Table 1
Patients' characteristics.

Baseline characteristics	
Age (y)	63.2 ± 13.6
Male/female	39/13
BMI	24.7 ± 3.4
Systolic blood pressure (mmHg)	139.4 ± 26.1
Diastolic blood pressure (mmHg)	79.8 ± 17.5
History	
Diabetes mellitus, *n* (%)	17 (32.7)
Current smoker, *n* (%)	30 (57.7)
Dyslipidemia, *n* (%)	34 (65.4)
Hypertension, *n* (%)	32 (61.5)
Medications	
Aspirin, *n* (%)	52 (100)
Clopidogrel, *n* (%)	52 (100)
β-Blocker, *n* (%)	5 (9.6)
ACEI/ARB, *n* (%)	12 (23.1)
Statin, *n* (%)	13 (25.0)
Type of ACS	
STEMI, *n* (%)	34 (65.4)
NSTEMI/UAP, *n* (%)	18 (34.6)
Angiographic results and biological findings	
Culprit coronary artery	
Left anterior descending artery, *n* (%)	28 (53.8)
Left circumflex artery, *n* (%)	7 (13.5)
Right coronary artery, *n* (%)	17 (32.7)
Laboratory findings	
LDL-C (mg/dL)	135.9 ± 32.9
HDL-C (mg/dL)	46.3 ± 11.6
Triglyceride (mg/dL)	140.8 ± 99.4
Creatinine (mg/mL)	0.74 (0.56–0.90)
Troponin T (pg/mL)	1471 (427–6725)
Platelets ($\times 10^4/\mu L$)	21.9 (18.8–27.0)

To distinguish differences from systemic alterations in circulating biomarker levels, we calculated various concentrations of biomarkers by subtracting levels of individual biomarkers in PB from those in CB after logarithmic transformation. Table 2 shows differences in LPA and sCD40L concentrations. However, only the difference in LPA concentration was statistically significant ($p < 0.01$).

Table 2
Differences in marker values between coronary and peripheral blood after logarithmic transformation.

Variable	Coronary	Peripheral	Difference (C − P)	*p*
LPA (μM)				
Median	0.266	0.230	0.06	
LPA values after logarithmic transformation				
Mean	−1.41	−1.50	0.10	0.008
SD	0.413	0.384	0.242	
95% CI	−1.52 to −1.29	−1.61 to −1.40	0.030 to 0.164	
sCD40L (pg/mL)				
Median	2055	1410	308	
sCD40L values after logarithmic transformation				
Mean	7.49	7.33	0.13	0.276
SD	0.788	0.689	0.719	
95% CI	7.274–7.713	7.130–7.525	−0.076–0.337	

CI, confidence interval; LPA, lysophosphatidic acid; SD, standard deviation; sCD40L, soluble CD 40 ligand.

3.3. Comparison of clinical presentations among patients

Systemic circulation levels of plasma LPA did not significantly differ between males and females (median: 0.230 vs. 0.220 μM, $p = 0.716$), or between those with and without conventional risk factors such as diabetes (median: 0.190 vs. 0.230 μM, $p = 0.722$), current smoking (median: 0.210 vs. 0.235 μM, $p = 0.623$), dyslipidemia (median: 0.205 vs. 0.230 μM, $p = 0.492$) or hypertension (median: 0.205 vs. 0.230 μM, $p = 0.631$). Moreover, LPA levels did not significantly differ between patients with STEMI and those with non-STEMI and unstable angina pectoris (median: 0.230 vs. 0.210 μM, $p = 0.512$) (Fig. 2).

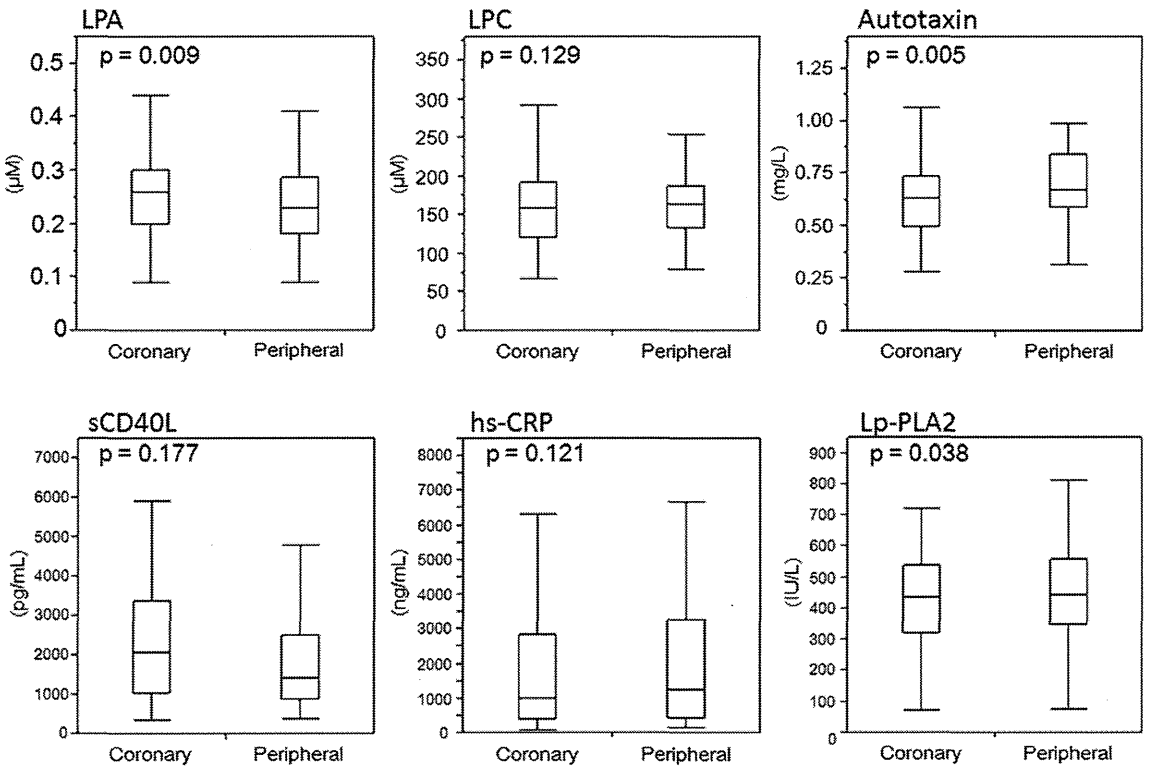

Fig. 1. Changes in markers in coronary and peripheral blood. Only median LPA levels were significantly higher in culprit coronary, than in peripheral blood (0.266 [IQR, 0.192–0.300] vs. 0.230 [IQR, 0.180–287] μM, $p = 0.009$).

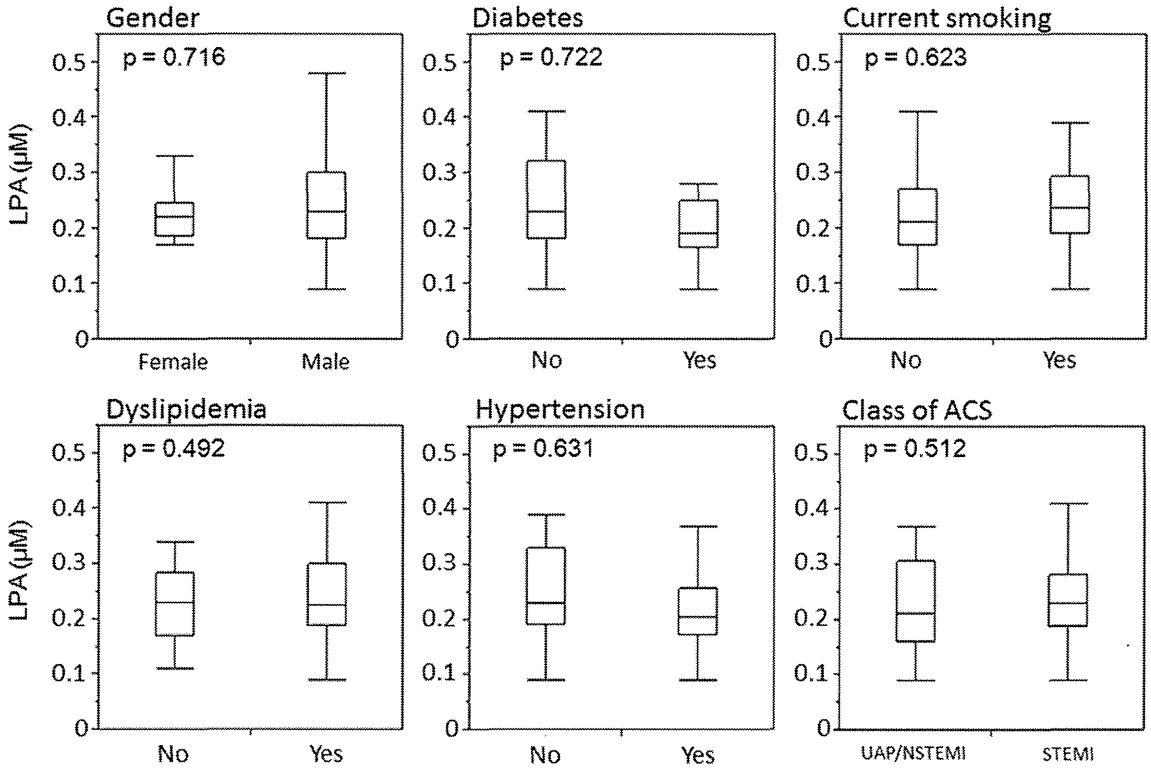

Fig. 2. Comparison of various clinical presentations with LPA levels ($n = 52$). Levels of LPA did not significantly differ according to gender (female, $n = 13$; male, $n = 39$), diabetes ($n = 17$), smoking ($n = 30$), dyslipidemia ($n = 34$), hypertension ($n = 32$) and class of ACS (STEMI, $n = 34$).

3.4. Relationships between LPA values and other markers

Table 3 shows correlations between LPA levels and other markers. Levels of LPA were analyzed along with other markers in CB and in PB. Levels of LPA were positively and significantly associated with those of LPC in both CB and PB (both $p < 0.001$), with serum levels of ATX in CB ($p = 0.009$) and with serum levels of sCD40L in CB and PB (both $p < 0.05$).

4. Discussion

We demonstrated that plasma LPA levels were significantly higher in coronary blood sampled from the culprit artery of ACS compared with peripheral blood (systemic arterial circulation). On the other hand, plasma LPC and serum autotaxin levels were significantly higher in the systemic, than in the coronary circulation. We therefore believe that these findings indicate that higher LPA concentrations are involved in the pathophysiology of ACS, which is based on unstable ruptured coronary plaque.

In general, LPA levels will easily increase if platelets are not completely inhibited. Although the absolute plasma LPA values were lower than those described by others, our LPA values might be higher than those in vivo. Indeed, LPA values in the present study were higher than those of our previous reports [15,18]. However our present and previous methods of measurement did not differ except for the omission of citrate-theophylline-adenosine-dipyridamole. In addition, we realize that ATX is the main source of increasing LPA levels in vitro. However, LPA levels did not change simply according to ATX in our present and previous studies [11]. We also speculate based on molecular species data that mechanisms other than an association with ATX and platelet activation can increase LPA levels in ACS. Regardless, we recognize that the possibility of increased LPA levels in vitro cannot be completely denied. However, we collected all blood samples under the same conditions. Therefore, we believe that we could compare coronary and peripheral samples, and that our conclusions can remain unaltered.

Table 3
Correlations between LPA and other variables in coronary and peripheral circulation.

	Coronary blood		Peripheral blood	
	r	*p*	*r*	*p*
LPC (μM)	0.632	<0.0001	0.465	< 0.0001
Autotaxin (mg/L)	0.354	0.009	0.136	0.343
sCD40L (pg/mL)	0.406	0.002	0.328	0.022
Hs-CRP (ng/mL)	−0.301	0.025	−0.337	0.016
Lp-PLA2 (IU/L)	0.064	0.656	0.052	0.716

Correlations searched using Spearman's rank correlation.

We previously reported that plasma LPA concentrations closely correlate with LPC, autotaxin and LDL-C, findings that are consistent with those of recent studies of LPA production mechanisms [2,3]. However, these studies could not define the source and details of local LPA production mechanisms in patients with ACS, or define LPA as an acute phase reactant and biomarker. Experimental studies have indicated an indirect primary role of platelets in LPA production, which might involve the localized generation of LPC as a substrate for LPA production via the lysophospholipase D activity of ATX [6,20–22]. Indeed, levels of LPA significantly correlated not only with LPC in both the coronary and systemic circulation, but also with those of ATX in the present study, and the correlation was closer with LPC than with ATX. We thus considered that LPC, which is closely associated with complex plaque composition, could be a major source of LPA production [23]. We also believe that the following can account for higher LPA levels when considering the above evidence. Plaques after rupture release either LPA into the

bloodstream that activates platelets, or LPC that is converted to LPA by plasma ATX; conversely, LPC produced by activated platelets may be converted to LPA by ATX bound to integrins on activated platelets [21,22]. In addition, the present finding that LPA levels are increased in the coronary artery, whereas ATX levels are not is notable.

Endothelial dysfunction is caused by LPA via a mechanism associated with decreased endothelial nitric oxide synthase expression and increased oxidative stress [24]. Zhou et al. recently reported that endothelial cells can release ATX and generate LPA locally [25]. We therefore speculate that higher LPA levels in a coronary artery are associated with endothelial dysfunction. We also consider that lipid phosphate phosphatase does not completely digest LPA in the culprit coronary artery of ACS because of damaged endothelial cells. Therefore, LPA levels might be significantly higher in sites of localized endothelial dysfunction such as plaque rupture than in peripheral arteries with more normal endothelial function. In addition, a local increase of LPA at the ruptured vessel wall might contribute to plaque healing or restenosis by recruiting circulating SMC progenitor cells [26].

With respect to the clinical pathophysiology of ACS, we believe that exposure to LPA in the lipid rich core or LPA production at sites of platelet activation also plays key roles in triggering or potentiating platelet responses after plaque rupture or erosion during the acute phase of ACS. Lysophosphatidic acid stimulates platelet–monocyte aggregation in whole blood [27]. Platelet aggregation induced by components of an atherosclerotic plaque lipid-rich core can be inhibited by LPA receptor antagonists, indicating the fact that LPA is a functional component of atherosclerotic plaques that causes platelet aggregation [28]. In addition, recent reports suggest that the selective inhibition of LPA5, which is the functional LPA receptor on platelets, could be an attractive novel target for antithrombotic therapy [29,30].

Soluble CD40L is a dual prothrombotic and proinflammatory member of the tumor necrosis factor superfamily [31]. As circulating sCD40L is mainly derived from activated platelets, its association with atherothrombosis could be primarily due to its ability to reflect platelet activation [31]. However, we measured sCD40L levels in serum samples. Although we recognize that these values did not completely reflect all sCD40L in the circulation, we found that only plasma LPA and serum sCD40L levels were higher in CB than in PB. Interactions between CD40 and its immunomodulating ligand (CD40L) that is expressed in various cell types including platelets and vascular wall cells are actively involved in atherogenetic and thrombotic mechanisms [32]. Furthermore, evidence suggests that CD40/CD40L interactions induce the expression of matrix metalloproteinases that degrade interstitial collagen and the thin fibrous cap of atheromatous plaques, leading to plaque instability and rupture [33]. In addition to the present findings, others have demonstrated that intracoronary sCD40L levels are higher in culprit CB than in PB [34,35]. Based on these factors, we consider that higher coronary sCD40L levels could be explained by increased platelet activation and inflammation in ruptured culprit coronary vessels. Therefore, further studies of plasma sCD40L levels are necessary to define differences in circulation levels and associations with LPA.

5. Limitations

Several limitations are associated with the present study. Firstly, circulating LPA levels might be modified by heparin and by antiplatelet agents such as aspirin and clopidogrel. However, all of our patients received similar standard anti-platelet therapy and heparin. Therefore, our findings still increase understanding of the clinical pathophysiology of ACS. Secondly, vascular damage due to coronary intervention, which might affect levels of LPA and other biomarkers, would vary depending on lesion characteristics, operators, guide wire manipulation and other factors. Although we could not rule out all confounding factors including these, we compared levels of biomarkers within a single population. Thirdly, blood handling might affect LPA values. Samples were placed on ice as soon as possible after collection and immediately separated by centrifugation to remove artifacts in vitro. However, artifacts might not have been completely eliminated.

6. Conclusions

We found higher LPA levels in blood at sites in culprit coronary arteries than in the peripheral circulation of patients with ACS. Furthermore, levels of LPA and LPC significantly correlated in the coronary, and systemic circulation. A higher LPA concentration might be associated with the pathophysiology of ACS.

Disclosures

None.

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Association between Myocardial Triglyceride Content and Cardiac Function in Healthy Subjects and Endurance Athletes

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Abstract

Ectopic fat accumulation plays important roles in various metabolic disorders and cardiovascular diseases. Recent studies reported that myocardial triglyceride (TG) content measured by proton magnetic resonance spectroscopy (^{1}H-MRS) is associated with aging, diabetes mellitus, and cardiac dysfunction. However, myocardial TG content in athletes has not yet been investigated. We performed ^{1}H-MRS and cardiac magnetic resonance imaging in 10 male endurance athletes and 15 healthy male controls. Serum markers and other clinical parameters including arterial stiffness were measured. Cardiopulmonary exercise testing was also performed. There were no significant differences in clinical characteristics including age, anthropometric parameters, blood test results, or arterial stiffness between the two groups. Peak oxygen uptakes, end-diastolic volume (EDV), end-systolic volume (ESV), left ventricular (LV) mass, peak ejection rates and peak filling rates were significantly higher in the athlete group than in the control group (all $P<0.02$). Myocardial TG content was significantly lower in the athlete group than in the control group (0.60 ± 0.20 vs. $0.89\pm0.41\%$, $P<0.05$). Myocardial TG content was negatively correlated with EDV ($r=-0.47$), ESV ($r=-0.64$), LV mass ($r=-0.44$), and epicardial fat volume ($r=0.47$) (all $P<0.05$). In conclusion, lower levels of myocardial TG content were observed in endurance athletes and were associated with morphological changes related to physiological LV alteration in athletes, suggesting that metabolic imaging for measurement of myocardial TG content by ^{1}H-MRS may be a useful technique for noninvasively assessing the "athlete's heart".

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Introduction

Ectopic fat accumulation is associated with various metabolic disorders and cardiovascular diseases [1–3]. Previous animal studies have shown that myocardial triglyceride (TG) accumulation triggers pathological changes, including myocardial apoptosis and ventricular systolic dysfunction [4,5]. However, the assessment of myocardial TG content is hampered by the difficulty of obtaining myocardial tissues in a clinical setting.

Recent studies have demonstrated that proton magnetic resonance spectroscopy (^{1}H-MRS) enables the noninvasive monitoring of TG accumulation in human myocardial tissue. Indeed, myocardial TG content, as measured by ^{1}H-MRS, has been associated with aging [6], diabetes mellitus [7], myocardial systolic dysfunction [4,8,9], and diastolic dysfunction [6,10]. In addition, caloric restriction induced a dose-dependent increase in myocardial TG content [11], whereas endurance training reduced myocardial TG content [12]. However, myocardial TG content in endurance athletes has not yet been investigated.

The purpose of this study is to evaluate the associations between myocardial TG content, cardiac morphology and left ventricular (LV) function assessed by ^{1}H-MRS and magnetic resonance imaging (MRI) in healthy subjects and endurance athletes.

Methods

Subjects

Fifteen healthy male subjects and 10 male endurance athletes were recruited by advertisements in a local area. All subjects were non-obese, aged 20–40 years, and without acute or chronic disease. Subjects receiving medical treatment, current smokers, and those with abnormal laboratory parameters were excluded. We defined an endurance athlete as a person who performed endurance training for more than 5 days a week, and was affiliated with a specific athletic association to participate in competitive sports such as cycling, track, or swimming. The international physical activity questionnaire (IPAQ) was used to assess each subject's activity level [13]. All protocols were approved by the ethical committee of the Juntendo University, and all participants

provided written informed consent before their participation in this study according to the guidelines established in the Declaration of Helsinki.

Measurements of Body Composition

Skeletal muscle mass and body fat weight were measured after overnight fasting by multi-frequency bioelectrical impedance analysis using eight tactile electrodes (MF-BIA8; In-Body 720, Biospace, Korea) [14] after overnight fasting. The subject stood on the footplate with barefoot and held the electrodes in both hands. This process takes 2 min, and measurement requires no specific skills. The apparatus then automatically displays measurements of fat-free mass, fat mass, and percentage body fat.

Blood Measurements

Standard laboratory tests including blood cell counts, fasting plasma glucose, lipids, creatinine, free fatty acid, and N-terminal pro-brain natriuretic peptide (NT-proBNP) were performed immediately before MRS after overnight fasting. Serum lipid profiles were measured using specific assays for total cholesterol (Symex Co, Kobe, Japan), triglyceride (Sekisui Medical, Tokyo, Japan), and high-density lipoprotein cholsterol (Sekisui Medical, Tokyo, Japan) by BioMajesty JCA-BM8060 analyzer (Japan Electron Optics Laboratory Ltd, Tokyo, Japan). Serum low-density lipoprotein cholesterol levels were calculated using the Friedewald's formula. Serum insulin was measured by chemiluminescent enzyme immunoassay using the Lumipulse presto II analyzer (Fujirebio Inc, Tokyo, Japan). A homeostasis model assessment index (HOMA-IR) was calculated to estimate insulin resistance from fasting insulin and glucose concentrations: insulin (μU/ml)×glucose (mmol/l)/22.5. Free fatty acid (FFA) was measured a standard enzymatic assay (Eiken chemical Co. Ltd, Tokyo, Japan) by BioMajesty JCA-BM2250 analyzer (Japan Electron Optics Laboratory Ltd, Tokyo, Japan). Serum NT-proBNP was determined using an electrostatic controlled linear inchworm actuator on Hitachi modular analytics (HITACHI Hi-Technologies Co. Ltd. Tokyo, Japan). HbA1c concentrations were measured in whole blood samples using latex-enhanced immunoassay (Fujirebio Co. Ltd. Tokyo, Japan).

MRI and MRS

All cardiac MRI and ^{1}H-MRS studies were performed using a MAGNETOM Avanto 1.5-Tesla MRI system (Siemens Medical Solution, Erlangen, Germany) with subjects resting in the supine position. To minimize the influence of breathing, a towel was strapped around the subject's upper abdomen. Dynamic cine images were used to determine LV mass, and LV functional parameters. Image analysis was performed using special evaluation software (Argus; Siemens Medical Systems, Erlangen, Germany) [15,16] on a separate work station. Endocardial and epicardial LV borders were traced manually at end-diastole and end-systole from short-axis cine images. End-diastolic volume (EDV), end-systolic volume (ESV), stroke volume, and ejection fraction were calculated by Simpson's method. In addition, the peak LV ejection and filling rates were automatically derived on the basis of LV volume-time curves. The area of epicardial fat was traced on consecutive end diastolic short axis images, beginning with the most basal slice at the level of the mitral valve, and moving apically through the stack until the most inferior margin of adipose tissue, as reported previously [17].

After the cine MRI imaging, myocardial TG content was determined by ^{1}H-MRS. A volume of interest (VOI = 2.0 cm^3 − 10×10×20 mm) was selected within the ventricular septum from cine dynamic cine-mode images of the heart (Figure 1). We adjusted the VOI size to the anatomy of the ventricular septum. The spectrum of water and lipid was acquired by point-resolved spectroscopy (PRESS) method using an echo time (TE) of 30 ms, and repetition time (TR) of at least 4,000 ms, myocardial TG signals were acquired at 1.4 ppm from spectra with water suppression, and water signals were acquired at 4.7 ppm from spectra without water suppression (Figure 1). Areas under the curves for water and lipid peaks were quantified using standard line-fitting procedures (Siemens Syngo Spectroscopy). Myocardial TG level was expressed as a ratio of lipid to water (%). Thus, ^{1}H-MRS evaluation of myocardial TG content was performed essentially as has been previously validated [18–21].

Measurement of Cardiopulmonary Fitness

All subjects underwent an incremental cycling test (Corival 400, Lobe B.V., Groningen, Netherlands) using an expiratory gas analyzer (Vmax-295, sensorMedics Co., Yorba Linda, CA, USA) to measure anabolic threshold (AT) and maximal oxygen consumption (VO_{2max}). After a 3-min rest period, a warm-up was performed for 3 minutes at 40 W, followed by ramp loading (15–30 W/min) until the subjective exhaustion, as described previously [22]. According to the ATS/ACCP guidelines, AT was determined by V-slope method. In cases when AT was not identified on the V-slope, we used the point at which V_E/VO_2 starts to increase while V_E/VCO_2 remains constant [23].

Evaluation of Atherosclerotic Parameters

The cardio ankle vascular index (CAVI) was measured as atherosclerotic parameters. CAVI was automatically calculated by VaSera VS-1500AN (Fukuda Denshi Co. Ltd., Tokyo, Japan) [24,25].

Statistical Analyses

Values are expressed as mean ± standard deviation (SD). For variables that did not show a normal distribution, the data were transformed into natural logarithmic values before statistical analyses. Correlations were calculated using Pearson's correlation co-efficient. Unpaired Student's *t*-test was used to compare groups. All statistical analyses were performed with SPSS version 20 (SPSS, Inc). A P value of less than 0.05 was considered significant.

Results

The clinical characteristics of study subjects are summarized in Table 1. There were no significant differences, in age, body composition, lipids, glucose, insulin levels, or NT-proBNP between the two groups. The levels of AT (29.2±6.6 ml/kg/min vs. 19.0±5.2 ml/kg/min, P = 0.0002), VO_{2max} (52.3±6.2 ml/kg/min vs. 43.2±8.0 ml/kg/min, P = 0.0057) and international physical activity questionnaire (IPAQ) score (2318±1605 vs. 5310±2869, P = 0.0048) were significantly higher in the athlete groups than in the control group.

MRI and MRS variables are shown in Table 2. The values of EDV (182±24 ml vs. 153±16 ml, P = 0.0011), ESV (96±16 ml vs. 73±8 ml, P = 0.0002), and LV mass (139±16 g vs. 120±13 g, P = 0.0034), were significantly higher in the athlete group than in the control group. Peak ejection rate (777±230 ml/sec vs. 551±206 ml/sec, P = 0.019) and peak filling rate (839±250 ml/sec vs. 619±177 ml/sec, P = 0.018) were significantly higher in the athlete group than in the control group. None of the subjects had an abnormal peak ejection or filling rate. Myocardial TG content was significantly lower in the athlete group than in the control group (0.60±0.20% vs. 0.89±0.41%, P = 0.045) (Figure 2).

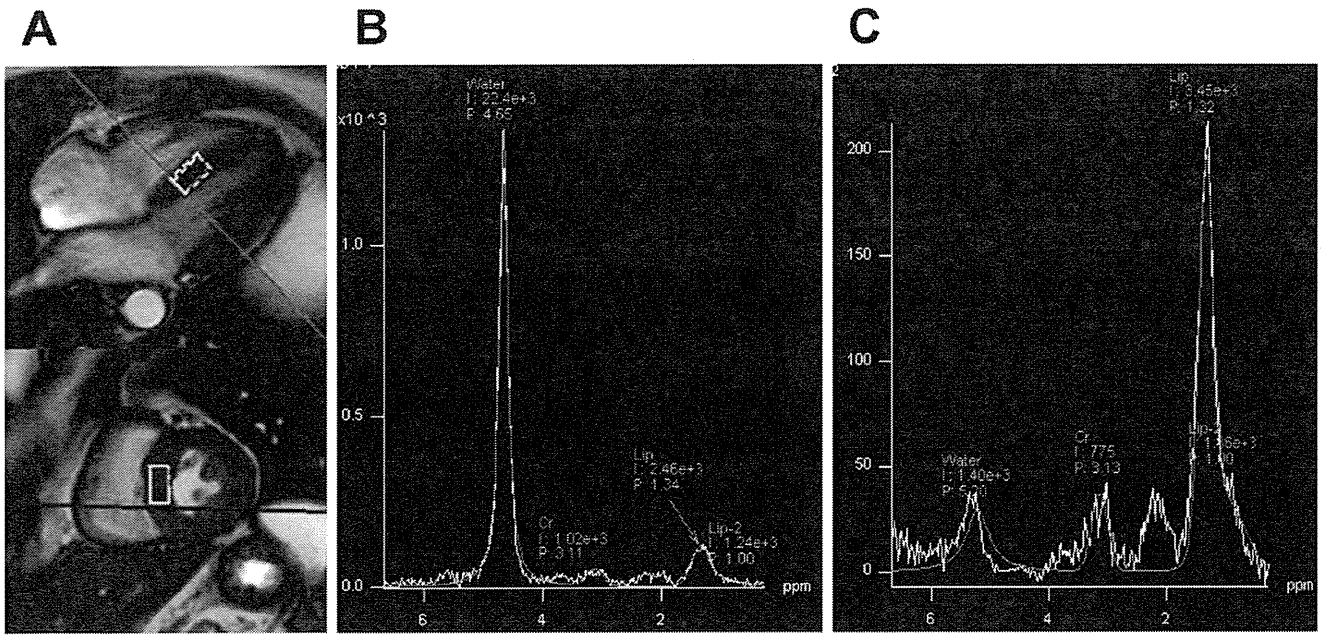

Figure 1. Representative results of H^1-MR spectra in a healthy subject. A: Myocardial voxel localization for H^1-MRS in 4-chamber and short axis views. B: H^1-MR spectra without water suppression. C: H^1-MR spectra without water suppression.
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Myocardial TG content was negatively correlated with EDV ($r=-0.47$, $P=0.018$), ESV ($r=-0.64$, $P=0.001$), LV mass volume ($r=-0.43$, $P=0.031$), and epicardial fat volume ($r=0.47$, $P=0.025$) (Figure 3). Although a significant correlation between myocardial TG content and VO_{2max} was not found ($r=-0.15$, $P=0.46$), epicardial fat volume was negatively correlated with EDV, a LV morphological parameter ($r=-0.44$, $P=0.022$).

Discussion

The present study demonstrated that myocardial TG content was significantly lower in the endurance athlete group than in the control group and that myocardial TG content was significantly correlated with EDV, ESV, LV mass, and epicardial fat volume. This study is, to the best our knowledge, the first report to demonstrate an association between TG content and physiological LV alteration in endurance athletes.

Much attention has been focused on the associations between ectopic fat accumulation, various metabolic disorders and cardiovascular diseases [1,2]. It has been reported that the myocardial TG content is associated with metabolic disorders [7,26]. The positive correlation between myocardial TG content and LV mass has also been reported among the diabetic patients as well as in obese individuals with insulin resistance [4,8]. Animal studies have demonstrated that myocardial TG content was associated with not only cardiovascular risk factors, but also with lipotoxicity-induced heart failure and premature death [20,27]. In addition, increased myocardial TG content induced pathological LV hypertrophy, cardiac dysfunction, and non-ischemic dilated cardiomyopathy [28]. However, the present study showed negative correlations between myocardial TG and LV mass as well as LV function. Several studies suggested that mitochondrial dysfunction in the myocardium exists in patients with diabetes and insulin resistance [29]. In contrast, the functional capacity of mitochondria in athlete's heart was reported to be increased by endurance training [30]. This difference in mitochondrial function may underlie the difference in myocardial TG content between the physiological modifications present in athlete's heart and the pathological changes that characterize the deteriorating heart in patients with diabetes and insulin resistance.

Previous studies reported the relationship between exercise and lipid content in skeletal muscle. High levels of intra-myocellular lipid (IMCL) were reported in the skeletal muscles of patients with diabetes mellitus [31] and elderly subjects [32]. On the other hand, it has also been reported that similar high levels of IMCL occur in skeletal muscles of athletes, despite the marked insulin sensitivity and the high oxidative capacity of these muscles, this is the so-called "athlete's paradox" [33]. Increases in IMCL content provide a substrate for energy metabolism during exercise [34]. A high availability of fatty acids is needed to augment TG resynthesis in skeletal muscle during and after exercise [34]. Diacylglycerol and/or ceramide, but not TG, may be directly associated with the development of insulin resistance [35,36]. In the present study, no "athlete's paradox" was observed in the subjects' cardiac muscles. Several potential reasons have been raised. One possibility is the difference in mitochondrial function with regard to fatty acid metabolism between skeletal muscle and cardiac muscle. Fatty acid metabolism may be more efficient in cardiac muscles, which has more abundant mitochondria than in skeletal muscles [37]. Another reason relates to the differences in regulation of fatty acid β-oxidation between the two types of muscle. To sustain contractile function in the heart requires a greater energy supply [38]. Therefore, the fatty acid β-oxidation system in cardiac muscle is very dynamic and sufficient to meet the energy demands of the heart. Alterations in lipoprotein lipase (LPL) synthesis as well as the activation, secretion, transportation, capillary luminal binding, and the degradation of fats in cardiac myocytes, contribute to myocardial fatty acid supply, uptake and fatty acid β-oxidation [38]. In addition, the heart muscle is reported to be less susceptible to developing insulin resistance than skeletal

Table 1. Clinical Characteristics.

	Control group (n = 15)	Athlete group (n = 10)	P value
Age, years	28.8±4.5	26.4±4.4	0.20
Body height, m	1.735±0.051	1.732±0.047	0.88
Body weight, kg	67.9±7.4	67.8±4.2	0.94
Body mass index, kg/m^2	22.5±1.9	22.6±1.9	0.90
Skeletal muscle mass, kg	30.7±2.6	32.5±2.0	0.083
Body fat weight, kg	13.6±3.8	10.6±3.6	0.066
Percent of body fat, %	18.6±5.0	15.4±4.8	0.14
Neck circumference, cm	36.9±2.4	36.8±1.8	0.92
Waist circumference, cm	80.5±6.8	78.1±4.0	0.36
Total cholesterol, mg/dl	174.6±26.3	182.5±24.5	0.45
Triglyceride, mg/dl	74.6±27.0	61.1±15.8	0.16
LDL-cholesterol, mg/dl	104.2±26.4	111.1±29.0	0.53
HDL-cholesterol, mg/dl	55.7±11.3	59.2±12.7	0.47
Fasting free fatty acid, μEq/L	299.1±132.3	364.7±211.5	0.32
Fasting blood glucose, mg/dl	90.7±8.6	90.9±5.0	0.93
Insulin, μU/ml	5.6±3.0	4.4±1.4	0.22
HOMA-IR	1.3±0.6	1.0±0.3	0.22
HbA1c, %	4.7±0.3	4.7±0.2	0.51
Creatinine, mg/dl	0.84±0.10	0.84±0.05	0.85
eGFR, ml/min/m^2	91.6±12.2	92.1±6.7	0.91
NT-proBNP, ng/l	18.6±18.0	10.1±3.9	0.15
Urinary acid, mg/l	6.0±0.9	5.4±1.3	0.15
Anaerobic threshold, ml/kg/min	19.0±5.2	29.2±6.6	0.0002
VO_2max, ml/kg/min	43.2±8.0	52.3±6.2	0.0057
CAVI	6.5±0.7	6.2±0.6	0.53
IPAQ score	2318±1605	5310±2869	0.0048

Values are mean ± SD. bpm = beats per minutes, LDL = low-density lipoprotein; HDL = high-density lipoprotein; eGFR = estimated glomerular filtration rate; HOMA-IR = homeostasis model assessment of insulin resistance, NT-proBNP = N-terminal pro brain natriuretic peptides, VO_2max = maximal oxygen intake, CAVI = cardio ankle vascular index, IPAQ = international physical activity questionnaire.
P value denotes significance of unpaired *t* test between athlete group and healthy control.
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muscle [39]. Therefore, insulin responsiveness and its consequences in the heart may be relatively high in endurance athletes.

A recent study has shown that acute endurance exercise leads to increased myocardial TG content depending on elevated plasma free fatty acid concentrations and the uptake of free acids in the heart. The mechanism is considered to be related to the increased availability of fatty acid during exercise in fasting healthy males [40]. The level of circulating free fatty acids concentration was low in the present study. Thus, fatty acid availability must be relatively low in these individuals. Indeed, myocardial TG content was not reported to change even after exercise in subjects with a suppressed state of free fatty acid synthesis [40]. In addition, endurance training regulates the activity of LPL [41], which provides the major source of free fatty acids derived from TG content lipoproteins. Endurance athletes manifesting physiological LV adaptations may be augmented to drive alterations in fatty acid metabolism on fasting state.

Table 2. MRI variables.

	Control group (n = 15)	Athlete group (n = 10)	P value
LV ejection fraction, %	50.6±5.5	48.1±6.3	0.32
LV end diastolic volume, ml	153±16	182±24	0.0011
LV end systolic volume, ml	73±8	95±16	0.0002
Stroke volume, ml	80±14	88±17	0.22
Cardiac output	4.8±0.8	5.2±1.2	0.29
LV myocardial mass, g	120±13	139±16	0.0034
Peak ejection rate, ml/sec	551±206	777±230	0.019
Peak filling rate, ml/sec	619±177	839±250	0.018
Epicardial fat volume, ml	48.8±14.8	38.3±8.2	0.057

Values are mean ± SD. LV = left ventricular.
P value denotes significance of unpaired *t* test between athlete group and healthy control.
doi:10.1371/journal.pone.0061604.t002

We measured several TG-associated enzymes and proteins, including adiponectin, pre-heparin LPL, apolipoprotein (apo) CII, and apo CIII. No significant difference was observed between the two groups for each parameter (data not shown). One of the major reasons, why these enzyme and proteins were not significantly different, is supposed to the study subjects consisting with healthy lean young men without any metabolic disorder. Myocardial lipid metabolism is regulated by a complex balance between fatty acid supply to the heart, competing energy substrates, energy demand and oxygen supply to the heart, uptake and esterification of fatty acid, and control of mitochondrial functions such as fatty acid oxidation and electron transport chain activity [38]. In addition, epicardial fat, which stores free fatty acid during excessive circulating free fatty acid accumulation and releases fatty acid when energy is needed, is directly connected to the myocardium.

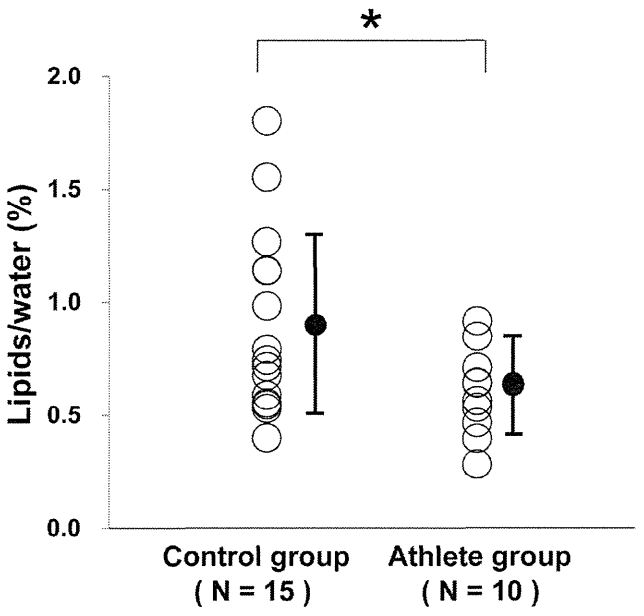

Figure 2. Comparison between myocardial TG content in the control group and the athlete group. * $P<0.05$ between the two groups.
doi:10.1371/journal.pone.0061604.g002

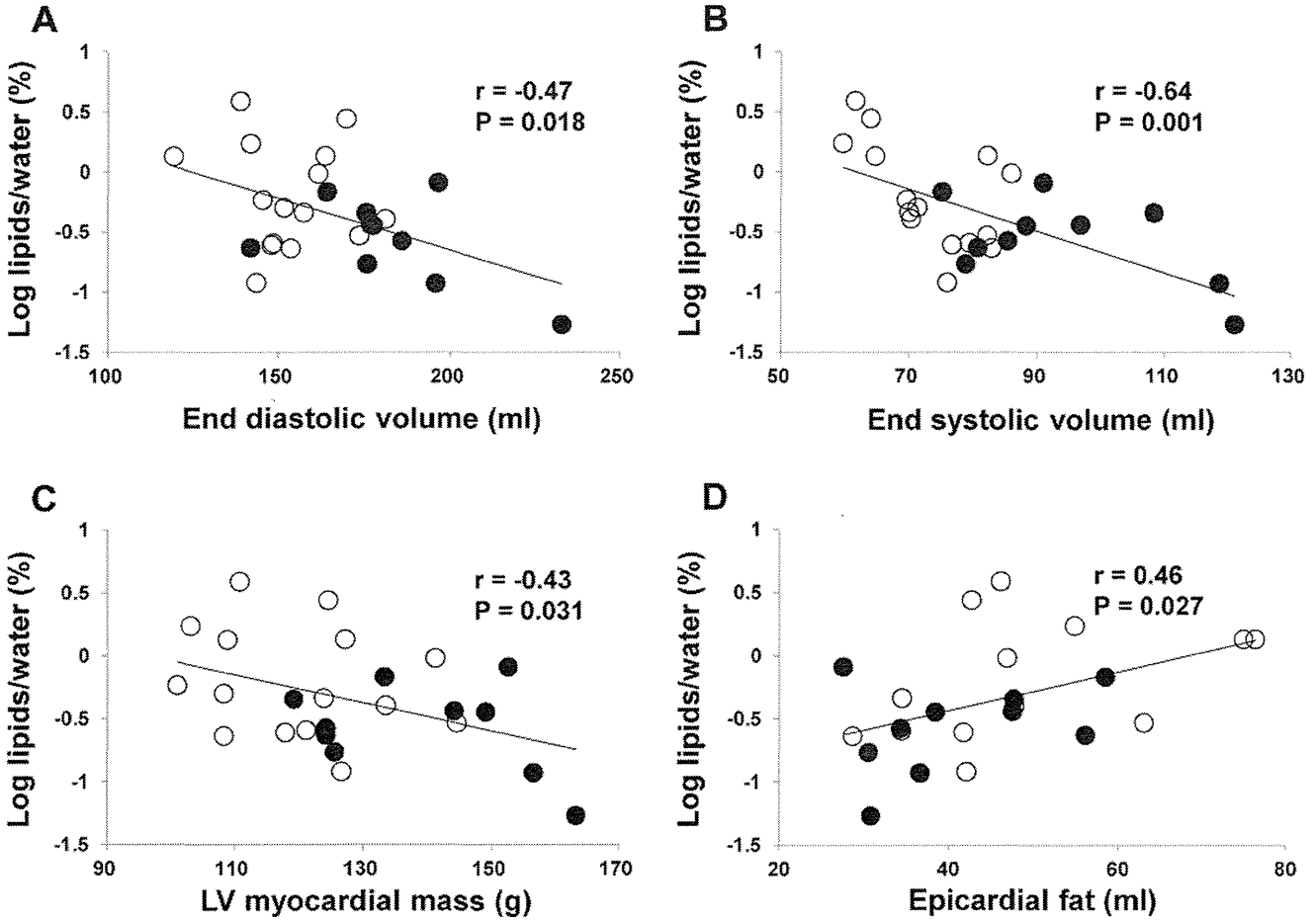

Figure 3. Correlations between myocardial TG content and MRI parameters. A: A correlation between myocardial TG content and end-diastolic volume. B: A correlation between myocardial TG content and end-systolic volume. C: Correlation between myocardial TG content and left ventricular (LV) mass. D: Correlation between myocardial TG content and epicardial fat volume. Open circle; control group. Closed circle; athlete group.
doi:10.1371/journal.pone.0061604.g003

Accordingly, we report a significant positive correlation between epicardial fat volume and myocardial TG content. It has reported that the metabolic rates of lipolysis and lipogenesis are 2-fold higher in epicardial fat than in other fat deposits. Indeed, we detected a negative correlation between epicardial fat volume and EDV, a LV morphology parameter. The precise mechanism underlying the low myocardial TG content in endurance athletes remains elusive. However, the significant positive correlation between epicardial fat volume and myocardial TG content may be related to the increase of utilizing fatty acid in endurance athletes. In our next step, we plan to clarify the impact of exercise on myocardial TG content and LV alterations in endurance athletes.

Limitations

The present study has several limitations. First, this was a single center study with a small sample size, studies of larger sample size are required to confirm these findings. Second, this study included only male subjects. Third, a previous study has demonstrated that a negative relationship between myocardial TG content and cardiopulmonary fitness in obese women [26]. This correlation between myocardial TG content and VO_{2max} was not found in our study. This discrepancy may have resulted from the difference between the subjects in these studies, as in the present study, all subjects of the present study were healthy males without metabolic disorders. Finally, athlete's heart is considered to be reversible [42], therefore, we will next evaluate the effect of detraining on myocardial TG content.

Conclusions

Low levels of myocardial TG content were observed in endurance athletes and were associated with the morphology of physiological LV alteration. These data suggest that metabolic imaging for measurement of myocardial TG content by ^{1}H-MRS may be a useful technique for noninvasively assessing the "athlete's heart".

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Author Contributions

Interpreted results of experiments: ES KS YT SA HW RK HD. Prepared figures: ES KS TM. Approved final version of manuscript: ES KS TY SS TM MH YT SA HW RK HD. Conceived and designed the experiments: ES KS TY. Performed the experiments: ES TY SS TM MH. Analyzed the data: ES KS. Contributed reagents/materials/analysis tools: ES KS TY. Wrote the paper: ES KS TY.

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ORIGINAL ARTICLE

Low high-density lipoprotein cholesterol is a residual risk factor associated with long-term clinical outcomes in diabetic patients with stable coronary artery disease who achieve optimal control of low-density lipoprotein cholesterol

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Abstract Diabetes mellitus is recognized an independent risk factor for coronary artery disease (CAD) and mortality. Clinical trials have shown that statins significantly reduce cardiovascular events in diabetic patients. However, residual cardiovascular risk persists despite the achievement of target low-density lipoprotein cholesterol (LDL-C) levels with statin. High-density lipoprotein cholesterol (HDL-C) is an established coronary risk factor that is independent of LDL-C levels. We evaluated the impact of HDL-C on long-term mortality in diabetic patients with stable CAD who achieved optimal LDL-C. We enrolled 438 consecutive diabetic patients who were scheduled for percutaneous coronary intervention between 2004 and 2007 at our institution. We identified 165 patients who achieved target LDL-C <100 mg/dl. Patients were stratified into two groups according to HDL-C levels (low HDL-C group, baseline HDL-C <40 mg/dl; high HDL-C group, ≥40 mg/dl). Major adverse cardiac events (MACE) that included all-cause death, acute coronary syndrome, and target lesion revascularization were evaluated between the two groups. The median follow-up period was 946 days. The rate of MACE was significantly higher in diabetic patients with low-HDL-C who achieved optimal LDL-C (6.9 vs 17.9 %, log-rank P = 0.030). Multivariate Cox regression analysis showed that HDL-C is significantly associated with clinical outcomes (adjusted hazard ratio for MACE 1.33, 95 % confidence interval 1.01–1.75, P = 0.042). Low HDL-C is a residual risk factor that is significantly associated with long-term clinical outcomes among diabetic patients with stable CAD who achieve optimal LDL-C levels.

Keywords Low HDL-C · Residual risk factor · Diabetes · Coronary intervention

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Introduction

Diabetes is associated with a marked increase in cardiovascular disease [1–3], and coronary artery disease (CAD) is the leading cause of death among diabetic patients [4, 5]. Several previous studies using different modalities demonstrated that diabetic patients had extensive coronary artery calcification, which is an independent predictor of cardiovascular events [6–8], and that diabetes is also associated with adverse cardiovascular events after coronary revascularization [9, 10].

Dyslipidemia in patients with diabetes is common and a strong predictor of cardiovascular risk, and a central strategy of dyslipidemia management is to reduce levels of low-density lipoprotein cholesterol (LDL-C) using statins. Clinical trials have shown a significant reduction in the rate of cardiovascular events among diabetic patients on statins [11, 12]. However, residual cardiovascular risk persists despite achieving of target LDL-C levels. Diabetic patients have mixed dyslipidemia including low levels of high-density lipoprotein cholesterol (HDL-C) levels, which is an established coronary risk factor that is independent of LDL-C levels [13, 14]. We therefore evaluated the impact of HDL-C on the long-term clinical outcome in diabetic patients with stable coronary artery disease (CAD) who achieve optimal LDL-C control.

Patients and methods

Study population and data collection

This single-center, observational historical cohort study analyzes data from 438 consecutive diabetic patients who underwent scheduled percutaneous coronary intervention (PCI) at Juntendo University Hospital between August 2004 (the date of our institution adopted the use of drug-eluting stents (DES)) and December 2007, and 438 consecutive diabetic patients were enrolled in this study. Diabetes mellitus was defined as having at least one of hemoglobin A1c (HbA1c) level ≥6.5 %, or being treated with antidiabetic agents (insulin or oral hypoglycemic drugs). The HbA1c is estimated as a National Glycohemogobin Standardization Program (NGSP) equivalent value (%) calculated by the formula HbA1c(%) = 1.02 × HbA1c (Japan Diabetes Society; %) + 0.25 % [15]. Among the 438 patients with diabetes, we analyzed data from 156 who achieved an optimal LDL-C level <100 mg/dl according to the National Cholesterol Education Program expert panel/Adult Treatment Panel III [16]. Patients with optimal LDL-C control were subdivided into groups according to baseline HDL-C levels of ≥40 mg/dl (high) or <40 mg/dl (low) [17].

Demographic data, coronary risk factors, and medication use were obtained from our institutional database. Blood samples were collected during the early morning after an overnight fast, and blood pressure (BP) was measured at the time of admission. Chronic kidney disease (CKD) was defined as an estimated glomerular filtration rate (eGFR) of <60 ml/min/1.73 m^2, calculated from the modification of diet in renal disease (MDRD) equation modified with a Japanese coefficient using baseline serum creatinine [18]. Patients with BP >140/90 mmHg or taking antihypertensive drugs were regarded as being hypertensive. Metabolic syndrome was defined using the following Japanese-specific criteria [19, 20]: waist circumference (WC) ≥90 cm in women or ≥85 cm in men; triglyceride (TG) ≥150 mg/dl; HDL-C ≤40 mg/dl; BP ≥130/85 mmHg; and fasting blood glucose ≥110 mg/dl. Written informed consent was obtained from all patients before undergoing coronary intervention. This study proceeded in accordance with the Declaration of Helsinki, under approval from our institutional review board.

Primary end point

The primary end point of this study was major adverse cardiac events (MACE) defined as a composite of all-cause death, acute coronary syndrome (ACS), and target lesion revascularization (TLR). Clinical follow-up comprised inspecting clinical visit charts, telephone contact, and questionnaires sent to patients or their families. Mortality data were collected from the medical records of patients who died or who were treated at our institution, and details and causes of death were requested from other hospitals where patients had been admitted. Mortality data were categorized as death from all causes or cardiovascular death including death from CAD, cardiogenic shock, stroke, and sudden death. Acute coronary syndrome was defined as acute myocardial infarction and unstable angina. Acute myocardial infarction was defined as the presence of ischemic symptoms accompanied by a two-fold increase in creatine kinase levels. Unstable angina was diagnosed in the presence of ischemic symptoms regardless of ST-T changes. TLR was defined as repeat revascularization clinically driven by any lesion in a stented segment.

Statistical analysis

Quantitative data are presented as mean ± standard deviation (SD). Patients' characteristics were compared between the low HDL-C group and the high HDL-C group. Continuous variables were compared using an unpaired *t* test or the Mann–Whitney *U* test. Categorical variables (presented as frequencies) were compared using Chi-square statistics or Fisher's exact probability test. Event-free survival rates of MACE were compared between the two groups using Kaplan–Meier curves and the log-rank test. Multivariate Cox regression analysis was applied to determine whether HDL-C is associated with adverse events even after adjusting for confounding factors. Models were initially adjusted for age and gender (Model 1). Second, we adjusted the models for TGs and LDL-C to identify the role of HDL-C independent of other lipid markers in addition to Model 1 (Model 2). Finally, Model 3 was adjusted for variables in Model 2 plus body mass index (BMI), hypertension, metabolic syndrome, current smoking, family history of CAD, prior myocardial infarction, left ventricular ejection fraction (LVEF), multivessel disease, use of DES, use of statin, use of aspirin, and use of angiotensin-converting inhibitors (ACE-I) or angiotensin receptor blockers (ARB). The latter covariates were added in Model 3 only if they were statistically significant predictors of MACE ($P < 0.05$). All variables were simultaneously adjusted in one step. Hazard ratios (HR) and 95 % confidence intervals (CI) were calculated. $P < 0.05$ was considered to indicate statistical significance. All data were analyzed using SPSS version 18.0 for Windows (SPSS, Chicago, IL, USA).

Results

Baseline and procedural characteristics

The baseline clinical characteristics compared between low HDL-C and high HDL-C groups in patients who achieved

optimal LDL-C (<100 mg/dl) are shown in Table 1. Baseline characteristics were similar between the two groups except for lower total cholesterol and HDL-C levels and higher diastolic pressure in the group with low HDL-C. The medication administered at discharge and the angiographic features of the patients are shown in Table 2. Medication including statins and insulin were comparable between the two groups. Implantation with DES was more frequent in the high HDL-C group, and the minimum lumen diameter and stent size were both larger in the group with low HDL-C.

Clinical outcomes

The median follow-up period was 963 days (interquartile range 675–1267 days), and prognostic data were fully documented during the entire follow-up period. The Kaplan–Meier survival curve for MACE is shown in Fig. 1. The cumulative incidence of events was significantly higher in the group with low HDL-C (6.9 vs 17.9 %, log-rank $P = 0.030$). Five patients in the group with low HDL-C died during long-term follow-up because of cardiac ($n = 2$), cancer ($n = 2$), and other causes ($n = 1$), whereas none of the group with high HDL-C died. The results of univariate and multivariate Cox hazard regression analysis are shown in Table 3. All adjusted models for baseline confounding factors demonstrated that HDL-C was significantly associated with clinical outcomes.

Discussion

The present observational study showed that HDL-C levels inversely correlated with major cardiovascular events in diabetic patients despite achieving optimal LDL-C control after coronary revascularization.

Diabetes is a leading cause of cardiovascular disease, and the incidence of diabetes is increasing in Asia, consistent with economic development and lifestyle changes [21]. The residual risk persists despite multifactorial intervention including intensive lipid-lowering therapy with statin. Previous observational study demonstrated that more than 50 % of diabetic patients with statin therapy have persistently low HDL-C [22, 23]. A TNT (Treating to New Targets) substudy including about 15 % of diabetes also demonstrated that low HDL-C, even with a lower LDL-C value, is a significant predictor of major cardiovascular events [24]. Taken together, a low HDL-C is one of the characteristics of diabetic dyslipidemia and is a potent risk factor for coronary artery disease independent of LDL.

Epidemiological surveys suggested that the prevalence of low HDL-C substantially differs among nations and ethnic groups [25–27] and that low HDL-C is more

Table 1 Baseline clinical characteristics of patients with optimal LDL-C control

	Low HDL-C ($n = 78$)	High HDL-C ($n = 87$)	P
Age (years)	65.5 ± 9.3	67.6 ± 8.8	0.13
Male gender, *n* (%)	72 (92.3)	74 (85.1)	0.15
Hypertension, *n* (%)	64 (82.1)	64 (73.6)	0.26
Metabolic syndrome, *n* (%)	53 (67.9)	50 (56.5)	0.17
Current smoker, *n* (%)	17 (21.8)	15 (17.2)	0.56
Family history, *n* (%)	21 (26.9)	27 (31.0)	0.61
Multivessel, *n* (%)	59 (77.6)	65 (74.7)	0.72
Prior CABG, *n* (%)	12 (15.4)	9 (10.3)	0.36
Prior MI, *n* (%)	36 (46.2)	30 (34.5)	0.15
BMI (kg/m^2)	25.1 ± 3.3	24.2 ± 3.2	0.09
Waist (cm)	89.9 ± 9.3	88.4 ± 9.4	0.31
SBP (mmHg)	129.6 ± 15.7	129.1 ± 17.0	0.83
DBP (mmHg)	70.7 ± 10.3	65.9 ± 11.7	<0.01
TC (mg/dl)	142.5 ± 21.3	161.2. ± 20.7	<0.01
LDL-C (mg/dl)	79.7 ± 15.5	83.7 ± 11.5	0.06
HDL-C (mg/dl)	32.2 ± 4.9	50.1 ± 9.6	<0.01
TG (mg/dl)	152.9 ± 96.6	131.1 ± 67.7	0.09
FBS (mg/dl)	120.4 ± 46.3	122.0 ± 40.0	0.81
HbA1c (%)	6.76 ± 1.09	6.69 ± 1.08	0.69
hsCRP (mg/dl)	0.29 ± 0.44	0.34 ± 1.69	0.84
eGFR (ml/min/1.73 m^2)	59.3 ± 21.0	63.3 ± 21.8	0.24
CKD, *n* (%)	34 (43.6)	33 (37.9)	0.46

BMI body mass index, *CABG* coronary artery bypass graft surgery, *CKD* chronic kidney disease, *DBP* diastolic blood pressure, *eGFR* estimated glomerular filtration rate, *FBG* fasting blood glucose, *HbA1c* hemoglobin A1c, *HDL-C* high-density lipoprotein cholesterol, *hsCRP* high-sensitivity C-reactive protein, *LDL-C* low-density lipoprotein cholesterol, *MI* myocardial infarction, *SBP* systolic blood pressure, *TC* total cholesterol, *TG* triglycerides

Table 2 Medication and angiographic profiles of patients with optimal LDL-C

	Low HDL-C (n = 78)	High HDL-C (n = 87)	P
Insulin, n (%)	16 (20.5)	24 (27.6)	0.36
OHA, n (%)	44 (56.4)	41 (47.1)	0.28
Aspirin, n (%)	76 (97.4)	87 (100)	0.22
Ticlopidine, n (%)	63 (80.8)	70 (80.5)	0.96
Clopidogrel, n (%)	13 (16.7)	15 (17.2)	0.92
ACE-I, n (%)	16 (20.5)	12 (13.8)	0.25
ARB, n (%)	41 (52.6)	35 (40.2)	0.11
β-Blocker, n (%)	47 (60.3)	41 (47.1)	0.09
Statin, n (%)	52 (66.7)	68 (78.2)	0.10
Type B2/C, n (%)	71 (91.0)	78 (89.7)	0.77
Target lesion, n (%)			0.21
LMT	4 (5.1)	1 (1.1)	
LAD	29 (37.2)	44 (50.6)	
LCx	12 (15.4)	16 (18.4)	
RCA	30 (38.5)	24 (27.6)	
SVG	3 (3.8)	2 (2.3)	
LVEF (%)	59.1 ± 11.8	60.8 ± 12.1	0.37
Reference lumen diameter (mm)	2.83 ± 0.49	2.68 ± 0.46	0.06
MLD postprocedure (mm)	2.82 ± 0.48	2.66 ± 0.44	<0.05
DES use, n (%)	61 (78.2)	82 (94.3)	<0.01
No. of stents, n	1.4 ± 0.6	1.3 ± 0.5	0.33
Mean stent size (mm)	2.97 ± 0.39	2.81 ± 0.35	<0.01
Total stent length (mm)	22.8 ± 6.0	21.5 ± 6.2	0.17
Lesion length (mm)	18.7 ± 8.4	17.5 ± 7.4	0.33

ACE-I angiotensin-converting enzyme inhibitors, *ARB* angiotensin receptor blockers, *DES* drug-eluting stents, *LAD* left anterior descending artery, *LCx* left circumflex artery, *LMT* left main trunk, *LVEF* left ventricular ejection fraction, *MLD* minimal lumen diameter, *OHA* oral hypoglycemic agents, *RCA* right coronary artery, *SVG* saphenous vein graft

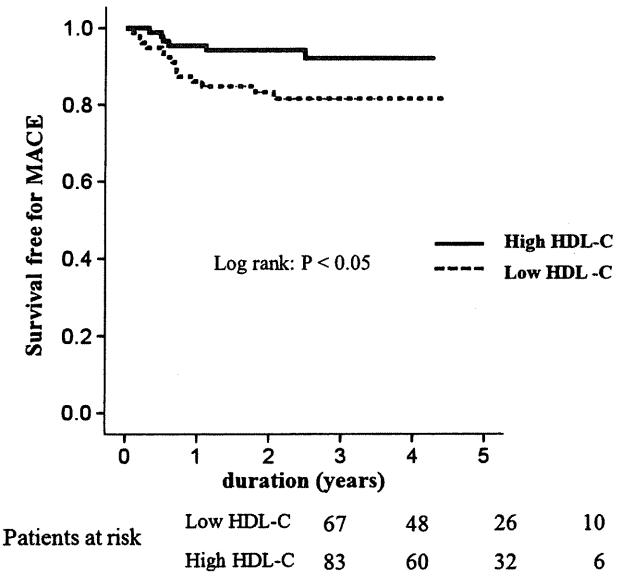

Fig. 1 Kaplan–Meier curves for major adverse cardiac events (*MACE*) in patients with optimal low-density lipoprotein cholesterol (*LDL-C*) control. The cumulative incidence of events was significantly higher in the group with low high-density lipoprotein cholesterol (*HDL-C*) (6.9 vs 17.9 %, log-rank P = 0.030)

Table 3 Hazard ratios of HDL-C levels (1-SD decrease) for MACE

	HR	95 % CI	P
Unadjusted	1.32	1.03–1.69	0.025
Model 1	1.35	1.05–1.72	0.021
Model 2	1.41	1.08–1.85	0.013
Model 3	1.33	1.01–1.75	0.042

Model 1, age and gender adjusted

Model 2, age, gender, LDL-C, TG adjusted

Model 3, adjusted for variables in Model 2 plus BMI, hypertension, metabolic syndrome, current smoking, family history of CAD, prior myocardial infarction, LVEF, multivessel disease, use of DES, use of statin, use of aspirin, and use of ACE-I/ARB. The latter covariates were added in Model 3 only if they were statistically significant predictors of MACE ($P < 0.05$)

MACE major adverse cardiac events, *HR* hazard ratio, *CI* confidence interval, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *TG* triglycerides, *BMI* body mass index, *DES* drug-eluting stents, *CAD* coronary artery disease, *ACE-I* angiotensin-converting enzyme inhibitors, *ARB* angiotensin receptor blockers

prevalent among Asian populations [28, 29]. The cohort study by Okamura et al. [30] revealed an inverse relationship between HDL-C and all-cause mortality in a large

Japanese general population that was followed up for approximately 10 years. Recent large Asian-Pacific meta-analysis demonstrated that isolated HDL-C (low HDL-C with normal LDL-C) is more prevalent and is strongly associated with risk of coronary heart disease in the Asian population than in non-Asians [31]. In addition, another observational study using a PCI cohort from Korea including 40 % diabetics also demonstrated that HDL-C is a potential predictor of clinical outcomes in patients achieving LDL-C targets with statins after PCI [32]. Patients with low HDL-C levels had a 40 % higher rate of MACE including all-cause death, nonfatal myocardial infarction, and target vessel revascularization (TVR) (adjusted HR 1.40, 95 % CI 1.11–1.77, $P < 0.01$), which was similar to our results (adjusted HR 1.33, 95 % CI 1.01–1.75, $P < 0.05$). In that study, TVR was included in the primary end point, in contrast to TLR included in the present study. The different definition of end points may contribute to balancing out the risk of MACE in differing prevalence of diabetes. Taken together, the present study found a significant association between low HDL-C and long-term clinical outcomes among Japanese patients with diabetes, after adjustment for covariates including LDL-C and statin use. Therefore, our results indicate that HDL-C is a residual risk factor in Japanese diabetic patients with optimal LDL-C control as secondary prevention after coronary intervention.

Definitive mechanisms accounting for the beneficial effects of HDL-C on cardiovascular disease have not been fully understood. HDL particles become triglyceride-enriched in the setting of insulin resistance and cleared more rapidly than normal HDL, resulting in reduced HDL-C levels in diabetes [33]. As suggested in experimental and clinical settings, plausible explanations included its ability to remove cellular cholesterol, as well as its anti-inflammatory, antioxidant, and antithrombotic properties, which improve endothelial function and inhibit atherosclerosis, thereby reducing cardiovascular risk [34, 35]. HDL-C becomes triglyceride-enriched in the setting of insulin resistance and cleared more rapidly than normal HDL particles, resulting in reduced HDL-C levels. Thus, diabetic patients with low HDL-C should be considered as being at sufficiently high risk as to warrant aggressive secondary prevention.

Recent clinical trials raising HDL-C therapy with cholesterylester transfer protein (CETP) inhibitors [36, 37], however, did not show any incremental clinical benefit (rather increased harm), in contrast to observational studies demonstrating HDL-C as a potent cardioprotective factor. Another study, the AIM-HIGH trial, which evaluated the raising of HDL-C levels through extended release of niacin in addition to statin in patients with a background of low HDL-C and high TG levels, also failed to show a clinical benefit [38]. In AIM-HIGH, however, the difference of HDL-C values between the niacin and placebo groups was unexpectedly only 4.0 mg/dl, mostly due to an unexpected increase in HDL-C by 9.8 % in the placebo group. In addition, niacin has an additional effect of lowering lipoprotein(a) beyond its effect on HDL-C, which seems to be still controversial in the HDL hypothesis. Previous large longitudinal observational study from diabetic cohorts demonstrated that an increase in HDL-C was associated with lower hospitalization for cardiovascular disease, suggesting the potential role of increasing HDL-C for prevention of cardiovascular disease [39]. Further ongoing clinical trials should offer more clues regarding HDL-C targeted therapy in addition to statin [40, 41].

Limitations

First, unknown confounding factors might have affected the outcomes regardless of the adjustments in this single-center, observational study of a small patient cohort. An observational cohort study of a larger diabetic patient population is thus necessary. Second, the number of events was relatively small in this study, which led to the absence of statistically significant differences in outcome measures. Since we classified patients as diabetic if they had been diagnosed or were under specific treatment for diabetes, patients with undiagnosed diabetes might have been misclassified as nondiabetic. In addition, the absence of information about the duration and control of diabetes might be another possible limitation. Finally, we do not include information about fibrate, which can potentially manage mixed dyslipidemia in diabetes and subsequently improve the clinical outcomes of diabetic patients according to post hoc analysis of previous randomized control trials [42, 43].

Conclusions

Low HDL-C might be a residual risk factor that is significantly associated with long-term clinical outcomes among diabetic patients with CAD who achieve optimal LDL-C levels.

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ORIGINAL ARTICLE
Metabolic Disorder

Effect of Darapladib on Plasma Lipoprotein-Associated Phospholipase A_2 Activity in Japanese Dyslipidemic Patients, With Exploratory Analysis of a *PLA_2G7* Gene Polymorphism of Val279Phe

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Background: Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is being evaluated as a therapeutic target for treatment of atherosclerosis. This is the first study to examine the effects of darapladib, a novel selective Lp-PLA_2 inhibitor, on Lp-PLA_2 activity in Japanese dyslipidemic patients with/without the Val279Phe (V279F) single-nucleotide polymorphism (SNP) of the *PLA_2G7* gene. Exploratory analysis to examine the effects of V279F on Lp-PLA_2 inhibition of darapladib was also performed.

Methods and Results: This was a 4-week, multicenter, randomized, double-blind, placebo-controlled, parallel-group, dose-ranging trial of darapladib in 107 Japanese patients with dyslipidemia receiving statins. Patients were randomized to placebo (n=25), darapladib 40 mg (n=28), 80 mg (n=28), or 160 mg (n=26). All darapladib doses produced sustained dose-dependent inhibition of Lp-PLA_2 activity of approximately 49%, 58%, and 67%, respectively ($P<0.001$ for all comparisons). The inhibitory effect achieved a plateau by 1 week. Patients with the V279F homogenous mutation who have no circulating levels of Lp-PLA_2, were excluded from the study. The Lp-PLA_2 activity was inhibited in both homozygous wild-type and heterozygote genotypes of the V279F polymorphism subjects to a similar extent, although the heterogeneous mutation has almost half the level of Lp-PLA_2 activity compared with that of wild-type in Japanese people. The most common adverse events were odor related. No major safety concerns were noted.

Conclusions: Darapladib produced sustained inhibition of Lp-PLA_2 activity in Japanese dyslipidemic patients with/without the V279F SNP of Lp-PLA_2. (*Circ J* 2013; **77:** 1518–1525)

Key Words: Atherosclerosis; Darapladib; Dyslipidemia; Lipoprotein-associated phospholipase A_2; V279F

Atherosclerosis, the most common cause of myocardial infarction, stroke, and cardiovascular death, is an inflammatory disease. Elevated circulating low-density lipoprotein (LDL) is well known to be a precursor of atherosclerosis and a risk factor for acute coronary syndrome.[1–4] When LDL is trapped in the subintimal space, apolipoprotein B (apoB) facilitates several steps in the oxidation of cholesterol, which signals the upregulation of adhesion molecules and cell surface chemoattractants that recruit monocytes and macrophages into the nascent atheroma. Macrophages play a key role in the ingestion of cholesterol, resulting in the release of free fatty acids and lysophospholipids, which provide metabolites for various inflammatory pathways. Phospholipase-driven inflammation is associated with endothelial dysfunction, plaque formation, and coronary artery disease (CAD), especially with acute coronary syndrome.[5–7] LDL oxidation results in a biochemical modification affecting phospholipid and apoB components. Lipoprotein-associated phospholipase A_2 (Lp-PLA_2), also known as platelet-activating factor acetylhydrolase (PAF-AH), is an enzyme that has pro-inflammatory properties thought to be involved during LDL oxidation in the development and progression of atherosclerosis,[5–7] and is currently being evaluated as a new therapeutic target.[8]

Lp-PLA_2 hydrolyzes oxidized phospholipids generated during the oxidation of LDL, and leads to formation of pro-inflammatory products,[6] such as lisophosphatidylcholine and oxidized non-esterified fatty acid. In contrast to other PLA_2 enzymes, Lp-PLA_2 acts preferentially on water-soluble polar

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phospholipids with oxidatively truncated sn-2 chains, lacking enzymatic activity on naturally occurring long-chain fatty acids in phospholipids found in cellular membranes.[6]

There are some polymorphisms of the Lp-PLA2 gene (*PLA2G7*). The Val279Phe (V279F) homogygous mutation (279FF) is the only polymorphism theoretically resulting in no circulating gene product of Lp-PLA2.[9,10] The prevalence of 279FF was reported to be 0.4%, 1.2%, and 3% in the Chinese, Korean, and Japanese populations, respectively,[11–13] but this variant is rare in non-Asian populations.[14]

In a recent meta-analysis including 79,036 participants in 32 prospective studies, Lp-PLA2 activity and mass each showed continuous associations with risk of CAD, similar in magnitude to that with non-high-density lipoprotein cholesterol and systolic blood pressure.[15] In addition, it has been shown that Lp-PLA2 is an independent predictor of cardiovascular disease (CVD) that is not influenced by traditional risk factors or other inflammatory markers such as high-sensitivity C-reactive protein (hs-CRP).[15–19] Thus, the development of small molecules specifically targeted against inflammatory mediators such as Lp-PLA2 can be seen as a new phase in cardiovascular drug development.

Darapladib is a novel, selective, reversible, orally active inhibitor of Lp-PLA2 activity, discovered by GlaxoSmithKline (GSK). Darapladib does not inhibit the other secretory PLA2 including sPLA2-IIA. It is in development by GSK for prevention of major adverse cardiovascular events in 2 large outcomes studies. In 1 non-clinical study, using a preclinical model of atherosclerosis in pigs with diabetes mellitus and hypercholesterolemia, despite the development of sustained severe hypercholesterolemia, selective inhibition of Lp-PLA2 by darapladib resulted in a significant decrease in atherosclerotic coronary lesion development in comparison to controls.[20] The primary route of elimination is via the feces and the compound is eliminated both as intact darapladib as well as oxidative metabolites, which are modified primarily with CYP3A4.

Two international Phase II studies have been completed. One study examined the effects of darapladib on biomarkers of cardiovascular risk in 959 CAD and CAD-risk equivalent patients who were previously randomized to atorvastatin 20 mg or 80 mg and then randomized to oral darapladib 40, 80, 160 mg, or placebo for 12 weeks. Overall dose-dependent inhibition of Lp-PLA2 activity was sustained over the study period and was present in both atorvastatin dose groups, at different baseline LDL cholesterol (LDL-C; < or ≥70 mg/dl), and high-density lipoprotein cholesterol (HDL-C) < or ≥40 mg/dl.[21] The other study (IBIS-2 study) compared the effects of 12-month treatment with darapladib 160 mg daily or placebo on coronary atheroma deformability and hs-CRP in 330 patients with angiographically documented coronary disease. The results showed that darapladib inhibits plasma Lp-PLA2 activity in a dose-dependent manner; no major safety concern was noted. In the IBIS-2 study, inhibition of Lp-PLA2 with darapladib also prevented necrotic core expansion of coronary plaque as measured on intravascular ultrasound.[22]

The present study is the first to examine the effects of darapladib on plasma Lp-PLA2 activity and to assess the safety and tolerability of darapladib in Japanese dyslipidemic patients. In addition, the effect of the V279F heterozygote on the inhibition activity of darapladib is investigated.

Methods

Patients

The subjects included dyslipidemic patients aged 20–80 years on statin therapy with no change in lipid-lowering medication or dose during the 4 weeks before randomization. Exclusion criteria included recent (≤6 months prior to screening) cardiovascular event and/or vascular procedure; Lp-PLA2 activity ≤10 nmol·min^{-1}·ml^{-1}; poorly controlled dyslipidemia (LDL-C ≥160 mg/dl); poorly controlled hypertension (blood pressure ≥160 mmHg systolic and/or ≥100 mmHg diastolic); severe renal dysfunction (estimated glomerular filtration rate [eGFR] <30 ml·min^{-1}·1.73 m^{-2}; eGFR [ml·min^{-1}·1.73 m^{-2}] = 0.741 × 175 × Age$^{-0.203}$ × Cr$^{-1.154}$ [×0.742 if patient is female]); chronic heart failure; or previous exposure to darapladib.

The study was approved by Institutional Review Boards of clinical trial sites. The study was conducted following the latest version of Declaration of Helsinki. All participants provided written informed consent.

Study Design

This was a 4-week, multicenter, randomized, double-blind, placebo-controlled, parallel-group, dose-ranging trial of darapladib in patients with dyslipidemia receiving statin therapy. The stratified randomization with plasma Lp-PLA2 activity was conducted in screened patients to receive placebo or enteric coated darapladib 40-mg, 80-mg, or 160-mg tablets once a day in a ratio of 1:1:1:1.

The study consisted of visit 1 (screening visit; weeks –6 to –2), visit 2 (baseline visit at which patients were randomized to treatment with darapladib or placebo; week 0), visits 3, 4, 5 (treatment visit; weeks 1, 2, 4), and visit 6 (follow-up visit; week 7). Patients were instructed to take the study medication once daily with food, generally after breakfast, to avoid risk of taking the study medication under a condition of low gastric pH. Patients were asked to swallow the tablets without chewing, because the tablets were enteric-coated.

Endpoints

The primary efficacy endpoint was change from baseline to week 4 in plasma Lp-PLA2 activity (log-transformed). The secondary endpoints were percent inhibition of Lp-PLA2 activity in plasma at week 4, and each-visit changes from baseline of Lp-PLA2 activity and percent inhibition of Lp-PLA2 activity.

To examine the effects of darapladib on plasma Lp-PLA2 activity, blood samples were taken, apart from pharmacogenetics samples, at each visit between screening and follow-up. Plasma Lp-PLA2 activity was measured using colorimetric assay.[23]

For exploratory endpoints, whole blood was taken from patients to detect a gene polymorphism of V279F for examination of the effect of darapladib on the V279F heterozygous (279FV) population. The effect of darapladib on biomarkers such as plasminogen activating inhibitor type 1 (PAI-1), hs-CRP, interleukin 6 (IL-6), P-selectin and urinary 11-dehydrothromboxane B2 was also evaluated.

Safety endpoints included the incidence and severity of adverse events (AEs); change from baseline in clinical laboratory data and urinalysis at week 2, week 4, and follow-up visit; change from baseline in 12-lead electrocardiogram (ECG) at week 4; and change from baseline in vital signs (blood pressure, heart rate) at each visit.

Statistical Analysis

The primary efficacy analysis group was the full analysis set (FAS): randomized patients except those who received no dose of study drug and those for whom Lp-PLA2 activity was not measured or evaluable. The secondary efficacy analysis

Table 1. Demographic and Baseline Subject Characteristics

Characteristics	Treatment groups				
	Placebo (n=25)	Darapladib 40 mg (n=28)	Darapladib 80 mg (n=28)	Darapladib 160 mg (n=26)	Total (n=107)
Sex					
Female	17 (68)	17 (61)	18 (64)	13 (50)	65 (61)
Male	8 (32)	11 (39)	10 (36)	13 (50)	42 (39)
Age category (years)					
<65	14 (56)	15 (54)	21 (75)	18 (69)	68 (64)
≥65	11 (44)	13 (46)	7 (25)	8 (31)	39 (36)
<75	25 (100)	27 (96)	26 (93)	25 (96)	103 (96)
≥75	0	1 (4)	2 (7)	1 (4)	4 (4)
Age (years)	59.5±11.71	59.8±10.17	58.3±9.54	58.3±10.48	59.0±10.34
Body weight (kg)	60.81±9.65	63.59±13.327	60.83±10.774	63.43±13.988	62.18±11.995
BMI (kg/m^2)	24.30±2.868	24.68±3.203	23.49±2.96	24.21±3.471	24.16±3.122
Height (cm)	158.0±8.09	159.8±9.58	160.5±9.90	161.0±9.52	159.9±9.26
TC (mmol/L)	5.2±0.70	5.2±0.64	5.3±0.71	4.8±0.57	
LDL-C (mmol/L)	3.1±0.59	2.9±0.61	3.2±0.71	2.8±0.46	

Data given as mean±SD or n (%). BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol.

Table 2. Plasma Lp-PLA$_2$ Activity Change From Baseline to Week 4

Treatment	Visit	Geometric mean	Adj. ratio†	vs. placebo (95% CI)‡	P-value
Placebo (n=25)	Baseline	127.56	0.961		
	Week 4/withdrawal	122.56			
Darapladib 40 mg (n=28)	Baseline	125.62	0.494	0.514 (0.449–0.590)	<0.001
	Week 4/withdrawal	62.12			
Darapladib 80 mg (n=28)	Baseline	136.06	0.404	0.421 (0.367–0.483)	<0.001
	Week 4/withdrawal	54.97			
Darapladib 160 mg (n=26)	Baseline	124.84	0.313	0.326 (0.284–0.375)	<0.001
	Week 4/withdrawal	39.12			

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$_2$.

group, the per-protocol set (PPS), excluded patients with major protocol violations. The safety group included patients receiving at least 1 dose of study drug.

The primary efficacy analysis compared each dose of darapladib vs. placebo for the change from baseline in log-transformed Lp-PLA$_2$ activity to week 4. A parametric analysis of covariance (ANCOVA) model included baseline Lp-PLA$_2$ activity as a covariate. Each of the 3 comparisons between active groups and placebo was made at the overall 2-sided significance level of 5% with adjustment for multiplicity using Dunnett correction. Missing values of Lp-PLA$_2$ activity after randomization were imputed using last observation carried forward. The sensitivity analysis to assess the robustness of the primary analysis was conducted using the PPS and observed case analysis, which is the non-imputation method for missing values.

For secondary efficacy analysis, to examine the dose response of darapladib on inhibition of plasma Lp-PLA$_2$ activity, change from baseline in log-transformed plasma Lp-PLA$_2$ activity to week 4 was analyzed using ANCOVA with contrast methods at the 1-sided 2.5% significance level. Subgroup analyses of V279F polymorphism were conducted by ANCOVA.

For each biomarker, the ANCOVA-adjusted baseline value was used for log-transformed change from baseline to week 4.

The number and percentage of serious AEs (SAEs), AEs leading to discontinuation of investigational product, and other safety endpoints were summarized by treatment group.

Results

Subject Baseline Characteristics

A total of 107 patients were randomized to placebo (n=25), darapladib 40 mg (n=28), 80 mg (n=28), or 160 mg (n=26). All 107 patients were included in the FAS and safety group. They received standard care for dyslipidemia and other diseases including diabetes mellitus and hypertension. The patients' lifestyle did not change during the 4-week treatment period. Patient demographic characteristics are listed in Table 1.

The V279F polymorphism genotype was generally similar across the treatment groups. There were 82/107 patients (77%) with the homozygous wild-type (279VV) and 25/107 (23%) with the heterozygous (279VF) genotype. There was no homozygous mutant (FF) subject because 3 patients who had Lp-PLA$_2$ activity ≤10 nmol·min^{-1}·ml^{-1} were excluded at