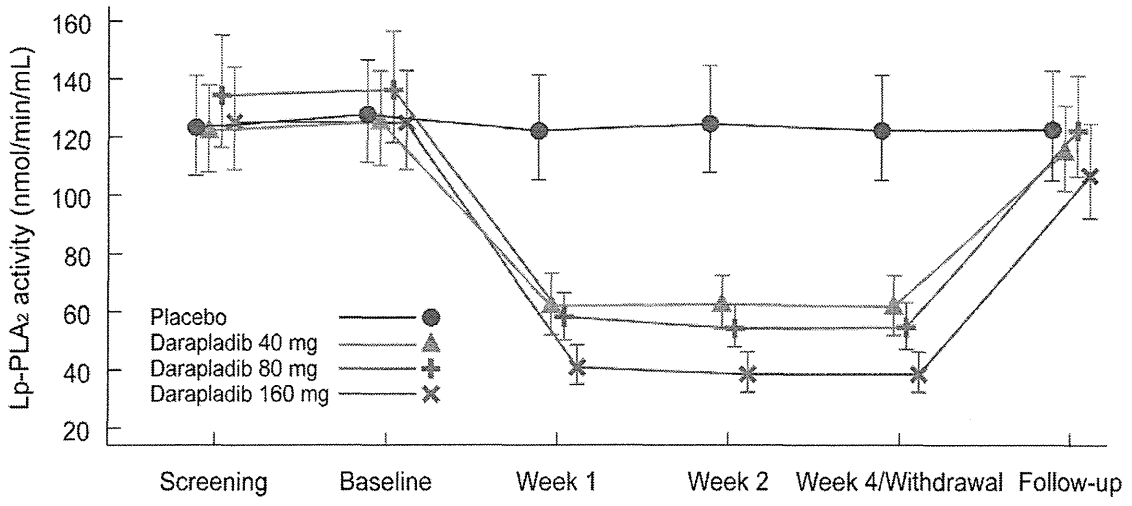

Figure. Geometric mean (95% confidence interval) plasma lipoprotein-associated phospholipase A_2 (Lp-PLA_2) activity.

Table 3. Plasma Lp-PLA_2 Activity Change vs. V279F Subgroup

Category/Treatment	Visit	Geometric mean	Adj. ratio†	vs. placebo (95% CI)‡	P-value
Homozygous wild-type (VV)					
Placebo (n=18)	Baseline	146.88	0.957		
	Week 4/withdrawal	140.30			
Darapladib 40 mg (n=22)	Baseline	145.62	0.488	0.510 (0.434–0.599)	<0.001
	Week 4/withdrawal	70.86			
Darapladib 80 mg (n=23)	Baseline	152.98	0.396	0.414 (0.353–0.485)	<0.001
	Week 4/withdrawal	60.80			
Darapladib 160 mg (n=19)	Baseline	148.82	0.316	0.330 (0.280–0.390)	<0.001
	Week 4/withdrawal	47.04			
Heterozygote (VF)					
Placebo (n=7)	Baseline	88.74	1.010		
	Week 4/withdrawal	86.59			
Darapladib 40 mg (n=6)	Baseline	73.09	0.510	0.505 (0.378–0.676)	<0.001
	Week 4/withdrawal	38.32			
Darapladib 80 mg (n=5)	Baseline	79.36	0.435	0.431 (0.321–0.578)	<0.001
	Week 4/withdrawal	34.56			
Darapladib 160 mg (n=7)	Baseline	77.49	0.303	0.301 (0.229–0.394)	<0.001
	Week 4/withdrawal	23.73			

ANCOVA was performed on the log-transformed data and back-transformed to provide statistics in original scale. Dunnett correction was used to adjust multiplicity. †Adjusted geometric mean ratio from baseline to week 4 of each of the darapladib groups. ‡Adjusted geometric mean ratio of each of the darapladib groups compared with the placebo. CI, confidence interval; Lp-PLA_2, lipoprotein-associated phospholipase A_2.

screening.

At baseline 21/107 patients (20%) had diabetes mellitus, 40/107 (37%) had hypertension, and 107/107 (100%) reported current dyslipidemia. Statins used concomitantly included atorvastatin 51/107 (48%), pravastatin 27/107 (25%), rosuvastatin 23/107 (21%), simvastatin 5/107 (5%), and pitavastatin 1/107 (<1%).

Efficacy

Primary Efficacy Analysis All darapladib doses (40 mg, 80 mg, and 160 mg) produced sustained inhibition of Lp-PLA_2 activity in a dose-dependent fashion (approximately 49%, 58%, and 67% inhibition, respectively; P<0.001 for all comparisons) from baseline to week 4 (Table 2). On the follow-up visit, Lp-PLA_2 activity level returned to the baseline level (Figure). Sensitivity analysis using the observed case dataset for the FAS and PPS came to the same conclusions and showed robustness of primary analysis.

Secondary Analysis All darapladib doses showed sustained inhibition in plasma Lp-PLA_2 activity over time. The dose

Table 4. Biomarker Change From Baseline to Week 4

	n	Adj. ratio†	vs. placebo (95% CI)‡	P-value
PAI-1 (ng/ml)				
Placebo	25	1.247		
Darapladib 40 mg	27	1.032	0.828 (0.673–1.019)	0.074
Darapladib 80 mg	28	1.047	0.840 (0.685–1.029)	0.091
Darapladib 160 mg	25	0.837	0.671 (0.542–0.832)	<0.001
hs-CRP (mg/L)				
Placebo	23	1.396		
Darapladib 40 mg	26	1.203	0.862 (0.560–1.327)	0.496
Darapladib 80 mg	25	0.797	0.571 (0.371–0.879)	0.012
Darapladib 160 mg	22	1.335	0.956 (0.613–1.492)	0.842
IL-6 (ng/L)				
Placebo	16	1.141		
Darapladib 40 mg	19	0.866	0.760 (0.508–1.135)	0.176
Darapladib 80 mg	17	0.782	0.686 (0.456–1.032)	0.070
Darapladib 160 mg	15	0.770	0.675 (0.439–1.036)	0.072
P-selectin (μg/L)				
Placebo	24	1.041		
Darapladib 40 mg	24	1.014	0.974 (0.855–1.110)	0.694
Darapladib 80 mg	27	0.981	0.942 (0.830–1.070)	0.354
Darapladib 160 mg	22	1.004	0.965 (0.844–1.103)	0.597
U-TxB_2 (pg/ml)				
Placebo	25	1.185		
Darapladib 40 mg	27	1.193	1.007 (0.697–1.454)	0.971
Darapladib 80 mg	28	1.178	0.994 (0.689–1.434)	0.974
Darapladib 160 mg	26	1.426	1.203 (0.828–1.748)	0.329

ANCOVA was performed on the log-transformed data and back-transformed to provide statistics in original scale. Dunnett correction was used to adjust multiplicity. †Adjusted geometric mean ratio from baseline to week 4 of each of the darapladib groups. ‡Adjusted geometric mean ratio of each of the darapladib groups compared with the placebo. CI, confidence interval; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; PAI-1, plasminogen activating inhibitor type 1; U-TxB_2, urinary 11-dehydrothromboxane B_2.

Table 5. Most Frequent Treatment-Emergency AEs†

Treatment-emergency AE	Placebo (n=25)	Darapladib 40 mg (n=28)	Darapladib 80 mg (n=28)	Darapladib 160 mg (n=26)
Any event	10 (40)	18 (64)	15 (54)	16 (62)
Abnormal feces	0	5 (18)	6 (21)	5 (19)
Nasopharyngitis	4 (16)	3 (11)	4 (14)	5 (19)
Urine odor abnormal	0	2 (7)	5 (18)	4 (15)
Diarrhea	1 (4)	1 (4)	2 (7)	3 (12)
Eczema	2 (8)	1 (4)	1 (4)	2 (8)
Headache	1 (4)	2 (7)	2 (7)	0
Skin odor abnormal	0	2 (7)	0	1 (4)
Abdominal pain upper	0	0	0	2 (8)
Constipation	0	2 (7)	0	0
Muscle spasms	0	0	2 (7)	0

†Any AE that occurred in more than 1 subject in any group. AE, adverse event.

response of darapladib on inhibition of plasma Lp-PLA_2 was analyzed using ANCOVA with contrast method at the 1-sided 2.5% significance level. Linear trend was the best fit of contrast for the dose-response analysis.

Exploratory Analysis There was no difference in proportional change of plasma Lp-PLA_2 activity between the 279VV and 279VF subjects (Table 3), in both genotypes showing a significant effect vs. placebo. Analyses of the genotypes were deemed exploratory.

Data for biomarkers are given in Table 4. PAI-1 in the darapladib 160 mg group had a significant reduction (P<0.001) compared with placebo. For the inflammatory biomarker hs-CRP, there was a reduction only in the darapladib 80-mg group (P=0.012) compared with placebo. Also, IL-6 showed a decreasing trend in the darapladib 80-mg and 160-mg groups compared with placebo. The platelet activation biomarkers

(P-selectin and U-Tx-B2) showed no significant change in all the darapladib treatment groups compared with placebo (Table 4).

Safety

Darapladib was generally well-tolerated. All AEs were reported as being mild or moderate in intensity. Table 5 lists the most frequently reported AEs (incidence ≥2) in any treatment group in the safety group. The most frequently reported AEs in the darapladib groups were odor-related disorders, primarily abnormal feces.

A total of 1/25 patients (4%) in the placebo group, 5/28 (18%) in the darapladib 40-mg group, 6/28 (21%) in the darapladib 80-mg group, and 7/26 (27%) in the darapladib 160-mg group experienced AEs that were considered related to study drug by the investigator.

There were no deaths reported during the study and follow-up. One subject in the darapladib 40-mg treatment group experienced a non-fatal SAE (pulmonary embolism) and was withdrawn from the study. The SAE was not considered to be related to darapladib by the investigator.

There was no evidence of a trend for dose-related effects in any laboratory parameter. In all treatment groups, the mean changes from baseline were small and were not of clinical importance.

No clinically meaningful change in vital signs over the period of study were reported, except for blood pressure increase reported in 1 subject (4%) in the darapladib 40-mg treatment group, which was reported as an AE. No pathognomonic findings in ECG results were seen during the study.

Discussion

Several large prospective epidemiological studies have suggested a positive association between plasma Lp-PLA2 level and CVD risk.[15] Lp-PLA2 plays a role during LDL oxidation and has been recognized as a predictor of CVD events, providing risk information beyond that provided by conventional CVD risk factors.[24] Furthermore, its potential as a therapeutic target for CVD risk reduction has been proposed.[24] The present study is the first to evaluate the efficacy and safety of darapladib, a novel inhibitor of Lp-PLA2 activity, in Japanese dyslipidemic patients receiving statin therapy, and to evaluate the influence of the V279F variant of the *PLA2G7* gene on the effect of darapladib.

All darapladib doses (40 mg, 80 mg, and 160 mg) produced sustained inhibition of Lp-PLA2 activity at approximately 49%, 58%, and 67%, respectively ($P<0.001$ for all comparisons). A linear dose response of darapladib on inhibition of plasma Lp-PLA2 activity was observed. The inhibitory effect of plasma Lp-PLA2 activity achieved a plateau by 1 week. The results are similar to those reported by Mohler et al, whose study recruited patients in 15 countries. In that 12-week study, inhibition of Lp-PLA2 activity was sustained at approximately 43%, 55%, and 66% for darapladib 40, 80, and 160 mg, respectively ($P<0.001$ for all comparisons) vs. placebo.[21]

Lp-PLA2 binds to the carboxyl terminus of human apolipoprotein B, and approximately 80% of circulating Lp-PLA2 is associated with apolipoprotein B-containing lipoproteins.[25,26] The remaining Lp-PLA2 is less firmly associated with phospholipid moiety of HDL-C and does not bind to apolipoprotein A-I. Higher Lp-PLA2 mass or activity is found in proatherogenic small dense LDL-C and electronegative LDL-C particles.[27–29] Previous studies have also shown that various hypolipidemic drugs (eg, statins, fenofibrate, ezetimibe) decrease plasma Lp-PLA2 mass or activity due to LDL-C lowering, without a direct effect on macrophage expression of the enzyme.[30,31] In general, statin treatment alone (eg, pravastatin, fluvastatin, simvastatin, atorvastatin) has been associated with approximately 20–30% reduction in the measurement of Lp-PLA2 (mass or activity) in stable CVD patients.[32–34] Similarly, other lipid-modifying drugs, such as ezetimibe and fenofibrate, modestly lower Lp-PLA2 (mass or activity).[35]

Mohler et al reported that darapladib produced substantial additional reductions in Lp-PLA2 activity when added to intensive atorvastatin therapy (up to 66%).[21] This effect was largely independent of atorvastatin dose and preserved in clinically relevant strata of LDL-C and HDL-C values. The present study also showed that darapladib gave additional reductions in Lp-PLA2 activity of up to 67% compared to the placebo group when added to statin therapy (atorvastatin, 43%; rosuvastatin, 27%; pravastatin, 26%; simvastatin, 4%; pitavastatin, 1%).

In the present study, patients whose Lp-PLA2 activity was $\leq 10\,nmol \cdot min^{-1} \cdot ml^{-1}$ at screening were regarded as lacking LpPLA2 activity, hence indicative of 279FF, and were excluded. Consequently, there were 82 patients (77%) with 279VV and 25 (23%) with 279VF.

Of the several polymorphisms of the *PLA2G7* gene, the V279F homogenous mutation is known to lack Lp-PLA2 activity.[9,10] Jang et al found that the V279F variant in the *PLA2G7* gene led to significant loss of enzyme activity in heterozygous subjects and no appreciable enzyme activity in homozygous subjects. The mechanism of the deficiencies of plasma Lp-PLA2 activity were suggested to be due to a loss-of-function mutation (V279F, exon 9, position 994; G3T; G>T) in the Lp-PLA2 gene, because Val-279 conserved in plasma Lp-PLA2 lies between the active site Ser-273 and Asp-296 residues in a region that is critical for proper folding of the enzyme.[12]

It has been reported that the mutant allele was present in 27% of the Japanese population as heterozygotes and in 4% as homozygotes.[10] In contrast, no heterozygous or homozygous deficient subjects were identified in a random North American population[10] and were rare in the Caucasian hapmap samples and absent in the African samples (www.hapmap.org). In addition, some reports showed that Lp-PLA2 activity in the 279VF population was approximately half of that in the 279VV population, and that 279FF completely lacked Lp-PLA2 activity.[9,13]

It should be noted that, in the present study, darapladib significantly reduced plasma Lp-PLA2 activity in comparison with placebo in both the 279VV and 279VF genotype subgroups. Moreover, there was no significant difference in change of plasma Lp-PLA2 activity in both the 279VF and 279VV subgroups after 4 weeks of treatment. This suggests that darapladib may have the potential to reduce risk of atherosclerosis and subsequent cardiovascular events even in individuals with the 279VF genotype that have almost half the level of Lp-PLA2 activity compared to that of the wild type. A further 2 phase III studies, to evaluate for the incidence of major adverse cardiovascular events in chronic CAD and acute coronary syndrome patients, are now going on. We await the results on cardiovascular outcome.

Several cardiovascular biomarkers were evaluated within a limited number of patients and a short period in this study, so the possible effect on cardiovascular biomarkers provided only exploratory data and may not be robust. The on-going phase III studies may identify the effect on CV biomarkers correctly.

No major safety concerns were noted, and darapladib appeared to be generally well-tolerated in patients. The majority of AEs were not related to the study medication. The most common AEs with a probable relationship to study drug were odor related. Odor-related events were not unexpected, in line with previous studies in which odor-related AEs have been associated with repeated doses of darapladib. None of the odor-related events resulted in withdrawal of patients from the study.

Study Limitations

This was a phase II study designed to examine the efficacy and safety of darapladib in a small group of patients. The effects of darapladib on clinically important outcomes are currently unknown; whether the inhibitory effect of darapladib shown in the present study leads to clinical efficacy in the prevention of major adverse cardiovascular events in patients with CAD or acute coronary syndrome is being examined in 2 ongoing international phase III outcomes studies.

If darapladib is proven to be effective in preventing major cardiovascular events and the progression of atherosclerotic disease, then it would be also applicable to the heterozygous (VF) genotype, which has almost half the level of Lp-PLA2 activity compared with that of the wild type.

Conclusion

Darapladib produced sustained inhibition of Lp-PLA2 activity with no major safety problems in Japanese dyslipidemic patients with or without a V279F variant who were receiving statin therapy.

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High levels of very long-chain saturated fatty acid in erythrocytes correlates with atherogenic lipoprotein profiles in subjects with metabolic syndrome

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ABSTRACT

Aim: Very long chain saturated fatty acid (VLCFA) levels in erythrocytes are associated with metabolic syndrome (MS). However, the relationship between levels of the VLCFA ligonoceric acid (C24:0) in erythrocytes and the atherogenic lipoprotein profiles and inflammatory state in MS remain unclear.

Methods: Based on the International Diabetes Federation (IDF) definition of MS, 195 apparently healthy males were assigned to either an MS group ($n = 38$) or a non-MS group ($n = 157$). Fatty acid composition of erythrocytes was determined by gas liquid chromatography.

Results: Erythrocytes from the MS group had a significantly higher level of C24:0 than cells from the non-MS group ($4.06 \pm 0.48\%$ versus $3.88 \pm 0.34\%$; $p = 0.03$). C24:0 levels were significantly correlated with several components of MS. The C24:0 levels showed a significant negative correlation with LDL and HDL particle size. Multivariate linear regression analysis showed that C24:0 levels were independently correlated with LDL particle size after adjusting for age and each MS criterion. C24:0 levels were also positively correlated with log-transformed high-sensitivity CRP levels ($p = 0.04$).

Conclusion: C24:0 levels in erythrocytes are associated with specific atherogenic lipoprotein profiles and inflammation status in subjects with MS.

1. Introduction

Metabolic syndrome (MS) is a constellation of metabolic risk factors that includes increased waist circumference, atherogenic dyslipidemia, elevated blood pressure, and elevated blood glucose associated with insulin resistance [1,2]. Several meta-analyses have shown that MS is associated with an approximately 2-fold increased risk of cardiovascular disease [3–5]. One of the characteristic phenotypes of MS is the accumulation of fat in adipose tissue and release of free fatty acids (FFAs) into the circulation. An excessive influx of FFAs

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into muscles and the liver leads to insulin resistance. Several studies have suggested a relationship between plasma fatty acid composition and each of the MS components, including insulin resistance, glucose intolerance, hypertension, and serum lipoprotein disorders [6,7].

Although saturated very long chain fatty acids (VLCFAs) are minor fatty acid components in human tissues and the bloodstream, associations between C26:0 levels, a VLCFA, in erythrocytes and risks of cardiovascular diseases have been observed [8]. We have also reported that absolute C26:0 levels in whole blood were significantly associated with several features of MS [9]. However, levels of C26:0 in the circulation are so low that it is relatively complicated to measure C26:0 levels in clinical settings. Therefore, we confirmed the association between MS and the levels of another saturated VLCFA, lignoceric acid (C24:0), which were measured by a simple established method [10].

Few studies have investigated possible correlations between saturated VLCFA levels and precise atherogenic lipoprotein profiles and systemic inflammatory states, both of which are important MS atherogenic features. Here, we found that a high level of C24:0, but not other fatty acids, in erythrocytes was significantly correlated with small LDL and HDL particles, which are specific components of atherogenic lipoprotein profiles, and high levels of high-sensitivity C-reactive protein (hs-CRP), which indicates systemic inflammation. These results suggest that measuring the level of C24:0 VLCFA in erythrocytes may be a useful marker to evaluate MS atherogenicity.

2. Materials and methods

2.1. Study subjects

We studied 195 consecutive and apparently healthy male subjects who underwent a medical check-up at the Nagasu-Kashiwado Clinic from December 2004 to January 2005. All subjects gave informed consent and the study was approved by the local ethical committee. We excluded patients who were receiving any medicines for diabetes mellitus or dyslipidemias and subjects with high levels of hs-CRP (more than 1 mg/l). Blood pressure (BP) was measured with a standard mercury sphygmomanometer after the subjects had rested for more than 5 min. The mean of two measurements of systolic and diastolic BP while sitting was used. Height and weight were measured using an automated scale, and body mass index was calculated as the weight in kilograms divided by the square of height in meters. Waist circumference was determined by measurements around the umbilical area while standing straight and after expiration.

Study subjects were divided into an MS group and a non-MS group according to the International Diabetes Federation (IDF) definition of MS [2]. Briefly, subjects with MS were defined as having a waist circumference of $\geq$85 cm plus two or more of the following factors: (1) elevated concentration of triglycerides (TG > 150 mg/dl) or specific treatment for this lipid abnormality; (2) reduced concentration of high density lipoprotein cholesterol (HDL-C < 40 mg/dl) or specific treatment for this lipid abnormality; (3) elevated BP: systolic BP > 130 mmHg or diastolic BP > 85 mmHg or treatment for previously diagnosed hypertension; and (4) elevated fasting plasma glucose (FPG concentration > 100 mg/dl) or previously diagnosed type 2 diabetes.

2.2. Blood sampling

Whole blood samples were drawn after overnight fasting. Serum levels of total cholesterol (TC), TG, and HDL-C were measured by standard enzymatic methods (Kainos, Tokyo, Japan) and low-density lipoprotein cholesterol (LDL-C) values were calculated using the Friedewald formula [11]. Plasma glucose concentrations were determined by the glucose oxidase method (Kainos, Tokyo, Japan) and serum insulin levels were measured according to a double antibody technique (Dainabot, Tokyo, Japan). HbA1c (%) was measured with previously standardized Japanese HbA1c and measurement methods (NGSP). The homeostasis model assessment for insulin resistance (HOMA-IR) was calculated using the following formula: fasting glucose (mmol/l) × fasting insulin (mU/l)/22.5, which was rearranged from the formula originally proposed by Matthews et al. [12].

2.3. Measurement of fatty acid composition in erythrocytes

Total lipids were extracted from erythrocytes by the method of Folch, and fatty acids were directly transmethylated with 14% boron trifluoride methanol solution (Sigma–Aldrich Japan, Tokyo, Japan) at 90 °C for 90 min. Fatty acids were measured using a GC-FID system (6890 N; Agilent Technologies, Tokyo, Japan) equipped with a fused silica capillary column (Omegamax 250; 30 m × 0.25 mm i.d.; 0.25 μm film thickness; Supelco, USA) using tricosanoic acid (C23:0) methyl ester as an internal standard. The injector and detector temperatures were both set at 270 °C and the column temperature was held at 205 °C. Helium was used as the carrier gas at a flow rate of 2.0 ml/min with a split ratio of 50:1 [10].

2.4. Measurement of lipoprotein profiles

Average LDL and HDL particle diameters (nm) were obtained from LDL and HDL peak times with a dual detector, high performance liquid chromatography (HPLC) system with two tandem-connected TSKgel LipopropakXL columns (300 mm × 7.8 mm; Tosoh, Japan) from Skylight Biotech, Inc. (Akita, Japan), as previously described [13,14].

2.5. Statistical analysis

Continuous variables were expressed as mean ± SD and categorical variables were reported as percentages. Statistical differences between the groups were analyzed by Welch's test and chi-square tests. Levene's test was used to assess the equality of variances in different samples. Correlations between two variables were determined by simple linear regression analysis. Multiple linear regression analysis was used to determine the associations between erythrocyte C24:0

levels and LDL particle sizes, HDL particle size or hs-CRP levels independently related to the MS components. Statistical analysis used StatView software (Version 5.0 for Windows, SAS Institute, Cary, NC). p-Values < 0.05 were considered statistically significant.

3. Results

3.1. Characteristics of the study subjects

The characteristics of the subjects in the present study are shown in Table 1. The two groups were not significantly different in terms of age. Compared with the non-MS group, the MS group had significantly higher body mass index (BMI) ($p < 0.001$), waist circumference ($p < 0.001$), systolic BP ($p < 0.001$), diastolic BP ($p < 0.001$), and mean BP ($p < 0.001$). In the MS group, plasma TG levels were significantly increased ($p = 0.005$) and HDL-C levels were significantly decreased ($p < 0.001$) compared with the non-MS group. Plasma TC and LDL-C levels were not significantly different between the two groups. In the MS group, FPG ($p < 0.001$), insulin ($p < 0.001$), HbA1c ($p = 0.01$), HOMA-IR ($p < 0.001$), and hs-CRP ($p = 0.03$) were significantly increased compared with the non-MS group.

3.2. Comparison of erythrocyte fatty acid composition in the MS and non-MS groups

Table 2 shows the fatty acid composition of erythrocytes. In the MS group, erythrocyte levels of C18:0 (stearic acid), and C24:0 (lignoceric acid) were significantly higher than in the non-MS group ($17.6 \pm 1.4\%$ versus $17.2 \pm 1.0\%$, $p = 0.04$; and $4.06 \pm 0.48\%$ versus $3.88 \pm 0.34\%$, $p = 0.03$, respectively). Conversely, MS group erythrocytes had significantly lower levels of C18:1n-7 (vaccenic acid) than erythrocytes from the non-MS group ($1.30 \pm 0.16\%$ versus $1.38 \pm 0.15\%$, $p = 0.005$).

3.3. Correlations between C24:0 and MS risk factors

The correlations between erythrocyte C24:0 levels and the components of MS are shown in Table 3. C24:0 levels were positively correlated with BMI ($r = 0.227$, $p < 0.001$) and systolic BP ($r = 0.158$, $p = 0.03$), plasma LDL-C ($r = 0.167$, $p = 0.02$), and TG ($r = 0.176$, $p = 0.01$). C24:0 levels were negatively correlated with, HDL-C ($r = -0.186$, $p = 0.009$). There was no significant correlation between C24:0 levels and age, plasma TC, fasting plasma glucose, fasting insulin levels, or HOMA-IR.

3.4. Correlation of C24:0 with LDL and HDL particle size

We explored the correlation between erythrocyte C24:0 levels and LDL and HDL particle size (Fig. 1). C24:0 levels were inversely correlated with both LDL and HDL particle diameter. The levels of other fatty acids however, were not significantly correlated with LDL and HDL particle size. After adjusting for each MS criterion (waist circumference, systolic BP, FPG, TG, and HDL-C) and age, C24:0 levels were still independent variables associated with LDL particle size ($p = 0.04$), but not HDL particle size ($p = 0.12$).

3.5. Correlation between C24:0 and hs-CRP

Fig. 2 shows that the level of C24:0 in erythrocytes was significantly correlated with log-transformed hs-CRP levels ($r = 0.15$, $p = 0.04$). After adjusting for each MS criterion (waist circumference, systolic BP, FPG, TG, and HDL-C) and age, there was no significant association between log-transformed C24:0 levels and hs-CRP levels ($p = 0.36$).

4. Discussion

The level of C24:0 was significantly higher in erythrocytes from the MS group than the non-MS group, and C24:0 levels

Table 1 – Characteristics of study subjects.

	Non-MS	MS	F statics	p value
	$n = 157$	$n = 38$		
Age (years)	50.2 ± 9.9	50.1 ± 8.6	0.11	NS
Body mass index (kg/m^2)	23.2 ± 2.5	27.2 ± 3.3	0.29	<0.001
Waist circumference (cm)	83.4 ± 6.5	95.0 ± 6.3	0.32	<0.001
Systolic blood pressure (mmHg)	127 ± 15	139 ± 15	0.58	<0.001
Diastolic blood pressure (mmHg)	80 ± 10	87 ± 11	0.53	<0.001
Current smoker (%)	69 (44)	16 (43)		NS
Total cholesterol (mg/dl)	194 ± 35	190 ± 39	0.05	NS
Triglycerides (mg/dl)	111 ± 44	130 ± 32	0.33	0.005
HDL-cholesterol (mg/dl)	58 ± 12	47 ± 8	0.009	<0.001
LDL-cholesterol (mg/dl)	77 ± 18	82 ± 15	0.15	NS
Blood glucose (mg/dl)	99 ± 13	108 ± 14	0.46	<0.001
Insulin (mU/l)	4.6 ± 2.7	9.4 ± 6.7	<0.001	<0.001
HOMA-IR	1.2 ± 0.9	2.6 ± 2.1	<0.001	<0.001
HbA1c (NGSP)(%)	5.7 ± 0.4	6.0 ± 0.8	0.004	0.01
Hs-CRP (mg/l)	0.86 ± 1.41	1.66 ± 2.09	0.002	0.03

Values are mean ± SD. MS, metabolic syndrome; HDL, high density lipoprotein; LDL, low density lipoprotein; HOMA-IR, homeostasis model assessment for insulin resistance; Hs-CRP, high sensitive C reactive protein.

Table 2 – Proportion of fatty acid (%) in erythrocytes from MS and non-MS subjects.

	Non-MS $n = 157$	MS $n = 38$	F statics	p value
C14:0 (myristic acid)	0.21 ± 0.05	0.22 ± 0.06	0.04	NS
C16:0 (palmitic acid)	20.4 ± 1.3	20.9 ± 1.6	0.12	0.08
C16:1*n*-7 (palmitoleic acid)	0.44 ± 0.07	0.46 ± 0.08	0.70	NS
C18:0 (stearic acid)	17.2 ± 1.0	17.6 ± 1.4	0.004	0.04
C18:1*n*-9 (oleic acid)	12.4 ± 0.82	12.3 ± 0.79	0.80	NS
C18:1*n*-7 (vaccenic acid)	1.38 ± 0.15	1.30 ± 0.16	0.87	0.005
C18:2*n*-6 (linoleic acid)	8.30 ± 0.97	8.05 ± 1.06	0.57	NS
C18:3*n*-3 (α-linolenic acid)	0.13 ± 0.03	0.12 ± 0.03	0.86	NS
C20:0 (arachic acid)	0.35 ± 0.04	0.34 ± 0.04	0.78	NS
C20:1	0.42 ± 0.09	0.43 ± 0.10	0.28	NS
C20:3*n*-6 (dihomo-γlinolenic acid)	1.16 ± 0.18	1.22 ± 0.17	0.80	NS
C20:4*n*-6 (arachidonic acid)	10.9 ± 1.6	10.5 ± 1.8	0.44	NS
C20:5*n*-3 (eicosapentaenotic acid)	1.66 ± 0.77	1.57 ± 0.74	0.78	NS
C22:0 (behenic acid)	1.22 ± 0.15	1.25 ± 0.16	0.58	NS
C22:1	0.13 ± 0.15	0.11 ± 0.12	0.63	NS
C22:4*n*-6	1.56 ± 0.41	1.53 ± 0.51	0.03	NS
C22:5*n*-6 (*n*-6 docosapentaenoic acid)	0.28 ± 0.11	0.26 ± 0.07	0.71	NS
C22:5*n*-3 (*n*-3 docosapentaenoic acid)	2.27 ± 0.38	2.16 ± 0.42	0.69	NS
C22:6*n*-3 (docosahexaenoic acid)	7.01 ± 1.43	6.84 ± 1.78	0.23	NS
C24:0 (lignoceric acid)	3.88 ± 0.34	4.06 ± 0.48	0.02	0.03
C24:1*n*-9 (nervonic acid)	4.17 ± 0.39	4.19 ± 0.44	0.37	NS

Values are mean ± SD.

were significantly associated with several components of MS. Additionally, we found that the increased level of C24:0, but not other fatty acids, in erythrocytes was significantly correlated with small LDL and HDL particle size, which are specific components of atherogenic lipoprotein profiles. Increased C24:0 levels were also positively correlated with systemic inflammation as indicated by hs-CRP levels.

Fatty acid beta-oxidation occurs in both mitochondria and peroxisomes. Long chain fatty acids (C16–C20) are primarily oxidized in mitochondria, whereas peroxisomes are involved in the beta-oxidation of VLCFAs (>C20) [15]. Peroxisomal dysfunction gives rise to an over-accumulation of VLCFAs in the body as a whole [16]. VLCFAs accumulate in the plasma, membranes of erythrocytes, and/or tissues of patients with inherited peroxisomal diseases, which are characterized by progressive demyelination and adrenal insufficiency [17–19]. X-adrenoleukodystrophy (X-ALD), the most common peroxisomal disorder, is associated with increased levels of saturated VLCFAs (>C22:0) [16]. Treatment with the potent and selective histone deacetylase inhibitor normalized the levels of VLCFAs in skin fibroblasts from X-ALD patients by increasing the peroxisomal C24:0 beta-oxidation activity [20]. Peroxisomal dysfunction plays an important role in aging-related diseases [21], and, furthermore, a recent report suggested that peroxisome-related alterations and increased VLCFAs may contribute to the progression of Alzheimer's disease [22].

Table 3 – Correlations of the proportion of C24:0 with risk factors of metabolic syndrome.

	r	t-Score	p-Value
Age	0.084	1.17	NS
Body mass index	0.227	3.24	0.001
Waist circumference	0.258	3.71	<0.001
Systolic blood pressure	0.158	2.23	0.03
Total cholesterol	0.139	1.95	NS
HDL-cholesterol	−0.186	−2.62	0.009
LDL-cholesterol	0.167	2.31	0.02
Triglycerides	0.176	2.49	0.01
Fasting plasma glucose	0.066	0.92	NS
Fasting serum insulin	0.125	1.75	NS
HOMA-IR	0.099	1.38	NS

HDL, high density lipoprotein, LDL, low density lipoprotein, HOMA-IR, homeostasis model assessment for insulin resistance.

The expression of enzymes involved in fatty acid synthesis and elongation may contribute to the accumulation of VLCFAs. In particular, ELOVL1 and ELOVL3 have chain length specificity toward VLCFA [23,24], and silencing of ELOVL1 reduces elongation of C22:0–C26:0 and lowers C26:0 levels in X-ALD fibroblasts [23]. ELOVL3 mediates the elongation of C22:0–C24:0 and C24:0–C26:0 in vivo [24]. It has been reported that ELOVL3 expression regulates diet-induced obesity, hepatic lipogenic gene expression, and hepatic TG content [25]. Several studies, including our own, have demonstrated the association of saturated VLCFA levels with the risks of cardiometabolic syndrome [8,9]. In this study, the accumulation of C24:0 was also associated with MS. Taken together, MS may be associated with an imbalance between the synthesis and metabolism of saturated VLCFAs.

LDL and HDL particles associated with MS tend to be small and dense [26]. Smaller LDL particles are more atherogenic than larger LDL as they may filter more readily into the arterial wall and are more prone to atherogenic modifications [27]. Small, dense HDL sub-fractions are increased in MS and are associated with elevated oxidative stress and insulin resistance [28]. However, the association between various fatty acid

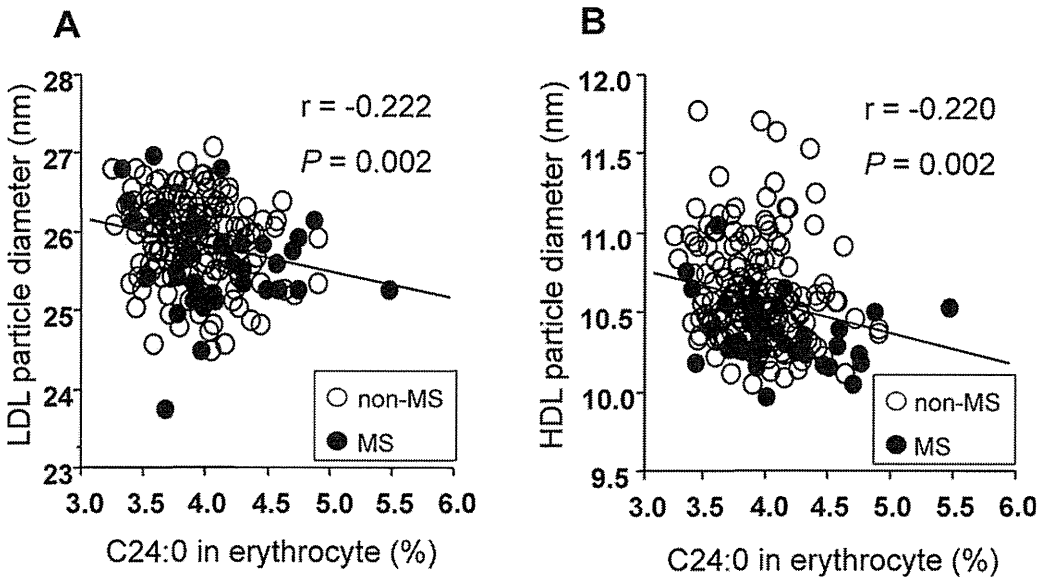

Fig. 1 – Correlation of the level of C24:0 in erythrocyte with low density lipoprotein (LDL) particle diameter (A) and high density lipoprotein diameter (B).

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.

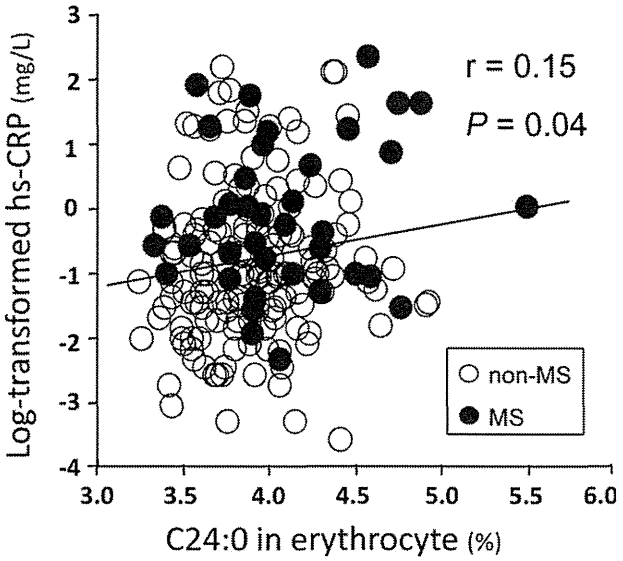

Fig. 2 – Correlation of the level of C24:0 in erythrocyte with log-transformed high sensitive C reactive protein (hs-CRP).

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

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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.

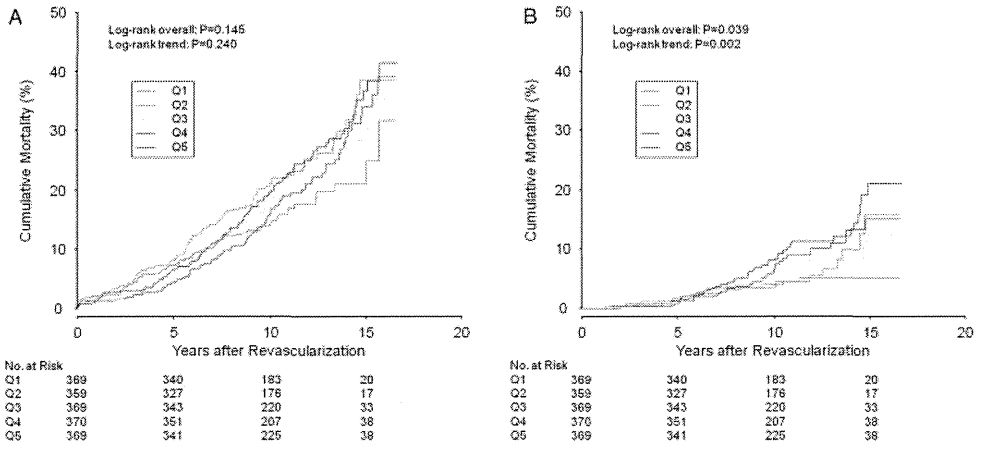

Figure 1 Cumulative mortality curves according to the quintiles of triglyceride levels for (A) all-cause deaths and (B) cardiac deaths. p Values for overall log-rank tests indicate whether there is a difference in the five different mortality curves (p=0.145 for all-cause death, p=0.039 for cardiac death). 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.

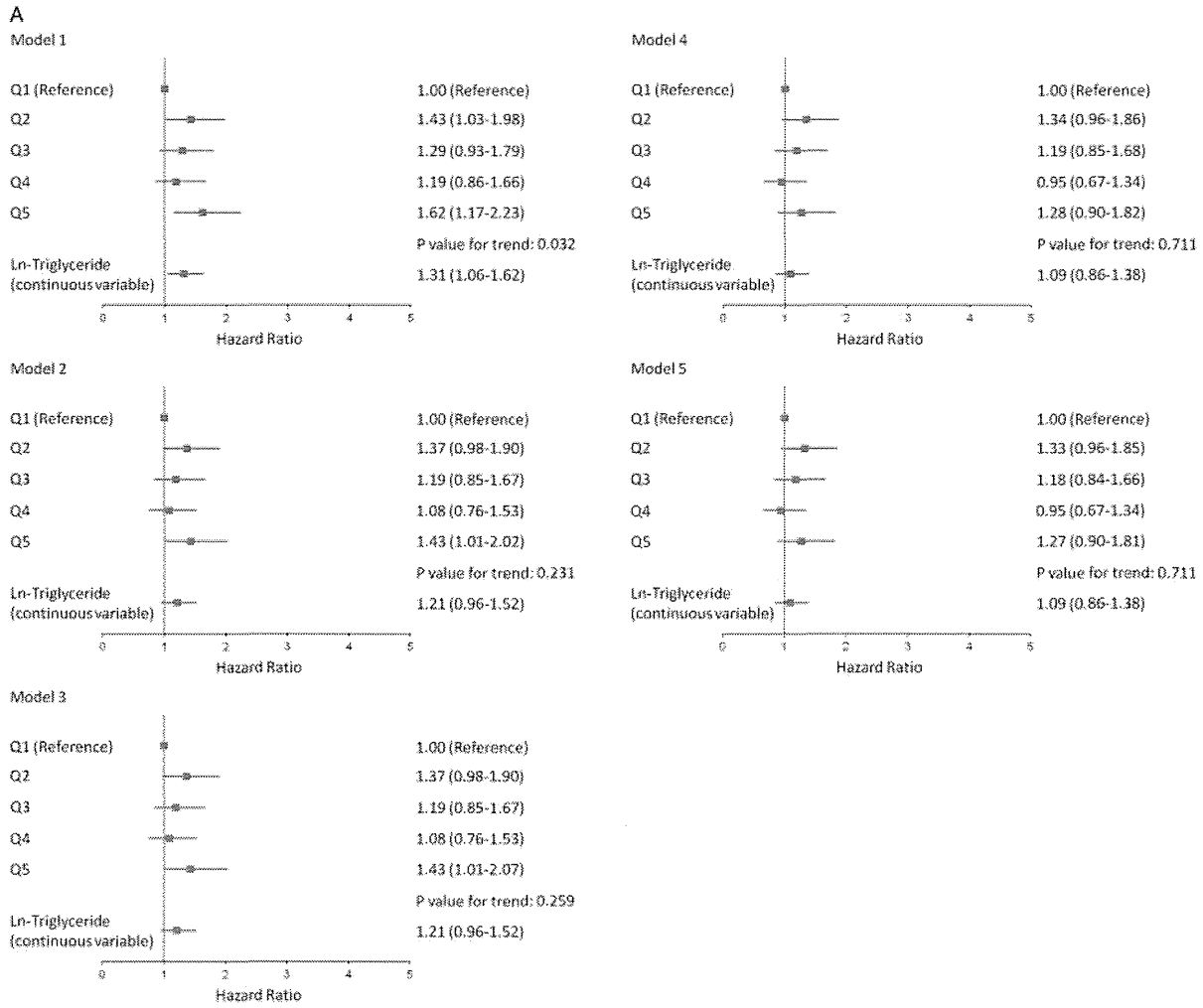

Figure 2 HR for mortality according to the quintiles of triglyceride levels: (A) all-cause deaths; (B) cardiac deaths. Model 1 adjusted for age and gender; Model 2 adjusted for age, gender, total and HDL cholesterols; Model 3 adjusted for age, gender, non-high-density lipoprotein (HDL) and HDL cholesterol; 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]

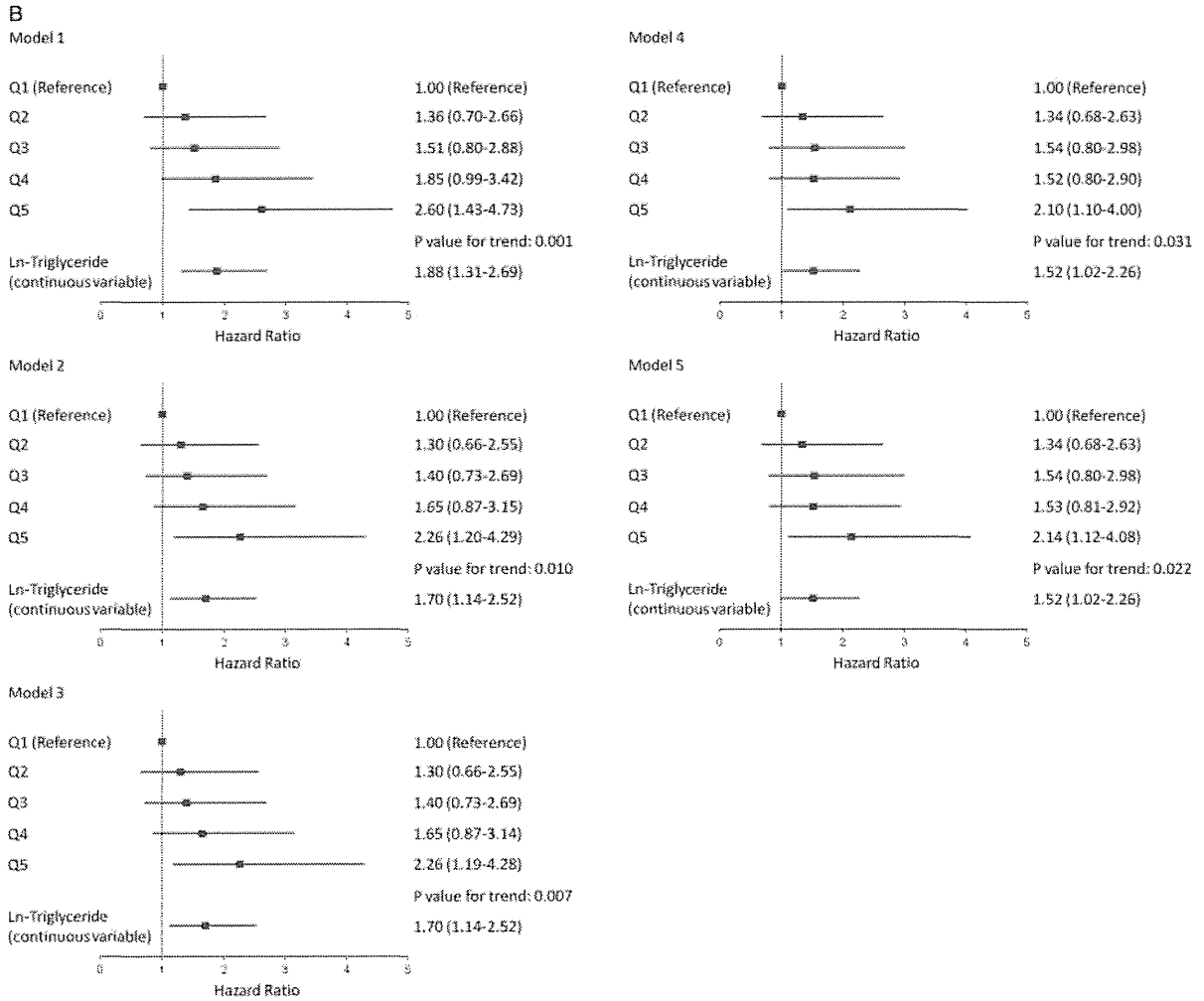

Figure 2 Continued.

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,

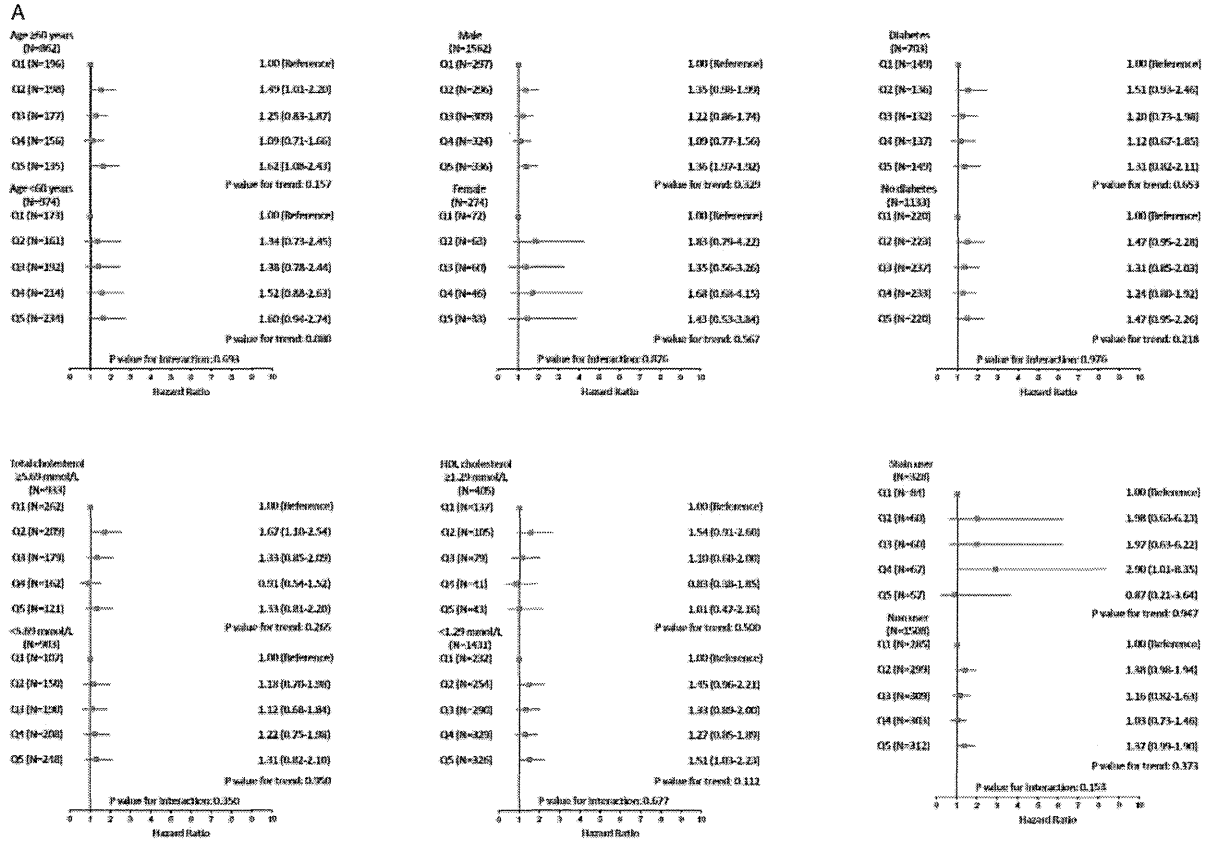

Figure 3 Subgroup analyses of (A) all-cause deaths and (B) cardiac deaths. HDL, high-density lipoprotein. This figure is only reproduced in colour in the online version.

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

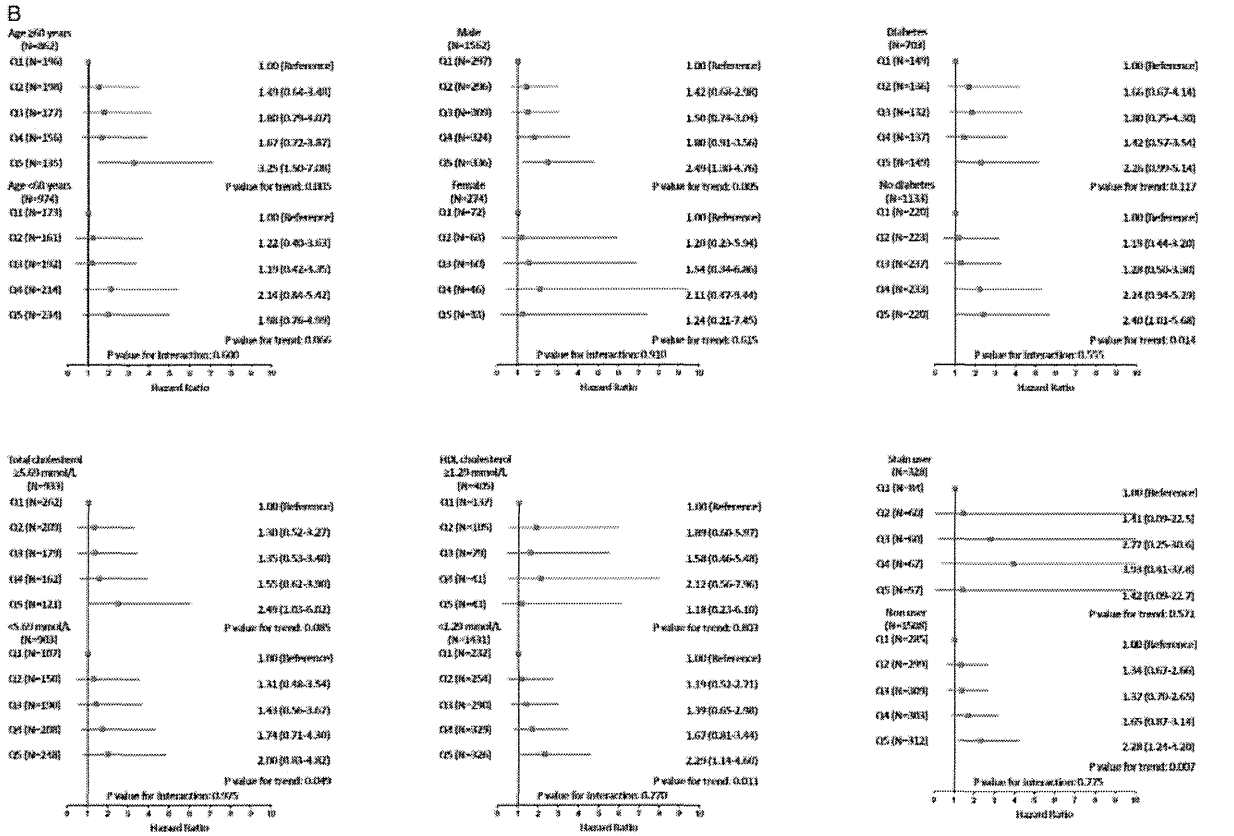

Figure 3 Continued.

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.

Provenance and peer review Not commissioned; externally peer reviewed.

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