronary artery bypass grafting (CABG), at Juntendo University Hospital (Tokyo, Japan) between January 1984 and December 1992. We included patients who had achieved complete revascularisation—that is, patients in whom no unbypassed major vessels had a stenosis of ≥50%.[9 10] Patients with an untreated neoplasm at baseline and those with associated complex cardiac procedures such as valve replacement or aneurysm repair at the time of surgical revascularisation were excluded. The study was approved by the institute's internal review board and was performed according to the principles expressed in the Declaration of Helsinki and the ethics policy of the institute.

Data collection and definitions of variables

Demographic data including age, gender, body mass index (BMI), coronary risk factors, medication use, revascularisation procedure-related factors and comorbidities were prospectively collected. Blood samples were obtained in the early morning after an overnight fast. Hypertension was defined as systolic blood pressure ≥140 mm Hg and diastolic blood pressure ≥90 mm Hg or treatment with antihypertensive medications. Diabetes mellitus (DM) was defined as fasting plasma glucose level of ≥6.99 mmol/l or treatment with oral hypoglycaemic drugs or insulin injections. A current smoker was defined as a patient who smoked at the time of complete revascularisation or who had quit smoking within 1 year prior to the procedure. Patients with isolated PCI had achieved complete revascularisation by PCI without bypass grafting.

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Outcomes

The follow-up period ended on 30 September 2000. Survival data were collected by establishing serial contact with the patients or their families or from the medical records of deceased patients or those who continued to be followed up at our hospital. Information about the circumstances and date of death was obtained from the families of patients in cases where the patient died at home, and details of the events or the cause of death was supplied by other hospitals or clinics where the patients were admitted. Mortality data were categorised according to the causes of death (eg, all-cause or cardiac deaths) using the International Classification of Diseases, Ninth Revision codes 410–414, 785.51 and 798.

Statistical analysis

For the main analysis, all patients were categorised according to the quintiles of triglyceride levels. Continuous variables are expressed as mean±SD and categorical data are tabulated as frequencies and ratios. Differences between the baseline characteristics of patients within each triglyceride category were analysed by analysis of variance for continuous variables and the Cochrane–Armitage test for trend for proportions. To determine whether the results differed with the cut-off points, we performed secondary analyses in which triglyceride levels were treated as a natural logarithm-transformed continuous variable.

Cumulative mortalities were plotted using Kaplan–Meier curves and differences between quintiles of triglyceride levels were determined using log-rank tests. p Values for log-rank trend tests were also estimated. Cox proportional hazard models were used to compute HR and 95% CI for each quintile of triglyceride level using the lowest quintile as the reference group. Linear trend analyses were performed by using linear contrast coefficients (−2, −1, 0, 1, 2) in Cox proportional hazard models. The assumption of proportional hazards was assessed using a log-minus-log survival graph. Models were initially adjusted for age and gender (Model 1). To determine the role of triglycerides independent of other lipid markers, we adjusted the models for total and HDL cholesterol levels (Model 2). Furthermore, multivariable models were adjusted for non-HDL and HDL cholesterol levels (Model 3) and for BMI, presence of hypertension, presence of DM, current smoking, family history of CAD, prior myocardial infarction (MI), prior stroke, presence of atrial fibrillation, under dialysis, left ventricular ejection fraction, number of diseased vessels, presence of an arterial bypass to left anterior descending artery, presence of a left main trunk lesion, whether complete revascularisation was achieved by isolated PCI and use of drugs (aspirin, angiotensin-converting enzyme (ACE) inhibitors, β-blockers, statins, fibrates and niacin) in addition to the variables used in Model 2 (Model 4). To avoid overadjustment, the latter covariates were added only if they were significant predictors of death from all-cause or cardiac death at an α level of 0.1. Finally, multivariable models were further adjusted for non-HDL cholesterol levels plus the same variables as in Model 4 other than total cholesterol (Model 5).

To assess the potential heterogeneity of the effect of triglyceride levels on cardiac mortality we performed subgroup analyses. The subgroups included age groups (cut-off 60 years), gender, presence/absence of DM, total cholesterol (cut-off 5.69 mmol/l), HDL cholesterol (cut-off 1.29 mmol/l) and use of statins. The first-order interactions in multivariable Cox proportional hazards models were examined by entering interaction terms between triglyceride levels and the abovementioned subgroup variables. We also determined the effect of triglyceride levels on cardiac mortality in each subgroup.

A p value of <0.05 was considered statistically significant unless indicated otherwise. All data were analysed using SAS V.9.2 (SAS Institute, Cary, North Carolina, USA).

RESULTS

We assessed data from 1836 eligible patients who had undergone complete coronary revascularisation during the study period. Baseline and clinical event data were fully documented during a median follow-up period of 10.5 years. All patients underwent PCI with simple balloon angioplasty; no patient received stent implantation since stents were not available when complete revascularisation was achieved. All CABG procedures were performed using a conventional cardiopulmonary bypass; arterial grafts were used in 51.4% of cases. None of the patients had type 1 DM. During the follow-up period 412 patients (22.4%) died from any cause and 131 patients (7.2%) died from cardiac causes.

The baseline characteristics of patients by quintiles of triglyceride levels are shown in table 1. Patients with high triglyceride levels were likely to be young, male and current smokers with a high BMI and total cholesterol level, a low HDL cholesterol level and frequently had prior MI. Among patients with high triglyceride levels, a smaller percentage of patients underwent revascularisation by isolated PCI, a high percentage were taking aspirin and a low percentage were taking statins.

The cumulative survival curves of patients according to the quintiles of triglyceride levels are shown in figure 1. Patients with high triglyceride levels were more likely to have high cumulative cardiac mortality rates (figure 1B) but they did not show any trend to high cumulative all-cause mortality (figure 1A).

The results of Cox proportional hazard regression analyses for all-cause and cardiac mortality are summarised in figure 2. Linear trends for all-cause mortality according to the quintiles of triglyceride levels were not significant in any models except for Model 1. However, among each quintile of triglyceride level in all models, HR increased significantly with the triglyceride levels in a dose-dependent manner for cardiac mortality.

The results of Cox proportional hazard regression analyses, in which triglyceride levels were treated as natural logarithm-transformed continuous variables, are also shown in figure 2. For all-cause mortality, only Model 1 showed a significant association between logarithm-transformed triglyceride level and mortality. However, for cardiac mortality, all models showed significant associations between these two factors.

We also conducted a subgroup analysis separately from the age, gender, presence of DM, total and HDL cholesterol levels and the use of statins for all-cause and cardiac death. Although associations of triglyceride level with mortality were more prominent in men, patients with low HDL and patients not receiving statins, all p values for interaction were not significant (figure 3).

DISCUSSION

In this study we made several important findings that provide insights into the relationship between triglyceride levels and cardiovascular diseases. First, we found that patients in the highest triglyceride quintile had a significantly greater risk of cardiac mortality than those in the lowest triglyceride quintile. Further, HR increased with the triglyceride quintile in a significant and dose-dependent manner, and high logarithm-transformed triglyceride levels were associated with increased long-term cardiac

Table 1 Baseline characteristics

	Triglyceride quintile, mmol/l					
	Q1 (≤1.11) N=369	Q2 (1.12–1.46) N=359	Q3 (1.47–1.83) N=369	Q4 (1.84–2.45) N=370	Q5 (≥2.46) N=369	p Value*
Age, years	60±9	61±9	59±8	58±9	58±8	<0.001
Men, n (%)	297 (81)	296 (82)	309 (84)	324 (88)	336 (91)	<0.001
BMI, kg/m^2	23±3	23±3	24±3	24±3	24±2	<0.001
Diabetes mellitus, n (%)	149 (40)	136 (38)	132 (36)	137 (37)	149 (40)	0.913
Hypertension n (%)	243 (66)	238 (66)	245 (66)	257 (70)	255 (69)	0.211
Total cholesterol, mmol/l	5.12±1.22	5.53±1.11	5.82±1.19	5.92±1.34	6.23±1.22	<0.001
HDL cholesterol, mmol/l	1.24±0.36	1.16±0.34	1.11±0.31	1.03±0.28	0.98±0.28	<0.001
Non-HDL cholesterol, mmol/l	3.88±1.16	4.37±1.09	4.71±1.13	4.89±1.27	5.25±1.20	<0.001
Current smoker, n (%)	260 (71)	243 (68)	272 (74)	280 (76)	304 (82)	<0.001
Family history of CAD, n (%)	126 (34)	119 (33)	102 (28)	103 (28)	125 (34)	0.446
Prior MI, n (%)	147 (40)	175 (49)	170 (46)	185 (50)	204 (55)	<0.001
Prior stroke, n (%)	15 (4)	24 (7)	11 (3)	14 (4)	18 (5)	0.720
Atrial fibrillation, n (%)	49 (13)	47 (13)	43 (12)	38 (10)	54 (15)	0.985
On dialysis (%)	6 (1.6)	1 (0.2)	6 (1.6)	7 (1.9)	7 (1.9)	0.283
No of diseased vessels	2.17±0.84	2.27±0.83	2.24±0.85	2.32±0.78	2.28±0.82	0.128
LMT lesion, n (%)	34 (9)	21 (6)	41 (11)	29 (8)	24 (7)	0.437
Arterial bypass to LAD, n (%)	117 (32)	112 (31)	126 (34)	124 (34)	129 (35)	0.791
LVEF (%)	65.3±12.7	64.0±12.9	65.3±11.9	63.9±13.5	63.4±13.4	0.136
Revascularisation-isolated PCI, n (%)	139 (38)	117 (33)	96 (26)	98 (27)	85 (23)	<0.001
Medications, n (%)						
Aspirin	279 (76)	264 (74)	266 (72)	253 (68)	251 (68)	0.006
ACE inhibitors	20 (5)	16 (4)	16 (4)	24 (7)	14 (4)	0.729
β-blockers	88 (24)	84 (23)	113 (31)	123 (33)	111 (30)	0.003
Statins	84 (23)	60 (17)	60 (16)	67 (18)	57 (16)	0.035
Fibrates	3 (0.8)	8 (2.2)	10 (2.7)	5 (1.4)	7 (1.9)	0.551
Niacin	37 (10)	29 (8)	28 (8)	37 (10)	23 (6)	0.214

*p Value for trend for all comparisons across triglyceride quintiles.
BMI, body mass index; CAD, coronary artery disease; HDL, high-density lipoprotein; LAD, left anterior descending; LMT, left main trunk; LVEF, left ventricular ejection fraction; MI, myocardial infarction; PCI, percutaneous coronary intervention.

mortality even after adjustment for cholesterol levels and other covariates in secondary prevention of CAD. Second, the mortality risk of triglyceride was observed in patients with significant CAD who had achieved complete revascularisation. Finally, there were no interactions in each subgroup, although associations of fasting triglyceride level with cardiac mortality after complete revascularisation were obvious in men, patients with low HDL cholesterol levels and patients not receiving statins. Our findings therefore suggest that fasting triglyceride levels indicate mortality risk in the secondary prevention of CAD regardless of the presence or absence of other concomitant cardiovascular risks.

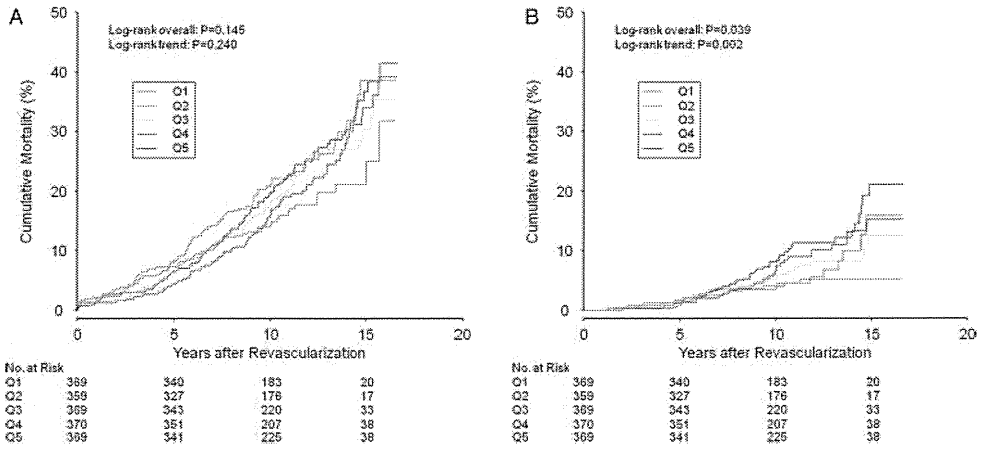

Figure 1 Cumulative mortality curves according to the quintiles of triglyceride levels for (A) all-cause deaths and (B) cardiac deaths. p Values for overall log-rank tests indicate whether there is a difference in the five different mortality curves (p=0.145 for all-cause death, p=0.039 for cardiac death). p Values for log-rank trend test indicate whether increased levels of triglycerides are associated with increased cumulative survival (p=0.240 for all-cause death, p=0.002 for cardiac death). This figure is only reproduced in colour in the online version.

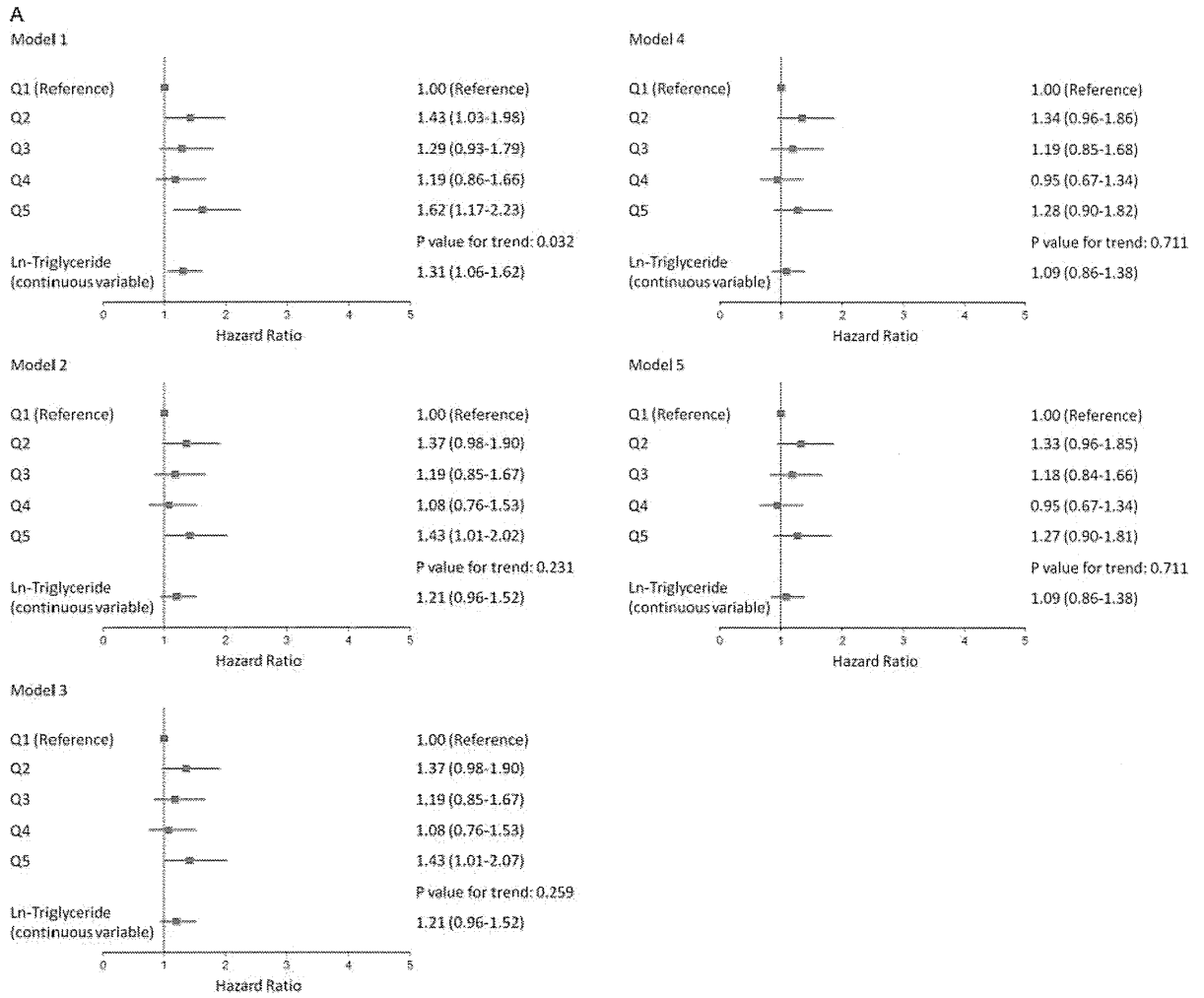

Figure 2 HR for mortality according to the quintiles of triglyceride levels: (A) all-cause deaths; (B) cardiac deaths. Model 1 adjusted for age and gender; Model 2 adjusted for age, gender, total and HDL cholesterols; Model 3 adjusted for age, gender, non-high-density lipoprotein (HDL) and HDL cholesterol; Model 4, adjusted for variables in model 2 plus hypertension, diabetes mellitus, prior myocardial infarction, prior stroke, atrial fibrillation, dialysis, left ventricular ejection fraction, number of diseased vessel, left main trunk lesion, isolated percutaneous coronary intervention, use of aspirin, use of angiotensin-converting enzyme (ACE) inhibitors, use of statins and use of niacin for all-cause death and the same variables other than hypertension, use of aspirin and use of ACE inhibitors for cardiac death; Model 5, adjusted for non-HDL cholesterol plus same variables in model 4 other than total cholesterol. Ln, natural logarithm-transformed. This figure is only reproduced in colour in the online version.

In the primary prevention of CAD the independent association of triglyceride levels with the morbidity and mortality rates of CAD has long been a controversial issue.[11–13] In previous case-control studies, triglyceride levels have been identified as one of the risk factors for CAD even after adjustment for total and HDL cholesterol levels.[14–17] Although most population-based cohort studies have shown a univariable association between triglyceride levels and CAD, the relationship becomes non-significant or weak after adjustment for total and/or HDL cholesterol levels.[13] There are at least four meta-analyses of population-based prospective studies regarding the relationships between triglyceride levels and morbidity and mortality rates of cardiovascular disease.[5–8] Of these, three have similar conclusions. Hokanson and colleagues reported the results of a meta-analysis of 46 413 men and 10 864 women from the USA and European countries.[5] In the univariable analysis the relative risk of triglyceride (per 1 mmol/l) for the composite of fatal and non-fatal cardiovascular disease was 1.32 in men and 1.76 in women. After adjustment for HDL cholesterol, these relative risks were attenuated to the modest levels of 1.14 in men and 1.37 in women. A recent updated meta-analysis that examined 262 525 subjects from the USA and European countries revealed a 1.7 times higher risk for the composite of fatal and non-fatal CAD at the upper triglyceride tertile compared with the lower triglyceride tertile in the adjusted analysis.[7] Another meta-analysis that examined 96 224 men and women from the Asian and Pacific populations showed that the risk for the composite of fatal and non-fatal CAD in individuals in the top triglyceride quintile was 1.8 times greater than those in the bottom triglyceride quintile after adjustment for several established risk factors.[6] In the most recent and robust evidence from the Emerging Risk Factors Collaboration, 302 430 people were examined in 68 prospective studies.[8] With adjustment for age and sex, triglycerides showed a strong stepwise association with fatal and non-fatal CAD. However, after adjustment for standard risk factors and other lipid measures such as non-HDL and HDL cholesterol levels, the association between triglycerides and CAD was no longer significant.[8] The American Heart Association has recently suggested that the independence of the triglyceride level as a causal factor in developing CAD remains debatable, but triglyceride levels appear to provide unique information and can be used as a biomarker of risk.[18]

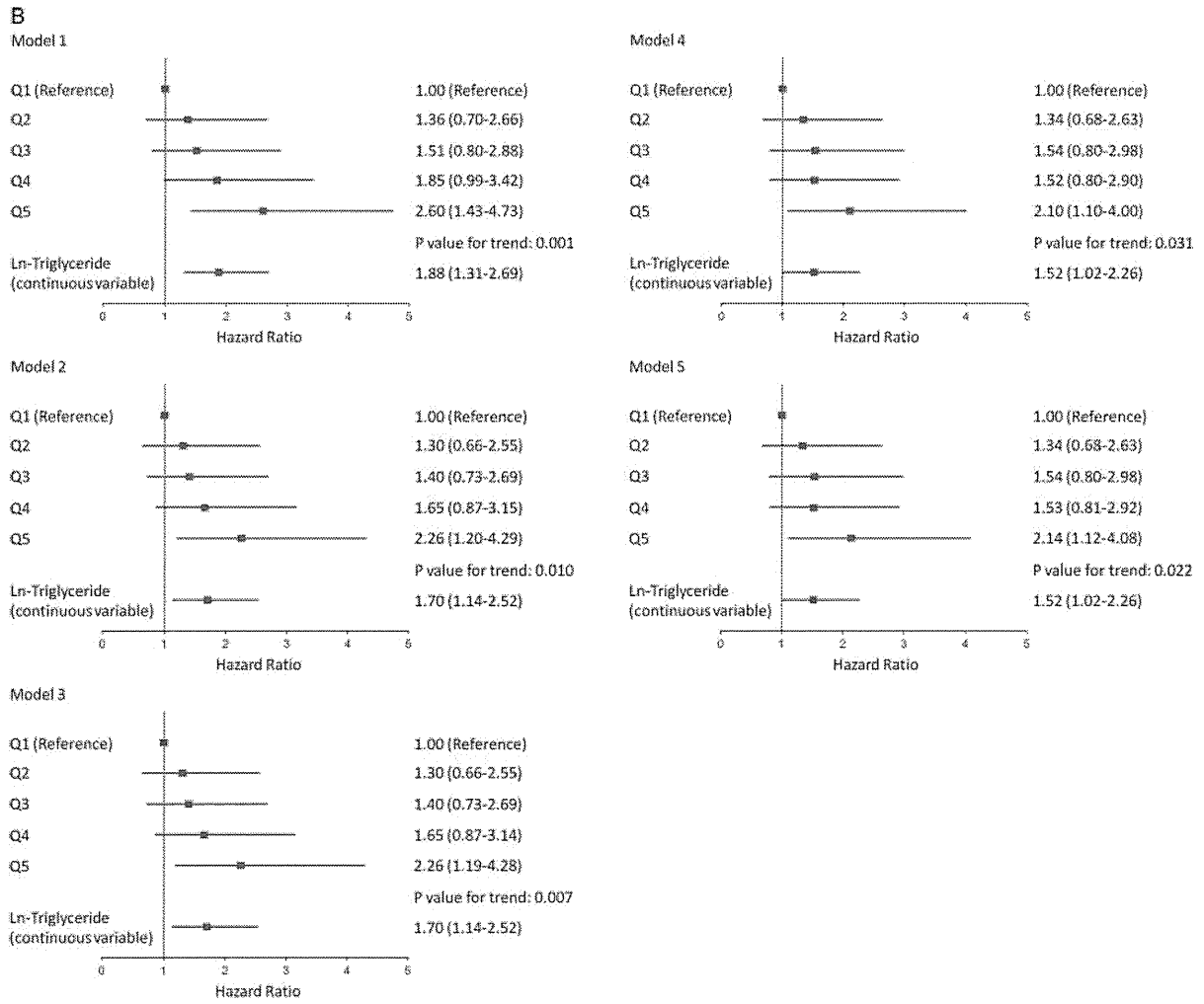

Figure 2 Continued.

Although individuals enrolled in some of the population-based prospective studies included in the abovementioned meta-analysis had a history of CAD, the percentage of such individuals was very low (<15%). Furthermore, there are no subdivided analyses regarding individuals with CAD. In the case of secondary prevention of CAD, only one report suggesting a significant relationship between triglyceride levels and long-term prognosis is available. von Eynatten and colleagues reported that, in patients with CAD, fasting triglyceride levels were associated with a high incidence of secondary cardiovascular events (ie, composite of cardiovascular death, non-fatal MI and stroke) during a median follow-up term of 57 months, even after adjustment for other lipid and adiponectin levels, with an HR of 1.5 which is identical to the results of our study (as a continuous variable).[19] However, the main purpose of their study was not to assess the relationship between triglyceride levels and prognosis but to investigate whether adiponectin is a useful prognostic predictor in patients with CAD and to compare the values of adiponectin for secondary risk stratification with the prognostic role of markers of dyslipidaemia (ie, triglyceride, low-density lipoprotein (LDL) and HDL cholesterol). Their patients were a mixture of those who had undergone non-invasive or invasive (PCI and CABG) treatment. Except for the severity of CAD, the details related to CAD and type of treatment were not specifically described and were not adjusted for in the multivariable analysis (eg, whether PCI was successful or whether complete revascularisation was performed were not mentioned and no adjustment was made for them). Our study shows that, in patients with complete revascularisation, fasting triglyceride levels were associated with increased cardiac mortality for a long-term follow-up period (>10 years). It was important to assess data only from patients who had achieved complete revascularisation because initial CAD events may be prevented or delayed by complete coronary revascularisation, even in patients with severe coronary atherosclerosis. This selection minimises the bias of treatment procedures for initial CAD events. Therefore, we specifically assessed the effect of fasting triglyceride levels on long-term mortality among the secondary prevention cohort of patients with CAD in this study.

We also assessed the possible interactions of triglyceride levels and cardiac mortality with age, gender, presence or absence of DM, total and HDL cholesterol and use of statins. There were no statistically significant interactions between the subgroups, which indicated that the relationship between triglyceride levels and high cardiac mortality was not affected by these factors. A strong relationship was found between triglyceride levels and cardiac mortality in men, patients with low HDL and patients not receiving statins. Although the Framingham Heart Study suggested that a high triglyceride level was a predictor of the incidence of cardiovascular disease in women,[20] the triglyceride level in our study did not show a significant relationship with increased cardiac mortality in women with CAD. In general,

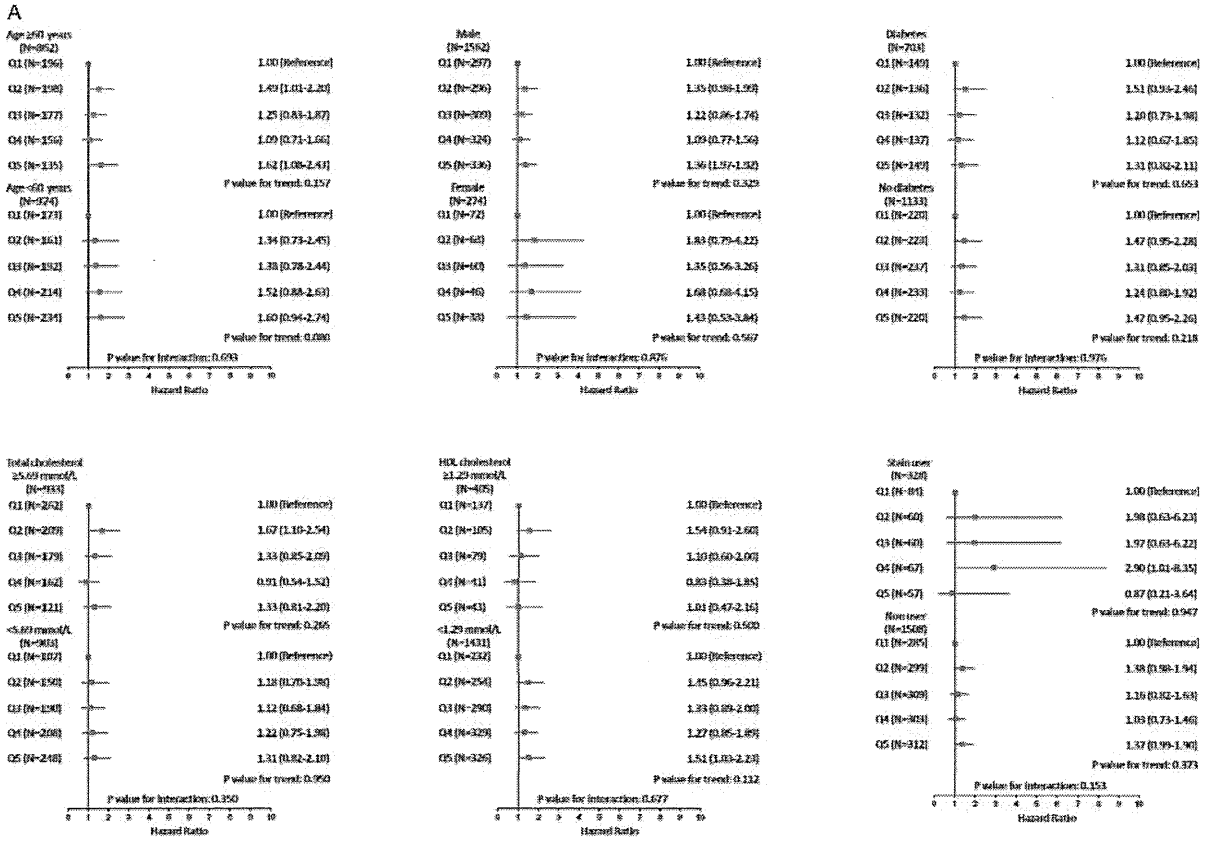

Figure 3 Subgroup analyses of (A) all-cause deaths and (B) cardiac deaths. HDL, high-density lipoprotein. This figure is only reproduced in colour in the online version.

women who receive coronary revascularisation are older and their baseline risk profiles are worse than men.[21 22] These factors might attenuate the significance of the association of triglyceride levels and cardiac mortality in women in our study population. Nevertheless, considering that no significant association was observed between triglyceride levels and cardiac mortality in all subgroups which included small numbers of patients (ie, women, patients with high HDL cholesterol levels and patients receiving statins), the results of analysis within individual subgroups should be interpreted with caution.

On the other hand, the relationship between triglyceride levels and mortality risks among patients with CAD receiving statin treatment has been investigated in several studies. For instance, Wolfram *et al*[23] reported that, in patients with acute coronary syndrome of whom 98% were on statin treatment, triglyceride levels were not associated with 1-year clinical outcomes. In addition, there was no significant relationship between triglyceride levels and short-term outcome in the Myocardial Ischaemia Reduction with Aggressive Cholesterol Lowering trial.[24] Analyses from the Incremental Decrease in End Point Through Aggressive Lipid Lowering trial and the Treating to New Targets trial showed that triglyceride levels are associated with a risk of cardiovascular events even after adjustment of other lipid parameters, but this relationship was no longer significant when other risk factors were included in further multivariable analysis.[25] In contrast, the Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis In Myocardial Infarction (PROVE IT-TIMI) 22 trial showed an independent relationship between triglyceride levels and cardiovascular events outcome, which is consistent with the results of the present study.[26] These conflicting results could be explained by the differences in baseline triglyceride levels across these studies, including ours. In studies which failed to show a significant relationship between triglyceride levels and outcomes,[23–25] patients had relatively low triglyceride levels compared with the PROVE IT-TIMI 22 trial[26] and the present study, suggesting that the degree of hypertriglyceridaemia may affect the effect of adjustment for covariates including other lipid parameters. Nevertheless, these results also indicate that elevated triglyceride levels can be a predictor of worse outcomes even in patients on statin treatment, and further adjunctive intervention for elevated triglyceride levels should be considered.[27]

It remains controversial whether there is a causal relationship between triglyceride levels and CAD morbidity and mortality. The triglyceride level is rather regarded as an important biomarker of cardiovascular disease because of its association with atherogenic remnant particles and apo CIII.[18] Randomised controlled studies on treatment for lowering triglyceride levels could provide a solution to this controversy. However, all available interventions for lowering triglyceride levels such as drugs (eg, fibrates, niacin and statins) and lifestyle modifications also affect the confounding parameters, including LDL cholesterol, HDL cholesterol and insulin resistance,[28–31] so we could not determine the causality in such studies. However, as the condition is characterised by an increased circulating triglyceride level, the triglyceride level can be considered as an interventional target. This hypothesis is supported by a recent report by Tirosh and colleagues[32] who followed 13 953 young men aged 26–45 years for 5.5 years and performed two measurements of fasting triglycerides 5 years apart. There were significant correlations between a good lifestyle and the reduction in triglyceride levels between the two measurements. Evaluation of the change

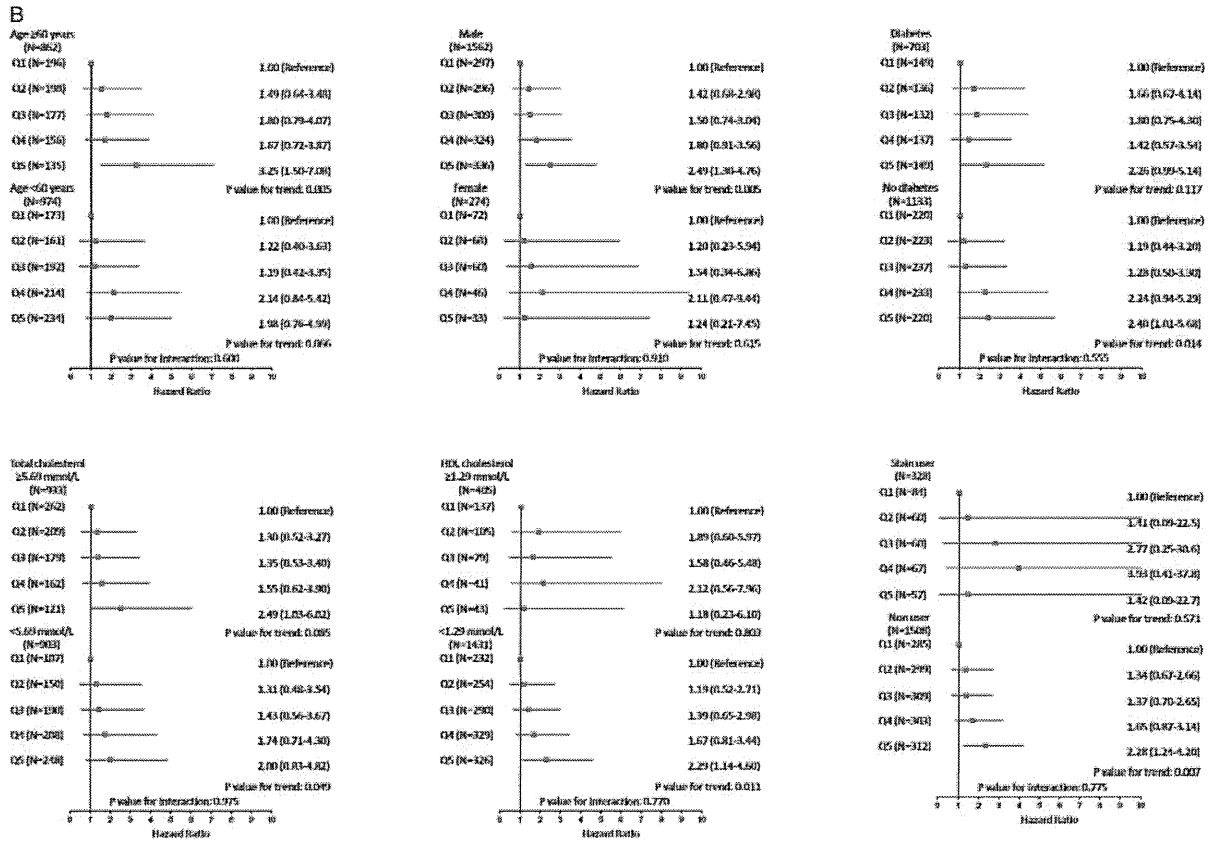

Figure 3 Continued.

in triglyceride levels over the first 5 years and incident CAD in the next 5.5 years showed a direct correlation between increases in triglyceride levels and the risk of CAD. These findings suggest a causal relationship between increased triglyceride level and CAD morbidity and mortality.

The present study has several limitations. First, balloon angioplasty was the only PCI used in all patients and 51.1% of the CABG procedures involved an arterial graft. It is difficult to determine whether the use of stents and arterial grafts could have improved the results in the recent era of revascularisation and to evaluate the relative importance of improvements in both operator skills and adjunctive drug therapy. Further investigation is needed to clarify whether triglyceride levels will affect the long-term mortality in the stent and arterial bypass era. Second, assessment of data only of patients who achieved complete revascularisation also introduced potential selection bias in terms of the overall mortality rate which should be taken into account. Third, several groups recently reported that the non-fasting triglyceride level is a superior predictor of cardiovascular risk than fasting levels.[33 34] If we can use non-fasting triglyceride levels as a predictor of cardiovascular morbidity and mortality in patients with CAD, it would have greater clinical applications as it is easier to obtain non-fasting than fasting triglyceride levels. Further studies and discussions regarding the importance of non-fasting triglyceride levels in the secondary prevention of CAD are therefore needed.

Conclusions

Fasting triglyceride levels were associated with an increase in cardiac mortality over a 10-year period after complete coronary revascularisation. This association was observed even after adjustment for the total and HDL cholesterol levels together with other covariates.

Contributors HD had full access to all data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Conception and design: HD, TK, KM. Acquisition of data: KK, NK, MO, ST. Analysis and interpretation of data: HD, TK, NY. Drafting of the manuscript: TK, NY. Critical revision of the manuscript for important intellectual content: HD, KM, AA. Final approval of the version to be published: HD, KM, KK, NK, MO, ST, AA.

Competing interests None.

Ethics approval Ethics approval was obtained from Juntendo University ethics committee.

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Heart

Mortality risk of triglyceride levels in patients with coronary artery disease

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Serum apolipoprotein B-48 levels are correlated with carotid intima-media thickness in subjects with normal serum triglyceride levels

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ABSTRACT

Background: Postprandial hyperlipidemia (PPHL) is an independent risk factor for coronary heart disease (CHD) which is based on the accumulation of chylomicrons (CM) and CM remnants containing apolipoprotein B-48 (apoB-48). Since atherosclerotic cardiovascular diseases are frequently observed even in subjects with normal serum triglyceride (TG) level, the correlation between fasting apoB-48 containing lipoproteins and carotid intima-media thickness (IMT) was analyzed in subjects with normal TG levels.
Methods: From subjects who took their annual health check at the Osaka Police Hospital ($n = 245$, male), one-hundred and sixty-four male subjects were selected to take part in this study; the excluding factors were: systolic blood pressure ≥140 mmHg, intake of antihypertensive or antihyperlipidemic drugs, or age >65 years. The association between biochemical markers and IMT was analyzed and independent predictors of max-IMT were determined by multiple regression analysis in all subjects and in groups N-1 (TG < 100 mg/dl, $n = 58$), N-2 (100 ≤ TG < 150 mg/dl, $n = 53$) and H (150 ≤ TG mg/dl, $n = 53$), respectively.
Results: Fasting total cholesterol, LDL-cholesterol, HDL-cholesterol, apoB-100 and ln RemL-C (remnant lipoprotein-cholesterol) levels were not correlated with max-IMT, but ln TG and ln apoB-48 were significantly correlated with max-IMT in all subjects. Ln apoB-48 and apoB-48/TG ratio were significantly correlated with max-IMT in group N-2. By multiple regression analysis, age and ln apoB-48 were independent variables associated with max-IMT in group N-2.
Conclusion: Serum apoB-48 level might be a good marker for the detection of early atherosclerosis in middle-aged subjects with normal-range levels of blood pressure and TG.

1. Introduction

Hypercholesterolemia, including high serum LDL-cholesterol (LDL-C) level, is strongly correlated to the development of atherosclerotic cardiovascular diseases [1]. Statins significantly decrease LDL-C levels and the morbidity of atherosclerotic cardiovascular diseases; however, they cannot completely prevent the occurrence of these diseases yet [2]. Epidemiologic studies have revealed that fasting hypertriglyceridemia is also associated with atherosclerosis, independent of other coronary risk factors such as high LDL-C level [3,4]. A case-control study showed that fasting and non-fasting TG levels were also superior among patients with coronary heart disease (CHD) as compared with control subjects [5]. A Japanese prospective study demonstrated that not only fast-

Abbreviations: BMI, body mass index; apoB-48, apolipoprotein B-48; PPHL, postprandial hyperlipidemia; CM, chylomicrons; CMR, chylomicron remnants; RemL-C, remnant lipoprotein-cholesterol; TG, triglycerides; LDL, low-density lipoprotein; HDL, high-density lipoprotein; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; HOMA-IR, homeostasis model assessment as an index of insulin resistance; IRI, immuno-reactive insulin; IMT, intima-media thickness.

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ing but also non-fasting TG levels were significantly correlated with CHD morbidity [6]. In this study, the authors also showed that an increase in TG levels was significantly correlated with an increase in CHD morbidity even though TG levels remained below 150 mg/dl, a level which has been recognized as borderline of high risk status for atherosclerotic cardiovascular diseases on the basis of Framingham Study [7]. Therefore, we need to evaluate the emerging risk of atherosclerotic cardiovascular diseases even in subjects with normotriglyceridemia (TG < 150 mg/dl).

Postprandial hyperlipidemia (PPHL) is caused by the impaired metabolism of lipoproteins, which is mainly characterized by a postprandial accumulation of intestine-derived lipoproteins, chylomicrons (CM) and their hydrolyzed lipoproteins, chylomicron remnants (CM-R). In subjects with normal lipoprotein metabolism, CM and CM-R are promptly hydrolyzed, diminished in size and cleared from the circulation by the liver within a few hours after a meal. PPHL does not indicate the postprandial increase of lipids and lipoproteins which are promptly cleared from the circulation in subjects with normal lipoprotein metabolism. However, in patients with PPHL, CM-R continue to accumulate for over 6–8 h after a meal, penetrating into the vessels to form foam cells. Many recent studies have proved that PPHL is an independent risk factor for the development of CHD and atherosclerosis of carotid arteries [8–10]. Many basic studies have suggested that accumulated CM-R particles may promote atherogenicity in the arterial wall [11]. An oral fat loading (OFL) test is sometimes used to assess PPHL levels; however, this it is not a suitable testing option for routine clinical use because it requires a lot of time (6–8 h). Further, consensus has not yet been reached regarding the indication and the interpretation of data from this test. We developed a novel enzyme-linked immuno-sorbent assay (ELISA) to measure serum levels of apolipoprotein B-48 (apoB-48) [12]. Since one apoB-48 molecule is included in one CM and CM-R particle up to the clearance by the liver, serum apoB-48 level represents the number of both CM and CM-R particles and is suitable for the quantitative evaluation of postprandial changes. In patients with suspected accumulation of CM and CM-R, serum apoB-48 levels are significantly higher at the fasting state and increased after OFL in normolipidemic subjects [12]. High levels of fasting serum apoB-48 suggest the existence of PPHL, without performing an OFL test [13], and are reportedly related to the development of atherosclerotic cardiovascular diseases [14–17]. These results suggest that fasting apoB-48 level is a good marker for the evaluation of atherogenic risk in patients with hypertriglyceridemia. However, very few studies have so far investigated the correlation between fasting serum apoB-48 levels and the development of atherosclerosis among subjects with normal fasting TG levels.

In the current study, we have investigated the correlations between profiles of apoB-48-containing lipoproteins and the progression of atherosclerosis in subjects with normal TG levels. For the evaluation of atherosclerosis progression, intima-media thickness (IMT) of carotid arteries was measured using a diagnostic ultrasound, which was shown to be significantly correlated with the development and prognosis of CHD and cerebrovascular diseases [18,19].

2. Subjects and methods

2.1. Subjects

A consecutive series of subjects ($n = 245$, male) who came to Osaka Police Hospital for the annual health checkup were picked up serially. One-hundred and sixty-four male subjects were finally enrolled by the following exclusion criteria: systolic blood pressure $\geq$140 mmHg, age over 65 years and intake of any drugs affecting lipid metabolism and blood pressure. This study was approved by the Ethical Committee of Osaka Police Hospital, and all participants gave their written informed consent.

2.2. Biochemical analyses

Height, weight, and waist circumference were measured in the standing position. Systolic and diastolic blood pressures were measured at rest in the sitting position. Blood samples were collected after an overnight fast, followed by an immediate separation of serum and plasma. Total cholesterol (TC), triglycerides (TG), HDL–C, fasting plasma glucose (FPG) and uric acid (UA) levels were measured by enzymatic methods, LDL–C levels by direct method, and serum apoB levels by immunoturbidity method, respectively (Sekisui Medical Co., Ltd., Tokyo, Japan). Hemoglobin A1c (HbA1c) levels were determined by high performance liquid chromatography (HPLC) method and immunoreactive insulin (IRI) levels by the immunoturbidity method (SRL Inc., Tokyo, Japan). Serum apoB-48 levels were measured by the chemiluminescent enzyme immunoassay (CLEIA) using anti-human apoB-48 monoclonal antibodies, which we developed previously with minor modification (Fujirebio Inc., Tokyo, Japan). Remnant lipoprotein–cholesterol (RemL–C) levels were measured by the homogenous assay (Kyowa Medex, Tokyo, Japan) [12]. ApoB-100 levels were calculated by subtracting the value of apoB-48 from the value of serum apoB. Plasma adiponectin levels were determined by the human adiponectin ELISA kit (Otsuka Pharmaceuticals, Tokyo, Japan). Subjects were divided into 3 groups by serum TG level: group N-1 ($n = 58$), TG < 100 mg/dl; Group N-2 ($n = 53$), 100 $\leq$ TG < 150 mg/dl and Group H ($n = 53$), 150 $\leq$ TG mg/dl.

2.3. Ultrasound measurements

The IMT of carotid arteries was determined using ultrasonography in the supine position. High-resolution B-mode ultrasound images were obtained (Toshiba Nemio, Toshiba Corp., Tokyo, Japan) with a 12 MHz linear array transducer. Three arterial wall segments in each carotid artery were imaged from a fixed lateral transducer angle at the far wall. All segments, including both sides of common carotid artery, the carotid bifurcation, and the internal carotid artery, were scanned. The thickest part of the IMT was recorded as max-IMT, and the IMT of the far wall was measured at 3 continuous sites at a 1.0-cm interval proximal to the thickest part of IMT in each side and then averaged to obtain mean-IMT. The mean-IMT value and greater max-IMT value obtained from scans of the right and left carotid arteries in each subject were used for statistical analyses.

2.4. Statistical analysis

Values were expressed as mean $\pm$ SD. ApoB-48 levels were normalized by logarithmic transformation. Between-group comparisons of the means and median were performed by Tukey's HSD test among group N-1, group N-2 and group H. The correlations between metabolic parameters and mean-/max-IMT were calculated by Pearson's correlation coefficients. Stepwise multiple regression analysis was used to determine independent predictors of max-IMT measurement with P value-to-enter set at 0.20. Age, sBP, dBP, total cholesterol, ln TG, LDL–C, HDL–C, apoB-48, apoB-100, ln RemL–C, FPG, HbA1c, ln HOMA-IR, and IRI were included as explanatory variables in the method. Data were analyzed with JMP8 software (SAS Institute, Cary, NC). All statistical significance was accepted at $P < 0.05$.

Table 1
Clinical profiles of subjects investigated.

	Total $n = 164$	Group N-1 TG < 100 $n = 58$	Group N-2 100 ≤ TG < 150 $n = 53$	Group H 150 ≤ TG $n = 53$
Age (year)	52 ± 6	53 ± 6	52 ± 6	52 ± 7
BMI (kg/m2)	24.7 ± 3.0	23.4 ± 2.3	24.6 ± 2.5	26.1 ± 3.4$^{\#}$
Waist circ. (cm)	87 ± 8	83 ± 6	88 ± 7**	91 ± 8$^{\#}$
sBP (mmHg)	120 ± 12	117 ± 12	120 ± 11	123 ± 12
dBP (mmHg)	82 ± 9	79 ± 9	82 ± 9	84 ± 9
TC (mg/dl)	208 ± 30	201 ± 27	211 ± 30	213 ± 32
HDL-C (mg/dl)	54 ± 13	60 ± 14	56 ± 11	47 ± 8$^{\#\#}$
LDL-C (mg/dl)	124 ± 28	123 ± 24	129 ± 26	119 ± 33
TG (mg/dl)	152 ± 120	77 ± 15	122 ± 15*	264 ± 156$^{\#\#}$
apoB-48 (mg/dl)	0.57 ± 0.55	0.28 ± 0.14	0.42 ± 0.19	1.03 ± 0.74$^{\#\#}$
apoB-100 (mg/dl)	97.8 ± 17.3	89.6 ± 15.1	100.8 ± 14.6*	103.7 ± 18.8
RemL-C (mg/dl)	12.2 ± 8.3	7.0 ± 5.0	9.5 ± 2.1	20.4 ± 8.9$^{\#\#}$
FPG (mg/dl)	96 ± 14	96 ± 13	98 ± 15	95 ± 15
HbA1c (%)	5.1 ± 0.5	5.1 ± 0.5	5.2 ± 0.4	5.1 ± 0.6
HOMA-IR	1.3 ± 0.9	1.0 ± 0.5	1.3 ± 0.8	1.6 ± 1.1
IRI (μU/ml)	5.2 ± 2.9	4.0 ± 1.9	5.1 ± 3.0	6.5 ± 3.4$^{\#}$
Adiponectin (μg/ml)	5.4 ± 3.1	6.4 ± 3.9	5.2 ± 2.5	4.5 ± 2.3

From male subjects who took their annual health checkup at Osaka Police Hospital, one-hundred and sixty-four male subjects (aged 52 ± 6 years) were divided into 3 groups by serum TG level; group N-1 ($n = 58$), TG < 100 mg/dl; group N-2 ($n = 53$), 100 ≤ TG < 150 mg/dl; group H ($n = 53$), 150 ≤ TG mg/dl, respectively. Values are the mean ± SD; between-group comparisons of the means and median were performed by Tukey's HSD test among group N-1, group N-2 and group H.

* $P < 0.05$ (group N-2 compared with group N-1).
** $P < 0.005$ (group N-2 compared with group N-1).
$^{\#}$ $P < 0.05$ (group H compared with group N-2).
$^{\#\#}$ $P < 0.005$ (group H compared with group N-2).

3. Results

3.1. Clinical profiles

Table 1 shows the clinical profiles of all patients ($n = 164$), group N-1 ($n = 58$, TG < 100 mg/dl), group N-2 ($n = 53$, 100 ≤ TG < 150 mg/dl) and group H ($n = 53$, 150 ≤ TG mg/dl). The subjects were 52 ± 6 years-old (mean ± SD), and apoB-48 level was 0.57 ± 0.55 mg/dl. Waist circumference, TG and apoB-100 levels in group N-2 were significantly higher than those of group N-1. BMI, waist circumference, TG, apoB-48, apoB-100 and RemL-C levels in group H were significantly higher, and HDL-C levels were significantly lower in group H than in group N-2. Mean- and max-IMT were measured in all subjects, and between-group comparisons of the means and median were performed by Tukey's HSD test among total subjects, group N-1, group N-2 and group H. There was no significant difference in mean-IMT (total subjects, 0.80 ± 0.18 mm; group N-1, 0.75 ± 0.13 mm; group N-2, 0.79 ± 0.17 mm; and group H, 0.84 ± 0.23 mm, respectively) and in max-IMT (total subjects, 0.87 ± 0.23 mm; group N-1, 0.81 ± 0.16 mm; group N-2, 0.86 ± 0.22 mm; and group H, 0.93 ± 0.29 mm, respectively).

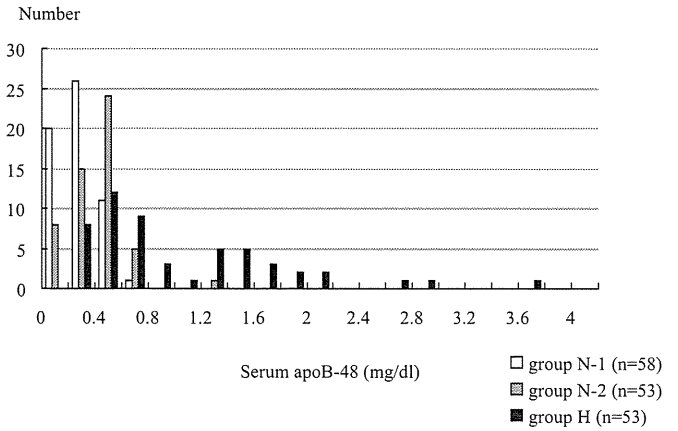

Fig. 1. Distribution of fasting serum apoB-48 levels. Geometric means were 0.24 mg/dl in group N-1, 0.41 mg/dl in group N-2 and 0.69 mg/dl in group H. The distribution of apoB-48 levels was significantly shifted to higher values; the data was normalized by logarithmic transformation for further statistical analysis.

3.2. Distribution of apoB-48 in each TG group

For the analysis of the correlation between apoB-48 levels and IMT, the distribution of apoB-48 levels was compared among groups N-1, N-2 and H (Fig. 1). The distribution of apoB-48 levels in group H was significantly shifted to higher values as compared with group N-1 and group N-2. ApoB-48 levels in group N-2 were also shifted to higher values compared with group N-1. In order to compare the apoB-48 levels in these TG groups, we normalized the apoB-48 levels by logarithmic transformation for further statistical analysis.

3.3. Correlation analysis in all subjects with max-IMT

Coronary risk factors such as TC, LDL-C, HDL-C, apoB-100 and ln RemL-C levels showed no significant correlations with mean- and max-IMT as assessed by Pearson's correlation coefficients in total subjects. To the contrary, ln TG and ln apoB-48 levels were significantly correlated with max-IMT (Fig. 2), and not significantly correlated with mean-IMT levels.

3.4. Correlation analysis in each TG group with max-IMT

The correlation of fasting apoB-100 levels and ln apoB-48 levels was analyzed with max-IMT in groups N-1, N-2 and H, respectively (Fig. 3). ApoB-100 levels were not significantly correlated with max-IMT in each TG group. Ln apoB-48 levels were significantly correlated with max-IMT only in group N-2.

3.5. Correlation analysis of apoB-48/TG ratio in each TG group with max-IMT

The significant correlation between ln apoB-48 levels and max-IMT means that the increase in apoB-48-containing lipoproteins might promote atherosclerosis in the carotid artery. The correla-

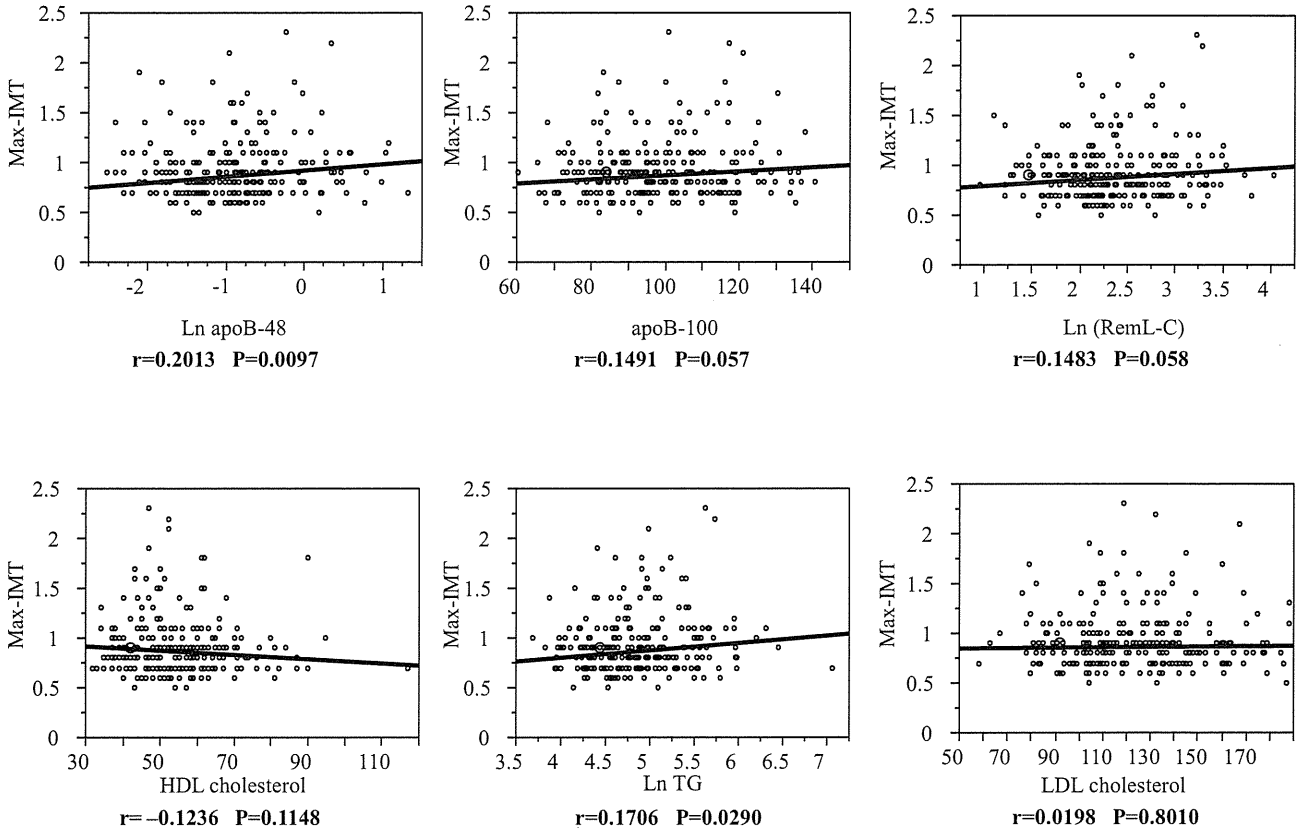

Fig. 2. Correlations between max-IMT and fasting lipid profiles. Because the distribution of apoB-48, TG and RemL–C was skewed to the left, the data were normalized by logarithmic transformation for statistical analysis. The fasting serum concentrations of TC, LDL–C, HDL–C, apoB-100 and ln RemL–C were not significantly correlated with max-IMT, but ln TG and ln apoB-48 were significantly correlated with max-IMT. The correlations were calculated by Pearson's correlation coefficients, and statistical significance was accepted at $P < 0.05$.

tions with max-IMT of fasting apoB-48/TG ratio, which refers to the number of CM-R lipoprotein particles, were evaluated and shown to be significant in group N-2, but not in N-1 and H (Fig. 4).

3.6. Stepwise multiple regression analysis between max-IMT and biochemical parameters

By multiple regression analysis, the correlations between max-IMT and age, blood pressure, lipid profiles and glucose-related parameters were assessed. Age, systolic blood pressure (sBP), diastolic blood pressure (dBP), TC, ln TG, LDL–C, HDL–C, apoB-48, apoB-100, ln RemL–C, FPG, HbA1c, ln HOMA-IR, and IRI were independent variables. Among these parameters, age, sBP and ln apoB-48 were independent variables associated with max-IMT level in all subjects (Table 2). In group N-2, age and ln apoB-48 were independent variables associated with max-IMT, but sBP was not. HbA1c was an independent variable associated with max-IMT in group N-1.

4. Discussion

A positive correlation between fasting serum apoB-48 levels and IMT was observed in patients with hypertriglyceridemia or diabetes mellitus [14,17]. Significantly high TG level (TG >150 mg/dl) is correlated to an impaired metabolism of TG-rich lipoproteins in endogenous (VLDL and LDL) and exogenous (CM and CM-R) lipoprotein pathways, which are strongly related to the development of atherosclerosis and the morbidity of cardiovascular diseases. In the current study, our results showed that fasting serum apoB-48 levels are correlated with max-IMT in subjects with relatively high, but normal TG level (from 100 to 150 mg/dl).

4.1. Contribution of increased CM-R to atherosclerosis

A postprandial increase in remnants has been considered as atherogenic since Zilversmit proposed his postprandial hyperlipidemia concept over 30 years ago [20]. Several studies indicate that apoB-48-containing lipoproteins have various kinds of atherogenic

Table 2
Stepwise multiple regression analysis of max-IMT in relation to age, blood pressure, lipid profiles, and glucose-related parameters.

	All subjects		Group (N-1)		Group (N-2)		Group (H)	
	F value	*P* value	*F* value	*P* value	*F* value	*P* value	*F* value	*P* value
Age	18.889	<0.0001	Not remain		5.51	0.023	12.603	0.0009
sBP	6.467	0.0120	Not remain		Not remain		8.249	0.0060
ln apoB-48	5.542	0.0198	Not remain		5.106	0.0283	Not remain	
HbA1c	2.541	0.1129	6.123	0.0164	2.098	0.1538	Not remain	

Stepwise multiple regression analysis was used to determine independent predictors of max-IMT measurement with *P* value-to-enter and *P* value-to-retain set at 0.20. Age, sBP, dBP, TC, ln TG, LDL–C, HDL–C, apoB-48, apoB-100, ln RemL–C, FPG, HbA1c, ln HOMA-IR, and IRI were included as explanatory variables in the method.

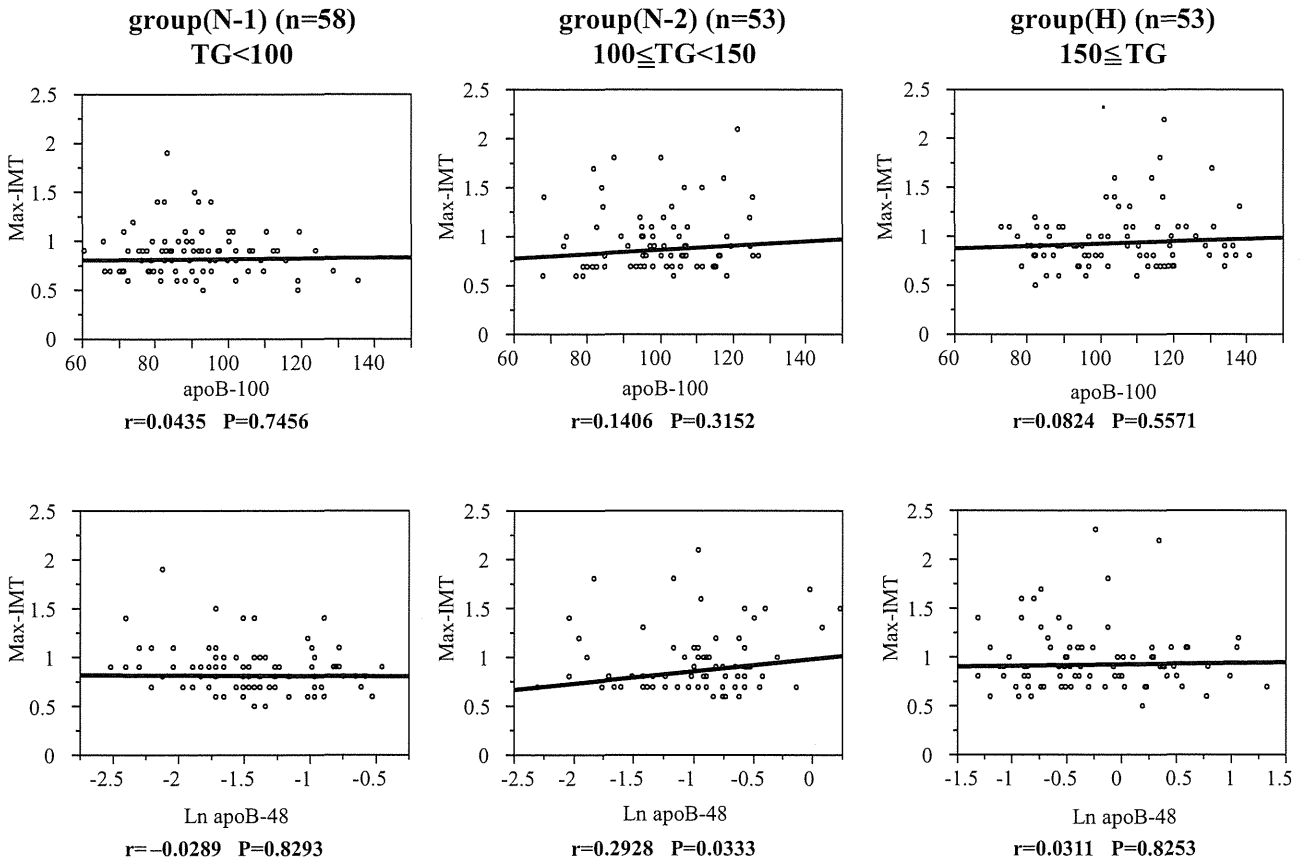

Fig. 3. Correlations between fasting apoB-100 levels or fasting Ln apoB-48 levels and max-IMT. There was no significant correlation between fasting apoB-100 levels and max-IMT in each TG group. Although the correlations between fasting apoB-48 levels and max-IMT in group N-1 and group H were not significant, there were a significant correlation between fasting apoB-48 levels and max-IMT in group N-2 as assessed by Pearson's correlation coefficients ($P < 0.05$).

features [11]. ApoB-48 was identified *in vivo* in human atherosclerotic plaques from femoral and carotid endarterectomy samples [21]. CM-R were shown to cause foam cell formation of mouse peritoneal and human monocyte-derived macrophages *in vitro* by both LDL-receptor-dependent and -independent mechanisms [11,22], stimulate MCP-1 expression in cultured vascular smooth muscle cells (VSMCs) [23], induce early growth response factor-1 (Egr-1) and proinflammatory cytokines, such as interleukin-2 (IL-2) and interferon-γ (IFN-γ) in VSMCs [24], increase the production of plasminogen activator inhibitor-1 (PAI-1) in endothelial cells via the MAPK pathway and redox system [25] and enhance endothelial cell apoptosis [26]. We found that fasting apoB-48 level was an independent risk factor for coronary stenosis assessed by coronary angiography (OR of apoB-48; 6.4, 95% CI; 3.64–1.79) (Masuda et al., unpublished observation). The increase in carotid IMT is significantly correlated with CHD and stroke [27]. Only a few studies have

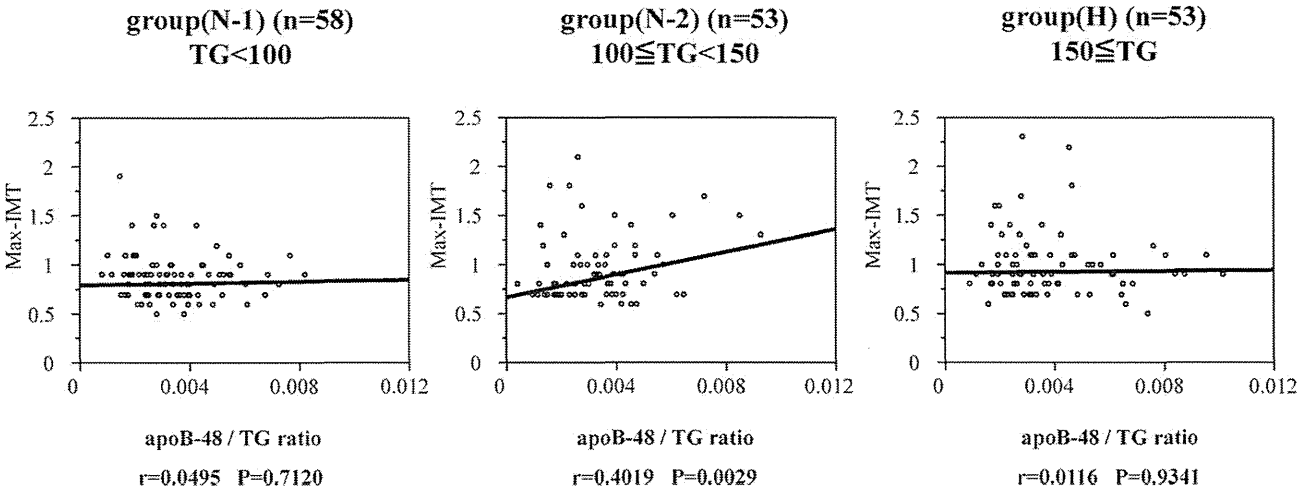

Fig. 4. Correlations between apoB-48/TG ratio and max-IMT.
The correlation between apoB-48/TG ratio and max-IMT was not significant in group N-1 and group H, but there was a significant correlation between apoB-48/TG ratio and max-IMT in group N-2 as assessed by Pearson's correlation coefficients ($P < 0.05$).

shown that there was a highly significant, independent correlation between the postprandial TG response and IMT [10], and that the presence of carotid plaque was associated with fasting apoB-48 and TG levels in age- and gender-adjusted analysis in type 2 diabetic patients [17]. As shown in the current study, the increase in apoB-48-containing lipoproteins, mainly CM-R, had a significant relationship with max-IMT. ApoB-48 level was also shown to be an independent variable of max-IMT in group N-2 (Fig. 3 and Table 2) which may significantly affect the development of systemic atherosclerosis associated with CHD and stroke. Ln apoB-48 level was associated with max-IMT, but LDL-C or apoB-100 levels were not correlated (Table 1 and Figs. 3 and 4). Tanimura et al. [17] also showed that the presence of carotid plaque was associated with high fasting apoB-48 levels but not with fasting TG levels in subjects with normal LDL-C (<140 mg/dl) levels. It was speculated that the impaired clearance and the accumulation of CM-R might be linked to carotid IMT and the development of atherosclerotic cardiovascular diseases, independent of the impaired clearance of VLDL and LDL.

4.2. CM-R particle size and atherogenic status

The size of CM produced by the small intestines is too large to penetrate the arterial wall; however, through the hydrolysis of TG by lipoprotein lipase (LPL) CM-R can become small enough to penetrate the arterial wall, be retained in the subendothelial space and affect the development of atherosclerotic plaques [11]. As shown in our former study, the size of CM-R changes from that of CM to that of HDL in the postprandial state [28]. Interestingly, in the current study, there was a strong correlation between apoB-48/TG ratio and max-IMT in group N-2 (Fig. 4). The high ratio of apoB-48/TG indicates that the number of apoB-48-containing lipoprotein particles increased while the number of TG components of these lipoproteins decreased, suggesting that the number of small-sized CM-R increased. The correlation between ln apoB-48 level and max-IMT in group N-2 whose TG levels were small indicates that the increase of small-sized CM-R was associated with the development of carotid atherosclerosis. Thus, serum apoB-48 level might be a good marker for the detection of early atherosclerosis in middle-aged, normotensive subjects with normal TG level.

4.3. Other metabolic phenotypes and apoB-48 levels

In subjects with high TG level (group H), there is a strong risk factor for the development of atherosclerosis. BMI, waist circumference, TG, apoB-48, RemL-C and IRI levels were significantly higher and HDL-C levels were significantly lower in group H than in group N-2 (Table 1), indicating that subjects in group H were capable of accumulating abdominal visceral fat which strongly affects insulin resistance or adipocytokine dysregulation. However, there was no significant correlation between ln apoB-48 level and max-IMT (Fig. 3). This discrepancy might be due to the clearance of CM and CM-R in subjects with abdominal visceral fat accumulation. The existence of insulin resistance deteriorates the lipoprotein metabolism of apoB-48-containing lipoproteins as well as apoB-100 -containing lipoproteins, which has been mainly explained by the impaired activity of LPL [29]. In these patients, low LPL activity causes an accumulation of large-sized CM-R or VLDL-R, resulting in an increase in TG and apoB-48. It was suggested that this buildup in large-sized lipoproteins was not precisely correlated with the enhancement of atherogenicity. There was no significant difference in LDL-C and apoB-100 levels among each TG group (Table 1), and fasting TG levels were mainly related to the accumulation of CM-R, less related to insulin resistance or apoB-100-containing lipoprotein metabolism. We could not find a positive correlation between RemL-C and max-IMT in our study subjects. The increase in CM-R did not properly reflect the increase in remnant lipoprotein cholesterol (RemL-C) levels which were shown to consist of CM-R and VLDL remnants. These CM-R and VLDL remnants have different origins, and their serum concentrations may vary depending upon the impairment of different pathways of lipoprotein metabolism. Above all, in the current study, there was a significantly positive correlation between apoB-48 level and max-IMT in group N-2, which was mainly associated with the increase in small-sized CM-R.

4.4. Limitation of the study

We aimed at evaluating the effectiveness of apoB-48 measurement in healthy subjects for the prediction of asymptomatic carotid atherosclerotic change. We recruited only men and focused on subjects coming to the Osaka Police Hospital, and therefore these factors are supposed to be the baseline of the bias.

5. Conclusion

In conclusion, these data suggest that the accumulation of CM-R might be an independent risk factor for the development of atherosclerosis among subjects with TG levels between 100 mg/dl and 150 mg/dl. The measurement of fasting apoB-48 level is very useful for the detection of early onset of atherosclerotic plaques.

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Disclosure

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ORIGINAL ARTICLE

Correlation of fasting serum apolipoprotein B-48 with coronary artery disease prevalence

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ABSTRACT

Background Postprandial hyperlipidemia partially refers to the postprandial accumulation of chylomicrons and chylomicron remnants (CM-R). Many *in vitro* studies have shown that CM-R has highly atherogenic properties, but consensus is lacking on whether CM-R accumulation correlates with the development of atherosclerotic cardiovascular diseases. We investigated the correlation between CM-R accumulation and the prevalence of coronary artery disease (CAD).

Design Subjects who received a coronary angiography and did not take any lipid-lowering drugs (n = 189) were enrolled. Subjects with coronary artery stenosis (≥ 75%) were diagnosed as CAD. Biochemical markers for glucose and lipid metabolism including fasting apolipoprotein (apo) B-48 concentration were compared between CAD patients (n = 96) and age-, sex-, and body mass index (BMI)-matched non-CAD subjects without overt coronary stenosis (< 75%) (n = 67). We tried to determine which metabolic parameters were correlated with the prevalence of CAD by multiple logistic regression analysis, and whether or not the combination of high apo B-48 and other coronary risk factors (high triglyceride, low HDL-C, high HbA1c or low adiponectin levels) increased the prevalence of CAD.

Results Fasting serum apo B-48 levels were significantly higher in CAD patients than in non-CAD subjects (3·9 ± 2·4 vs. 6·9 ± 2·6 μg/mL, P < 0·0001) and had the most significant correlation with the existence of CAD. The clustering of high fasting apo B-48 levels (> 4·34 μg/mL, the cut-off value) and other coronary risk factors were found to be associated with a stronger risk of CAD compared with single high fasting apo B-48 levels.

Conclusion Fasting serum apo B-48 levels significantly correlated with the prevalence of CAD.

Keywords Apolipoprotein B-48, chylomicrons, coronary artery disease, postprandial hyperlipidemia, remnant lipoproteins.

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Introduction

Fasting hypertriglyceridaemia and postprandial hyperlipidaemia (PH) are both closely related to the development of atherosclerotic cardiovascular diseases [1,2]. PH is characterized by postprandial accumulation of triglyceride (TG)-rich lipoproteins and their partially hydrolysed products, 'remnant lipoproteins', as suggested by Zilversmit [3] and supported by numerous subsequent studies [4,5]. Remnant lipoprotein cholesterol levels proved to be closely correlated with the prevalence of coronary artery disease (CAD) [6,7]. The atherogenicity of remnant lipoproteins has been the subject of numerous studies [5]. However, the atherogenicity of chylomicron remnants (CM-R) has been investigated less frequently than that of very-low-density lipoproteins (VLDL) remnants (VLDL-R) or intermediate-density lipoprotein (IDL). Investigators have developed an assay system for measuring serum apolipoprotein B-48 (apo B-48) concentration, which represents the