

# Role of Small Dense Low-density Lipoprotein Cholesterol in Cardiovascular Events in Patients with Coronary Artery Disease and Type 2 Diabetes Mellitus Receiving Statin Treatment

Takayuki Yamaji<sup>1</sup>, Takahiro Harada<sup>1</sup>, Masato Kajikawa<sup>2</sup>, Tatsuya Maruhashi<sup>3</sup>, Shinji Kishimoto<sup>3</sup>, Farina Mohamad Yusoff<sup>3</sup>, Kazuaki Chayama<sup>4</sup>, Chikara Goto<sup>5</sup>, Ayumu Nakashima<sup>2</sup>, Hirofumi Tomiyama<sup>6</sup>, Bonpei Takase<sup>7</sup>, Takahide Kohro<sup>8</sup>, Toru Suzuki<sup>9</sup>, Tomoko Ishizu<sup>10</sup>, Shinichiro Ueda<sup>11</sup>, Tsutomu Yamazaki<sup>12</sup>, Tomoo Furumoto<sup>13</sup>, Kazuomi Kario<sup>14</sup>, Teruo Inoue<sup>15</sup>, Kentaro Watanabe<sup>16</sup>, Yasuhiko Takemoto<sup>17</sup>, Takuzo Hano<sup>18</sup>, Masataka Sata<sup>19</sup>, Yutaka Ishibashi<sup>20</sup>, Koichi Node<sup>21</sup>, Koji Maemura<sup>22</sup>, Yusuke Ohya<sup>23</sup>, Taiji Furukawa<sup>24</sup>, Hiroshi Ito<sup>25</sup>, Akira Yamashina<sup>6</sup>, Shinji Koba<sup>26</sup> and Yukihiro Higashi<sup>2, 3</sup>

<sup>1</sup>Department of Cardiovascular Medicine, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan

<sup>2</sup>Division of Regeneration and Medicine, Medical Center for Translational and Clinical Research, Hiroshima University Hospital, Hiroshima, Japan

<sup>3</sup>Department of Cardiovascular Regeneration and Medicine, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan

<sup>4</sup>Department of Gastroenterology and Metabolism, Institute of Biomedical and Health Sciences, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan

<sup>5</sup>Department of Physical Therapy, Hiroshima International University, Hiroshima, Japan

<sup>6</sup>Department of Cardiology, Tokyo Medical University, Tokyo, Japan

<sup>7</sup>Division of Biomedical Engineering, National Defense Medical College Research Institute, Tokorozawa, Japan

<sup>8</sup>Department of Clinical Informatics, Jichi Medical University School of Medicine, Tochigi, Japan

<sup>9</sup>Cardiovascular Medicine, University of Leicester, Leicester, UK

<sup>10</sup>Cardiovascular Division, Institute of Clinical Medicine, University of Tsukuba, Ibaraki, Japan

<sup>11</sup>Department of Clinical Pharmacology and Therapeutics, University of the Ryukyus School of Medicine, Okinawa, Japan

<sup>12</sup>Department of Clinical Epidemiology and Systems, Faculty of Medicine, The University of Tokyo, Tokyo, Japan

<sup>13</sup>Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine, Hokkaido, Japan

<sup>14</sup>Division of Cardiovascular Medicine, Jichi Medical University School of Medicine, Tochigi, Japan

<sup>15</sup>Department of Cardiovascular Medicine, Dokkyo Medical University, Mibu, Tochigi, Japan

<sup>16</sup>Department of Neurology, Hematology, Metabolism, Endocrinology and Diabetology (DNHMED), Yamagata University School of Medicine, Yamagata, Japan

<sup>17</sup>Department of Internal Medicine and Cardiology, Osaka Metropolitan University Graduate School of Medicine, Osaka, Japan

<sup>18</sup>Department of Medical Education and Population-based Medicine, Postgraduate School of Medicine, Wakayama Medical University, Wakayama, Japan

<sup>19</sup>Department of Cardiovascular Medicine, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan

<sup>20</sup>Department of General Medicine, Shimane University Faculty of Medicine, Izumo, Japan

<sup>21</sup>Department of Cardiovascular and Renal Medicine, Saga University, Saga, Japan

<sup>22</sup>Department of Cardiovascular Medicine, Course of Medical and Dental Sciences, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan

<sup>23</sup>The Third Department of Internal Medicine, University of the Ryukyus, Okinawa, Japan

<sup>24</sup>Department of Internal Medicine, Teikyo University School of Medicine, Tokyo, Japan

<sup>25</sup>Department of Cardiovascular Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Japan

<sup>26</sup>Department of Medicine, Division of Cardiology, Showa University School of Medicine, Tokyo, Japan

**Aim:** There is little information on the relationships of serum small dense low-density lipoprotein cholesterol (sdLDL-C) levels and serum triglyceride (TG) levels with cardiovascular events in patients with coronary artery disease (CAD) and type 2 diabetes mellitus (DM) who are receiving statins. The aim of this study was to evaluate the relationships of serum TG levels and sdLDL-C levels as residual risks for cardiovascular events in patients with CAD and type 2 DM who were being treated with statins.

**Methods:** The subjects were divided into four groups based on TG levels and sdLDL-C levels: sdLDL-C of <40.0 mg/dL and TG of <150 mg/dL, sdLDL-C of  $\geq$  40.0 mg/dL and TG of <150 mg/dL, sdLDL-C of <40.0 mg/dL and TG of  $\geq$  150 mg/dL, and sdLDL-C of  $\geq$  40.0 mg/dL and TG of  $\geq$  150 mg/dL. During a median follow-up period of 1419 days, cardiovascular events occurred in 34 patients.

**Results:** The incidences of cardiovascular events were significantly higher in patients with sdLDL-C of  $\geq$  40.0 mg/dL and TG of <150 mg/dL and in patients with sdLDL-C of  $\geq$  40.0 mg/dL and TG of  $\geq$  150 mg/dL, but not in patients with sdLDL-C of <40.0 mg/dL and TG of  $\geq$  150 mg/dL, than in patients with sdLDL-C of <40.0 mg/dL and TG of <150 mg/dL.

**Conclusions:** Under the condition of treatment with statins, patients with CAD and type 2 DM who had sdLDL-C levels of  $\geq$  40.0 mg/dL had a high risk for cardiovascular events even though serum TG levels were controlled at <150 mg/dL.

**Key words:** Small dense low-density lipoprotein cholesterol, Triglycerides, Type 2 diabetes mellitus, Statin, Cardiovascular events

## Introduction

Diabetes mellitus (DM) is an established risk factor for atherosclerotic cardiovascular disease (ASCVD)<sup>1)</sup>. Cardiovascular events caused by ASCVD are the major cause of morbidity and mortality in patients with type 2 DM<sup>2)</sup>. It has been shown that patients with a combination of type 2 DM and a history of cardiovascular disease have a very high risk for cardiovascular events<sup>1)</sup>. Therefore, the American Diabetes Association (ADA) recommended treatment with high-intensity statins in patients with ASCVD and type 2 DM<sup>2)</sup>.

It has been shown that statin treatment reduces the risk of cardiovascular events in patients with coronary artery disease (CAD) by about 20%<sup>3, 4)</sup>. However, residual risks for cardiovascular events remain in patients who were receiving statins. It is well known that high serum triglyceride (TG) levels are residual risks for cardiovascular events<sup>5)</sup>. Hypertriglyceridemia is an established cardiovascular residual risk worldwide<sup>6-8)</sup>. The cut-off value of serum TG for hypertriglyceridemia in guidelines is 150 mg/dL<sup>9-11)</sup> since previous studies showed that the risk for

CVD increased above a cut-off value of 150 mg/dL<sup>12, 13)</sup>. Serum TG levels are increased in most patients with type 2 DM and are predictors of cardiovascular disease<sup>14)</sup>.

Several studies have shown that a high level of small dense low-density lipoprotein cholesterol (sdLDL-C) is also a residual risk for cardiovascular disease in patients being treated with statins<sup>15)</sup>. sdLDL particles are defined as particles with a diameter of  $\leq$  25.5 nm and with a density from 1.044 to 1.063 g/mL<sup>16)</sup>. In patients with type 2 DM, serum LDL-C levels are usually not increased, but serum sdLDL-C levels are increased as well as serum TG levels<sup>17)</sup>. In addition, a previous study showed that LDL particle size has a significant correlation with serum TG levels<sup>18)</sup>. sdLDL-C levels are correlated with blood pressure, blood glucose levels, and TG levels<sup>19)</sup>.

However, there has been no study on the relationships of serum sdLDL-C levels and TG levels with cardiovascular events in patients with CAD and type 2 DM who are receiving statins.

## Aim

Therefore, we evaluated the relationships of

Address for correspondence: Yukihito Higashi, Department of Cardiovascular Regeneration and Medicine, Research Institute for Radiation Biology and Medicine (RIRBM), Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan E-mail: yhigashi@hiroshima-u.ac.jp

Received: June 4, 2023 Accepted for publication: September 26, 2023

Copyright©2023 Japan Atherosclerosis Society

This article is distributed under the terms of the latest version of CC BY-NC-SA defined by the Creative Commons Attribution License.

**Advance Publication Journal of Atherosclerosis and Thrombosis**

**Accepted for publication: September 26, 2023 Published online: November 3, 2023**

serum sdLDL-C levels and TG levels with cardiovascular events in those patients in a multicenter clinical trial.

## Methods

### Study Patients

Between May 1, 2010 and August 31, 2012, a total of 679 subjects who had been diagnosed with CAD and who had been under regular follow-up at any of the participating institutions for at least 6 months were eligible for enrollment in Flow-mediated Dilation-Japan (FMD-J) Study A. CAD was defined as myocardial infarction, angina pectoris with organic stenosis of at least one coronary artery confirmed by diagnostic imaging (coronary angiography, cardiac nuclear scintigraphy, or coronary computed tomography), or previous percutaneous coronary intervention. The exclusion criteria were as follows: a history of coronary bypass surgery; severe valvular heart disease; arrhythmia that requires treatment (i.e., atrial fibrillation, atrial flutter, permanent pacemaker implantation or frequent ventricular premature beats); severe chronic heart failure (New York Heart Association level of >III); malignancy; undergoing treatment with steroids, nonsteroidal anti-inflammatory drugs, or immunosuppressive drugs; serum creatinine level of >2.5 mg/dL; history of stroke, aortic disease (except peripheral artery disease), or serious liver disease; and judgment of an attending physician that an individual is ineligible for inclusion in the study.

### Study Design

This study was a prospective multicenter observational cohort study conducted at 22 university hospitals and affiliated clinics in Japan to examine the usefulness of FMD assessment for management of patients with CAD with a 3-year follow-up period<sup>20, 21</sup>. In a total number of 679 patients, outcome data were available for 662 patients. Of those patients, 198 patients were included in this study after excluding patients without organic coronary artery stenosis ( $n=32$ ), without diabetes mellitus ( $n=388$ ), without statin treatment ( $n=33$ ) and without sdLDL-C information ( $n=11$ ) (**Supplementary Fig. 1**). This study was executed in accordance with the Good Clinical Practice guidelines. All subjects gave written informed consent for participation in the study. The protocol was registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN000012950).

### Study Procedures

Blood examinations and medical examinations were conducted at the start of this study. Cardiovascular events were monitored annually during the 3-year follow-up period. The participants were managed by their attending physicians who were encouraged to treat cardiovascular risk factors including hypertension, dyslipidemia and DM.

### Measurements of Blood Samples and Assessment of Cardiovascular Risk Factors

The subjects fasted the previous night and abstained from drinking alcohol, smoking and consuming caffeine and vitamins for at least 12 hours prior to the study. Each subject was kept in a supine position in a quiet, dark, airconditioned room (23°C to 26°C) throughout the study. A 23-gauge polyethylene catheter was inserted into the left antecubital vein. Levels of serum total cholesterol, TG, high-density lipoprotein cholesterol (HDL-C), and LDL-C were enzymatically measured (JCA-BM6010, JEOL, Tokyo, Japan). Although the accuracy of the calculation was reduced in patients with diabetes<sup>22</sup>, sdLDL-C was measured by the direct homogenous assay that was previously described<sup>23</sup>. Glucose level was measured by the glucose oxidase immobilized oxygen electrode method (GA09II; A&T, Yokohama, Japan). DM was identified using the ADA criteria<sup>24</sup>. Hypertension was defined as systolic blood pressure of  $\geq 140$  mm Hg or diastolic blood pressure of  $\geq 90$  mm Hg, on at least three different occasions in a sitting position, or currently taking antihypertensive medication<sup>25</sup>. Dyslipidemia was identified using the Third Report of the National Cholesterol Education Program<sup>26</sup>. We defined smokers as those who were current smokers. The Framingham risk score was calculated by points of risk factors: age, total cholesterol level, HDL-C level, systolic blood pressure, and smoking status<sup>27</sup>.

### Cardiovascular Outcomes

All cardiovascular events were reported to the Efficacy Endpoint Review Committee annually from each institution. The committee adjudicated the end points of death from cardiovascular causes, non-fatal acute coronary syndrome (ACS), non-fatal stroke, angina pectoris, and death from any cause<sup>20, 28</sup>. Primary cardiovascular events were defined as death from cardiovascular disease, non-fatal ACS, angina pectoris, and non-fatal stroke. Angina pectoris was defined as coronary artery restenosis or de novo coronary artery stenosis, confirmed by diagnostic imaging (coronary angiography, cardiac nuclear scintigraphy, or coronary computed tomography). The

committee, consisting of members blinded to any information with regard to vascular function, assessed the appropriateness of clinical judgement of cardiovascular events according to prespecified criteria. The committee could request physicians to provide additional clinical information on cardiovascular events if needed. Any differences in opinion under assessment were resolved by discussion, and the committee finally determined whether the cardiovascular events would be included as outcome events in the analysis. We first assessed the associations of serum sdLDL-C levels and serum TG levels with primary cardiovascular events and then we assessed the associations with first cardiovascular events in four groups according to serum sdLDL-C levels and TG levels.

### Statistical Analysis

Results are presented as mean  $\pm$  SD for continuous variables and as percentages for categorical variables. All reported *p* values were two-sided, and a *p* value of  $<0.05$  was considered statistically significant. Continuous variables were compared by using Student's *t*-test. Univariate linear regression analyses were performed to assess the relationships among the variables. Categorical variables were compared by means of the  $\chi^2$  test. Receiver-operator characteristic (ROC) curve analysis was performed to assess the sensitivity and specificity of measurements of sdLDL-C and LDL-C for predicting cardiovascular events. Time-to-event end point analysis was performed by using the Kaplan-Meier method. We categorized subjects into two groups according to the cut-off values of serum sdLDL-C levels and TG levels. Cut-off values for serum sdLDL-C were determined according to the highest Youden index from the ROC curves for predicting cardiovascular events. Cut-off values for serum TG were determined according to cut-off values in guidelines: normal ( $<150$  mg/dL) and hypertriglyceridemia ( $\geq 150$  mg/dL)<sup>9-11</sup>. The log-rank test was used to compare survival in the groups. We evaluated the associations between serum sdLDL-C level and TG level and primary major cardiovascular events after adjustment for age, sex, and cardiovascular risk factors by using Cox's proportional hazard regression analysis. The data were processed using JMP pro version 16 (SAS Institute, Cary, NC).

## Results

### Baseline Clinical Characteristics

The baseline clinical characteristics of the 198 patients are summarized in **Table 1**. The mean age of the patients was  $64 \pm 8$  years. Of the 198 patients, 161 patients were men and 37 were women. The mean

levels of glucose and hemoglobin A1c (HbA1c) in the patients were  $141 \pm 45$  mg/dL and  $7.2 \pm 1.0\%$ , respectively. Mean levels of total cholesterol, TG, HDL-C, LDL-C and sdLDL-C were  $166 \pm 30$  mg/dL,  $147 \pm 111$  mg/dL,  $50 \pm 14$  mg/dL,  $88 \pm 25$  mg/dL and  $32.4 \pm 14.8$  mg/dL, respectively. Of the 198 patients, 187 (94.4%) had hypertension, 26 (13.4%) were current smokers, 165 (83.3%) were being treated with anti-diabetic drugs, 149 (75.3%) were being treated with oral anti-diabetic drugs, and 33 (16.7%) were receiving insulin therapy. All of the patients were on statins, 1 (0.5%) was on fibrates, 14 (7.1%) were on eicosapentaenoic acids, and 18 (9.1%) were on ezetimibe.

### Relationships of sdLDL-C Levels with Cardiovascular Events

The cut-off value of sdLDL-C levels derived from ROC curve for predicting primary cardiovascular events was 40.0 mg/dL (**Supplementary Fig. 2**). Therefore, we divided the subjects into two groups according to the cut-off sdLDL-C level of 40.0 mg/dL. Clinical characteristics of the subjects in the two groups according to serum sdLDL-C levels are summarized in **Table 1**. Age, diastolic blood pressure, total cholesterol, TG, HDL-C, LDL-C, glucose, HbA1c, creatine, estimated glomerular filtration rate (eGFR) and Framingham risk score were significantly different in the two groups. There were no significant differences in other parameters between the two groups.

Univariate analysis revealed that serum sd-LDL-C levels were positively correlated with diastolic blood pressure, heart rate, total cholesterol, TG, LDL-C, glucose, HbA1c, eGFR, and Framingham risk score and were negatively correlated with age and HDL-C (**Supplementary Table 1**).

During the follow-up of period, 34 patients had primary cardiovascular events, 3 died from cardiovascular disease, 3 had non-fatal stroke, 22 had angina pectoris and 6 had non-fatal ACS (**Supplementary Table 2**). The incidences of primary cardiovascular events, non-fatal stroke, angina pectoris, and non-fatal ACS were significantly higher in the high sdLDL-C group than in the low sdLDL-C group.

Kaplan-Meier analysis showed that patients with sdLDL-C above the cut-off value of 40.0 mg/dL had significantly higher incidences of primary cardiovascular events (log-rank  $p < 0.001$ ; **Fig. 1A**), non-fatal ACS (log-rank  $p = 0.03$ ; **Fig. 1B**), non-fatal stroke (log-rank  $p = 0.005$ ; **Fig. 1D**), and angina pectoris (log-rank  $p = 0.048$ ; **Fig. 1E**) than those for patients with sdLDL-C below the cut-off value.

**Table 1.** Clinical Characteristics of Subjects in Groups According to sdLDL-C Levels

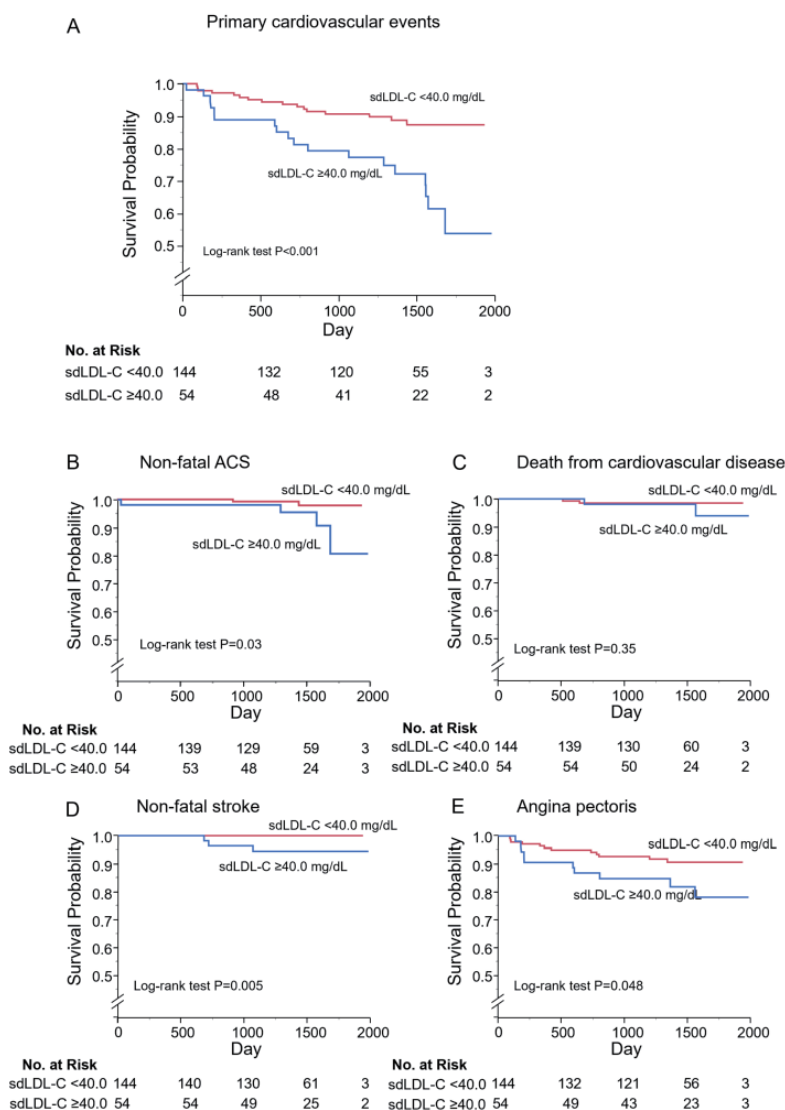
Variables	Total (n = 198)	sdLDL-C < 40.0 mg/dL (n = 144)	sdLDL-C ≥ 40.0 mg/dL (n = 54)	P value
Age, yr	64 ± 8	65 ± 8	61 ± 8	0.01
Gender, men/women	161/37	116/28	45/9	0.66
Body mass index, kg/m <sup>2</sup>	25.9 ± 4.2	25.9 ± 4.3	26.0 ± 3.7	0.95
Systolic blood pressure, mmHg	133 ± 18	132 ± 18	135 ± 15	0.28
Diastolic blood pressure, mmHg	74 ± 10	73 ± 10	78 ± 10	0.01
Heart rate, bpm	66 ± 12	65 ± 11	69 ± 12	0.053
Total cholesterol, mg/dL	166 ± 30	158 ± 25	187 ± 30	< 0.001
TG, mg/dL	147 ± 111	113 ± 55	236 ± 163	< 0.001
HDL-C, mg/dL	50 ± 14	51 ± 15	46 ± 11	0.02
LDL-C, mg/dL	88 ± 25	84 ± 21	99 ± 30	< 0.001
sdLDL-C, mg/dL	32.4 ± 14.8	24.9 ± 7.1	52.4 ± 10.7	N/A
Glucose, mg/dL	141 ± 45	136 ± 38	155 ± 58	0.01
Hemoglobin A1c, %	7.2 ± 1.0	7.0 ± 0.9	7.5 ± 1.3	0.003
Creatinine, mg/dL	0.86 ± 0.24	0.88 ± 0.26	0.80 ± 0.15	0.03
eGFR, mL/min per 1.73 m <sup>2</sup>	71 ± 18	69 ± 18	76 ± 17	0.02
Current Smoking, n (%)	26 (13.4)	21 (14.8)	5 (9.6)	0.35
Exercise habit, n (%)	116 (60.4)	86 (60.6)	30 (60.0)	0.94
Alcohol drink, n (%)	66 (34.2)	46 (32.2)	20 (40.0)	0.31
Medical history, n (%)				
Hypertension	187 (94.4)	137 (95.1)	50 (92.6)	0.49
Dyslipidemia	198 (100)	144 (100)	54 (100)	
Previous myocardial infarction	92 (46.5)	63 (43.8)	29 (53.7)	0.21
Previous angina pectoris	111 (56.1)	83 (57.6)	28 (51.9)	0.47
Medications, n (%)				
Antiplatelet drugs	193 (97.5)	140 (97.2)	53 (98.2)	0.71
Statins	198 (100)	144 (100)	54 (100)	
Fibrates	1 (0.5)	1 (0.69)	0 (0)	0.54
Eicosapentaenoic acids	14 (7.1)	11 (7.6)	3 (5.6)	0.61
Ezetimibe	18 (9.1)	12 (8.3)	6 (11.1)	0.54
ACEIs/ARBs	144 (72.7)	105 (72.9)	39 (72.2)	0.92
β-blockers	89 (44.9)	63 (44.8)	26 (48.2)	0.58
Calcium-channel blockers	100 (50.5)	75 (52.1)	25 (46.3)	0.47
Diuretics	31 (15.7)	25 (17.4)	6 (11.1)	0.28
Any-antidiabetic drugs	165 (83.3)	124 (86.1)	41 (75.9)	0.09
Oral antidiabetic drugs	149 (75.3)	111 (77.1)	38 (70.4)	0.33
Insulin	33 (16.7)	27 (18.8)	6 (11.1)	0.20
Framingham risk score, %	16 ± 8	15 ± 8	20 ± 9	< 0.001

sdLDL-C indicates small dense low-density lipoprotein cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; ACEIs, angiotensin-converting enzyme inhibitors; ARBs, angiotensin receptor blockers

Multivariate Cox proportional hazard analysis revealed that sdLDL-C above the cut-off value of 40.0 mg/dL was a significant predictor of higher risk of primary cardiovascular events after adjustment for cardiovascular risk factors including serum TG levels (hazard ratio, 2.89; 95% confidence interval, 1.11-7.48;  $p=0.03$ ) ([Supplementary Table 3](#)).

### Relationships of TG Levels with Cardiovascular Events

We divided the subjects into two groups according to the cut-off value of serum TG levels of 150 mg/dL. Clinical characteristics of the subjects in the two groups according to serum TG levels are summarized in [Supplementary Table 4](#). Age, total cholesterol, TG, HDL-C, LDL-C, glucose, HbA1c, use of insulin, and Framingham risk score were

**Fig. 1.**

Kaplan-Meier curves of cumulative event-free survival of first major cardiovascular events (A), non-fatal acute coronary syndrome (B), death from cardiovascular disease (C), non-fatal stroke (D), and angina pectoris (E) according to serum small dense low-density lipoprotein cholesterol (sdLDL-C) levels.

significantly different in the two groups. There were no significant differences in other parameters between the two groups.

Univariate analysis revealed that serum TG levels were positively correlated with body mass index, total cholesterol, sdLDL-C, glucose, HbA1c, and Framingham risk score and were negatively correlated with age and HDL-C (**Supplementary Table 5**).

During the follow-up of period, the incidences of primary cardiovascular events and non-fatal ACS were significantly higher in the high TG group than in the low TG group (**Supplementary Table 6**).

Kaplan-Meier analysis showed that patients with

serum TG above the cut-off value of 150 mg/dL had significantly higher incidences of primary cardiovascular events (log-rank  $p=0.01$ ; **Supplementary Fig. 3A**) and non-fatal ACS (log-rank  $p=0.001$ ; **Supplementary Fig. 3B**) than those for patients with serum TG below the cut-off value. Multivariate Cox proportional hazard analysis revealed that hypertriglyceridemia was a significant predictor of a higher risk of primary cardiovascular events after adjustment of cardiovascular risk factors (hazard ratio, 2.56; 95% confidence interval, 1.29-5.13;  $p=0.008$ ), but after adjustment of sdLDL-C, hypertriglyceridemia was not a significant predictor of primary cardiovascular events (hazard ratio, 1.64; 95% confidence interval,

**Table 2.** Clinical Characteristics of Subjects in Groups According to Cut-off Values of sdLDL-C and TG Levels

Variables	Total (n = 198)	Group 1 (n = 112)	Group 2 (n = 15)	Group 3 (n = 32)	Group 4 (n = 39)	P value
Age, yr	64 ± 8	65 ± 8	61 ± 8	62 ± 9	61 ± 8	0.02
Gender, men/women	161/37	89/23	10/5	27/5	35/4	0.22
Body mass index, kg/m <sup>2</sup>	25.9 ± 4.2	25.7 ± 4.2	24.7 ± 3.0	26.7 ± 4.7	26.5 ± 3.9	0.31
Systolic blood pressure, mmHg	133 ± 18	133 ± 19	137 ± 13	128 ± 16	134 ± 16	0.32
Diastolic blood pressure, mmHg	74 ± 10	73 ± 11	77 ± 8	71 ± 7	78 ± 10	0.03
Heart rate, bpm	66 ± 12	66 ± 11	71 ± 11	65 ± 11	68 ± 12	0.19
Total cholesterol, mg/dL	166 ± 30	158 ± 26	186 ± 28	160 ± 23	188 ± 32	<0.001
TG, mg/dL	147 ± 111	90 ± 27	119 ± 22	197 ± 44	281 ± 172	N/A
HDL-C, mg/dL	50 ± 14	53 ± 14	54 ± 8	45 ± 14	43 ± 11	<0.001
LDL-C, mg/dL	88 ± 25	86 ± 21	109 ± 23	75 ± 21	95 ± 32	<0.001
sdLDL-C, mg/dL	32.4 ± 14.8	23.0 ± 6.3	50.1 ± 9.4	31.4 ± 5.9	53.2 ± 11.1	N/A
Glucose, mg/dL	141 ± 45	132 ± 35	154 ± 36	149 ± 44	155 ± 65	0.01
Hemoglobin A1c, %	7.2 ± 1.0	7.0 ± 0.8	7.6 ± 1.3	7.3 ± 0.9	7.5 ± 1.3	0.007
Creatinine, mg/dL	0.86 ± 0.24	0.87 ± 0.24	0.77 ± 0.20	0.92 ± 0.33	0.81 ± 0.13	0.11
eGFR, mL/min per 1.73 m <sup>2</sup>	71 ± 18	69 ± 18	78 ± 22	69 ± 19	75 ± 15	0.11
Current Smoking, n (%)	26 (13.4)	13 (11.7)	0 (0)	8 (25.8)	5 (13.5)	0.08
Exercise habit, n (%)	116 (60.4)	68 (61.3)	9 (64.3)	18 (58.1)	21 (58.3)	0.97
Alcohol drink, n (%)	66 (34.2)	36 (32.1)	8 (57.1)	10 (32.2)	12 (33.3)	0.31
Medical history, n (%)						
Hypertension	187 (94.4)	108 (96.4)	14 (93.3)	29 (90.6)	36 (92.3)	0.55
Dyslipidemia	198 (100)	112 (100)	15 (100)	32 (100)	39 (100)	
Previous myocardial infarction	92 (46.5)	49 (43.8)	7 (46.7)	14 (43.8)	22 (56.4)	0.58
Previous angina pectoris	111 (56.1)	65 (58.0)	9 (60.0)	18 (56.3)	19 (48.7)	0.77
Medications, n (%)						
Antiplatelet drugs	193 (97.5)	110 (98.2)	14 (93.3)	30 (93.8)	39 (100)	0.25
Statins	198 (100)	112 (100)	15 (100)	32 (100)	39 (100)	
Fibrates	1 (0.5)	1 (0.89)	0 (0)	0 (0)	0 (0)	0.86
Eicosapentaenoic acids	14 (7.1)	9 (8.0)	1 (6.7)	2 (6.3)	2 (5.1)	0.94
Ezetimibe	18 (9.1)	10 (8.9)	0 (0)	2 (6.3)	6 (15.4)	0.30
ACEIs/ARBs	144 (72.7)	80 (71.4)	10 (66.7)	25 (78.1)	29 (74.4)	0.83
β-blockers	89 (44.9)	53 (47.3)	8 (53.3)	10 (31.3)	18 (46.2)	0.37
Calcium-channel blockers	100 (50.5)	60 (53.6)	8 (53.3)	15 (46.9)	17 (43.6)	0.71
Diuretics	31 (15.7)	20 (17.9)	2 (13.3)	5 (15.6)	4 (10.3)	0.72
Any-antidiabetic drugs	165 (83.3)	97 (86.6)	12 (80.0)	27 (84.4)	29 (74.4)	0.35
Oral antidiabetic drugs	149 (75.3)	86 (76.8)	10 (66.7)	25 (78.1)	28 (71.8)	0.77
Insulin	33 (16.7)	25 (22.3)	2 (13.3)	2 (6.3)	4 (10.3)	0.10
Framingham risk score, %	16 ± 8	14 ± 8	16 ± 6	16 ± 8	21 ± 9	<0.001

sdLDL-C indicates small dense low-density lipoprotein cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; ACEIs, angiotensin-converting enzyme inhibitors; ARBs, angiotensin receptor blockers; FMD, flow-mediated vasodilation.

Group 1 indicates TG of <150 mg/dL and sdLDL-C of <40.0 mg/dL; group 2, TG of <150 mg/dL and sdLDL-C of ≥ 40.0 mg/dL; group 3, TG of ≥ 150 mg/dL and sdLDL-C of <40.0 mg/dL; group 4, TG of ≥ 150 mg/dL and sdLDL-C of ≥ 40.0 mg/dL.

0.73-3.68;  $p=0.23$ ) (**Supplementary Table 7**).

### Relationships of the Combination of Serum sdLDL-C Level and TG Level with Cardiovascular Events

Next, we divided the subjects into four groups according to the sdLDL-C cut-off value of 40.0 mg/dL and TG cut-off value of 150 mg/dL: group 1, TG

of <150 mg/dL and sdLDL of <40.0 mg/dL; group 2, TG of <150 mg/dL and sdLDL of ≥ 40.0 mg/dL; group 3, TG of ≥ 150 mg/dL and sdLDL-C of <40.0 mg/dL; group 4, TG of ≥ 150 mg/dL and sdLDL-C of ≥ 40.0 mg/dL. Clinical characteristics of the subjects and clinical events are summarized in **Table 2**. Age, diastolic blood pressure, total cholesterol, TG,

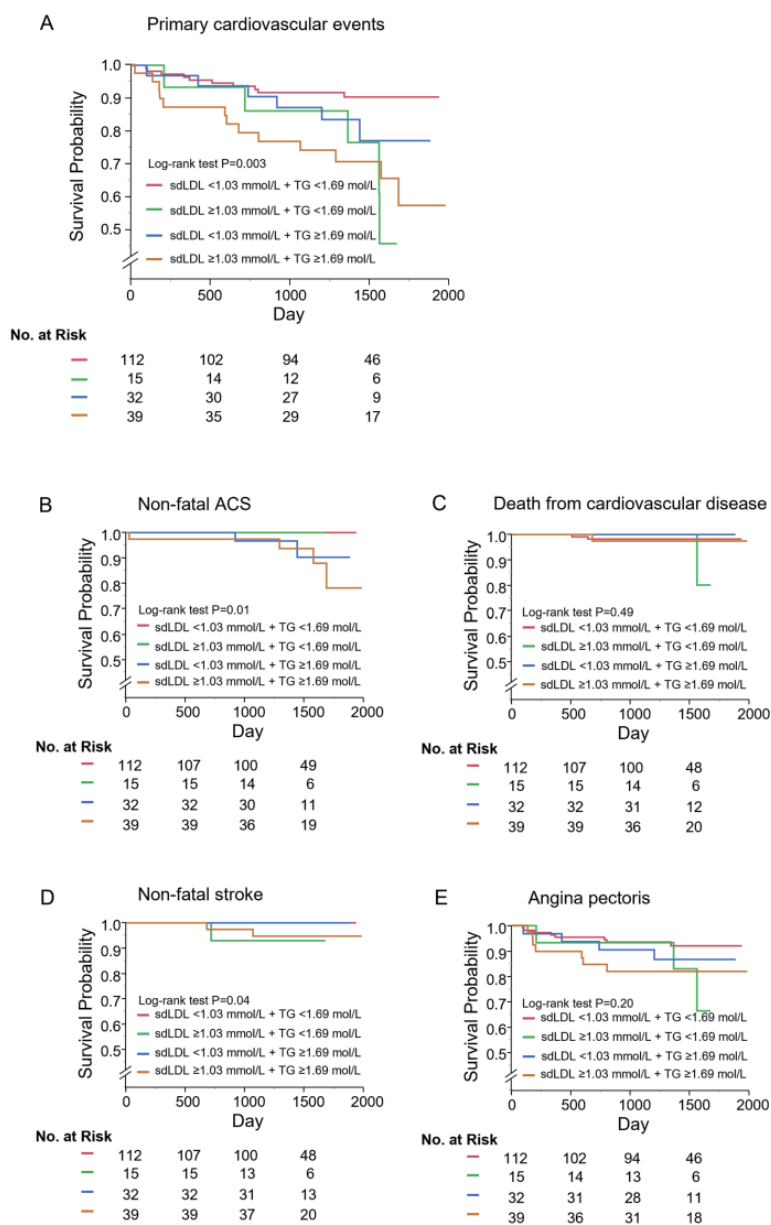


Fig. 2.

Kaplan-Meier curves of cumulative event-free survival of first major cardiovascular events (A), non-fatal acute coronary syndrome (B), death from cardiovascular disease (C), non-fatal stroke (D), and angina pectoris (E) among the four groups according to serum small dense low-density lipoprotein cholesterol (sdLDL-C) levels and triglycerides (TG) levels.

HDL-C, LDL-C, sdLDL-C, glucose, HbA1c, and Framingham risk score were significantly different among the four groups. There were no significant differences in other parameters among the four groups.

During the follow-up period, the incidences of primary cardiovascular events, non-fatal stroke, and non-fatal ACS were significantly higher in the high sdLDL group than in the low sdLDL group (Supplementary Table 8).

Kaplan-Meier curves for the four groups according to the cut-off values of sdLDL-C and TG showed that primary cardiovascular events (log-rank  $p=0.003$ ; Fig. 2A), non-fatal ACS (log-rank  $p=0.01$ ; Fig. 2B) and non-fatal stroke (log-rank  $p=0.04$ ; Fig. 2D) were significantly different. Multivariate Cox proportional hazard analysis revealed that patients with a combination of sdLDL-C of  $\geq 40.0$  mg/dL and TG of  $\geq 150$  mg/dL (hazard ratio, 4.38; 95% CI, 1.78-10.8;  $p=0.001$ ) and patients with a combination



**Table 3.** Associations of sdLDL-C Levels and TG Levels with Primary Cardiovascular Events During Follow-up

Variable	Unadjusted HR (95% CI) <i>P</i> value	Adjusted* HR (95% CI) <i>P</i> value	Adjusted† HR (95% CI) <i>P</i> value	Adjusted‡ HR (95% CI) <i>P</i> value
sdLDL-C mg/dL				
Group 1	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Group 2	4.06 (1.38-11.9) 0.01	4.22 (1.42-12.6) 0.01	4.72 (1.55-14.4) 0.006	5.32 (1.62-17.4) 0.006
Group 3	2.21 (0.80-6.09) 0.12	2.30 (0.83-6.40) 0.11	2.36 (0.84-6.58) 0.10	2.36 (0.83-6.72) 0.11
Group 4	3.97 (1.74-9.06) 0.001	4.17 (1.79-9.70) <0.001	4.47 (1.92-10.4) <0.001	4.38 (1.78-10.8) 0.001

sdLDL-C indicates small dense low-density lipoprotein cholesterol; TG, triglycerides; CI, confidence interval; HR, hazard ratio.

\* adjusted for age, gender.

† adjusted for age, gender, body mass index, hypertension and current smoking.

‡ adjusted for age, gender, body mass index, systolic blood pressure, LDL-C, glucose and current smoking.

Group 1 indicates TG of <150 mg/dL and sdLDL-C of <40.0 mg/dL; group 2, TG of <150 mg/dL and sdLDL-C of ≥ 40.0 mg/dL; group 3, TG of ≥ 150 mg/dL and sdLDL-C of <40.0 mg/dL; group 4, TG of ≥ 150 mg/dL and sdLDL-C of ≥ 40.0 mg/dL.

of sdLDL-C of ≥ 40.0 mg/dL and TG of <150 mg/dL (hazard ratio, 5.32; 95% CI, 1.62-17.4;  $p=0.006$ ) had significantly higher incidences of primary cardiovascular events than did patients with a combination of sdLDL-C of <40.0 mg/dL and TG of <150 mg/dL. There was no significant difference in the incidence of primary cardiovascular events between patients with a combination of sdLDL-C of <40.0 mg/dL and TG of ≥ 150 mg/dL and patients with a combination of sdLDL-C of <40.0 mg/dL and TG of <150 mg/dL (hazard ratio, 2.36; 95% CI, 0.83-6.72;  $p=0.11$ ) (Table 3).

### Relationship of Serum LDL-C Levels with Cardiovascular Events

Finally, we assessed the relationship between LDL-C levels and cardiovascular events. We divided the subjects into two groups according to the cut-off value of serum LDL-C levels of 70 mg/dL. Clinical characteristics of the subjects in the two groups according to LDL-C levels are summarized in Supplementary Table 9. Total cholesterol, HDL-C, sdLDL-C, use of insulin, and Framingham risk score were significantly different in the two groups. There were no significant differences in other parameters between the two groups.

Univariate analysis revealed that LDL-C levels were positively correlated with total cholesterol, sdLDL-C and Framingham risk score, and LDL-C levels was negatively correlated with glucose (Supplementary Table 10). Clinical events on the basis of serum LDL-C levels are summarized in Supplementary Table 11. During the follow-up period, there were no significant differences in the incidences of primary cardiovascular events between

the low LDL-C group and the high LDL-C group. Kaplan-Meier analysis showed that incidences of primary cardiovascular events (log-rank  $p=0.57$ ; Supplementary Fig. 4A), non-fatal ACS (log-rank  $p=0.80$ ; Supplementary Fig. 4B), death from cardiovascular disease (log-rank  $p=0.75$ ; Supplementary Fig. 4C), non-fatal stroke (log-rank  $p=0.33$ ; Supplementary Fig. 4D), and angina pectoris (log-rank  $p=0.99$ ; Supplementary Fig. 4E) were similar in the two groups.

We assessed the ROC curves of serum sdLDL-C and LDL-C levels for predicting primary cardiovascular events. The area under the ROC curve value for sdLDL-C to predict primary cardiovascular events was significantly higher than that for LDL-C (0.67 vs. 0.54,  $p=0.03$ ) (Supplementary Fig. 5).

### Discussion

In the present study, we demonstrated that high serum sdLDL-C levels were significant predictors of cardiovascular events in patients with established CAD and type 2 DM who were receiving statins. However, serum TG levels were not significant predictors of cardiovascular events in those subjects after adjustment for cardiovascular risk factors including serum sdLDL-C. In addition, the incidence of cardiovascular events was significantly higher in patients with CAD and type 2 DM who had serum sdLDL-C of ≥ 40.0 mg/dL and TG of <150 mg/dL, but not patients with sdLDL-C of <40.0 mg/dL and TG of ≥ 150 mg/dL, than in patients with serum sdLDL-C of <40.0 mg/dL and TG of <150 mg/dL. To our knowledge, this study is the first prospective multicenter study that was carried out to evaluate the

roles of sdLDL-C levels and TG levels in the prediction of cardiovascular events in patients with CAD and type 2 DM who were receiving statins.

First, to evaluate the association of serum sdLDL-C levels with cardiovascular events in patients with CAD and type 2 DM who were receiving statins, we divided the subjects into two groups of serum sdLDL-C levels. Sekimoto *et al.* showed that subjects after ACS with serum sdLDL-C of  $\geq 20.9$  mg/dL had significantly higher incidences of cardiovascular events than those for patients with sdLDL-C of  $< 20.9$  mg/dL<sup>29</sup>). Higashioka *et al.* showed that subjects without CAD with serum sdLDL-C of  $\geq 35.0$  mg/dL had significantly higher incidences of cardiovascular events than those for patients with sdLDL-C of  $< 35.0$  mg/dL<sup>30</sup>). However, the cut-off value of sdLDL-C levels has not yet been established in patients with CAD and type 2 DM. Therefore, we determined the cut off value of sdLDL-C levels by ROC curves for predicting cardiovascular events. Patients with serum sdLDL-C levels above the cut-off value of 40.0 mg/dL had a significantly higher incidence of primary cardiovascular events than that for patients with sdLDL-C levels below the cut-off value after adjustment for cardiovascular risk factors including serum TG. A previous study showed that serum sdLDL-C levels were correlated with mean intima-media thickness of the carotid artery in patients with type 2 DM<sup>31</sup>). Some studies have shown that increased serum sdLDL-C was significantly associated with a higher incidence of cardiovascular events. The adjusted hazard ratio (95% CI) for cardiovascular events per every 10 mg/dL increase in sdLDL-C was 1.210 (1.003-1.459) in patients with CAD<sup>32</sup>). Jin *et al.* reported that subjects with elevated serum sdLDL-C and DM had a higher incidence of cardiovascular events than that in subjects without elevated serum sdLDL-C and DM<sup>33</sup>). Interestingly, in the present study, serum LDL-C of  $\geq 70$  mg/dL was not a significantly higher risk for cardiovascular events. A high LDL-C level is an established cardiovascular risk factor and the benefit of statin treatment is also well established<sup>34</sup>). In the present study, all of the patients were treated with statins, and their mean serum LDL-C levels were controlled under 100 mg/dL and under 70 mg/dL in 44 patients. Serum LDL-C of  $\geq 70$  mg/dL might not be a significantly higher risk for cardiovascular events in patients with CAD and type 2 DM who are receiving statins. In addition, the area under the ROC curve value for sdLDL-C to predict primary cardiovascular events was significantly higher than that for LDL-C in these patients. A previous study showed that subjects after CAD with serum sdLDL  $> 35$  mg/dL had a

higher incidence of cardiovascular events than that in subjects with LDL-C  $> 100$  mg/dL<sup>35</sup>). This results of this study are consistent with the results of that study. Previous interventional studies showed that dietary interventions<sup>36</sup>), physical exercise<sup>37</sup>), and pharmacological therapy<sup>38</sup>) reduced sdLDL-C levels. Ishi *et al.* showed that high-dose statin therapy (4 mg pitavastatin) resulted in a 20% reduction in sdLDL-C levels compared to low-dose statin therapy (1 mg pitavastatin). Furthermore, in patients with sdLDL-C  $> 34.3$  mg/dL with established stable CAD, high-dose statin therapy (4 mg pitavastatin) significantly reduced the risk of major cardiovascular events (cardiovascular death, nonfatal ischemic stroke, nonfatal myocardial infarction, and unstable angina) compared to low-dose statin therapy (1 mg pitavastatin)<sup>38</sup>).

Finally, we divided the subjects into four groups according to the serum sdLDL-C cut-off value of 40.0 mg/dL and TG cut-off value of 150 mg/dL and assessed cardiovascular events. Interestingly, patients with serum sdLDL-C of  $\geq 40.0$  mg/dL and TG of  $< 150$  mg/dL had a higher incidence of cardiovascular events than did patients with serum sdLDL-C of  $< 40.0$  mg/dL and TG of  $< 150$  mg/dL. However, patients with serum sdLDL-C of  $< 40.0$  mg/dL and TG of  $\geq 150$  mg/dL did not have a higher incidence of cardiovascular events than that in patients with serum sdLDL-C of  $< 40.0$  mg/dL and TG of  $< 150$  mg/dL. These findings suggest that even though serum TG levels were controlled at  $< 150$  mg/dL, some subjects had serum sdLDL-C of  $\geq 40.0$  mg/dL and had a high risk for cardiovascular events.

This study has some limitations. First, we measured serum sdLDL-C levels only once when the subjects were enrolled. Repeated measurements of serum sdLDL-C levels and assessment of the change in serum sdLDL-C levels would enable more specific conclusions concerning the role of sdLDL-C levels in cardiovascular events to be drawn. In addition, the cut-off value of serum sdLDL-C for cardiovascular events in patients with CAD and type 2 DM has not been established. The results of the present study may be useful for determining the cut-off of value serum sdLDL-C for cardiovascular events in patients with CAD and type 2 DM. Second, it has been shown that statins decrease serum sdLDL-C levels<sup>38, 39</sup>). In the present study, we included patients with statin treatment and excluded patients without statin treatment to assess the residual risk for cardiovascular events under the condition of treatment with statins. Future studies are needed to evaluate the association of sdLDL-C levels with cardiovascular events in a general population. Third, we cannot completely exclude subjects with familial hypercholesterolemia

and combined hyperlipidemia. In the present study, we did not have information on untreated LDL-C levels, presence of tendon and skin xanthomas, family history of premature CAD, FH causative genes, and dose of the statin. Such information would enable more specific conclusions concerning the roles of sdLDL-C and TG in cardiovascular events to be drawn. Finally, all of the patients were Japanese. The doses of statins and effects of statins vary in different countries. Asians are often treated with lower statin doses than those for Caucasian. Indeed, the Japanese Lipid Intervention Trial showed that statin doses were about one-fourth of those in Western countries to obtain similar levels of LDL-C in Japanese with hypercholesterolemia<sup>40</sup>.

### Conclusions

In conclusion, patients with CAD and type 2 DM who had sdLDL-C levels of  $\geq 40.0$  mg/dL and were taking statins had a high risk for cardiovascular events even though serum TG levels were controlled at  $< 150$  mg/dL. We should pay attention to not only serum TG levels but also sdLDL-C levels as residual risks for cardiovascular events under the condition of treatment with statins in patients with CAD and type 2 DM.

### Acknowledgements

The authors would like to thank all of the subjects who participated in this study. In addition, we thank Megumi Wakisaka, Ki-ichiro Kawano and Satoko Michiyama for their excellent secretarial assistance.

### Role of Authors

Yukihito Higashi and Takayuki Yamaji drafting the article and conception of this study; Takahiro Harada, Masato Kajikawa, Tatsuya Maruhashi, Shinji Kishimoto, Farina Mohamad Yusoff, Chikara Goto, Ayumu Nakashima, Hirofumi Tomiyama, Bonpei Takase, Takahide Kohro, Toru Suzuki, Tomoko Ishizu, Shinichiro Ueda, Tsutomu Yamazaki, Tomoo Furumoto, Kazuomi Kario, Teruo Inoue, Shinji Koba, Kentaro Watanabe, Yasuhiko Takemoto, Takuzo Hano, Masataka Sata, Yutaka Ishibashi, Koichi Node, Koji Maemura, Yusuke Ohya, Taiji Furukawa, Hiroshi Ito, and Akira Yamashina, measuring the brachial artery diameter and FMD; Yukiko Nakano, Kenichi Yoshimura, and Kazuaki Chayama, revising the article critically for important intellectual content.

### Sources of Funding

Grant-in-Aid of Japanese Arteriosclerosis Prevention Fund (to Y.Higashi).

### Conflict of Interest

All authors have no conflicts of interests to report.

### Trial Registration Number

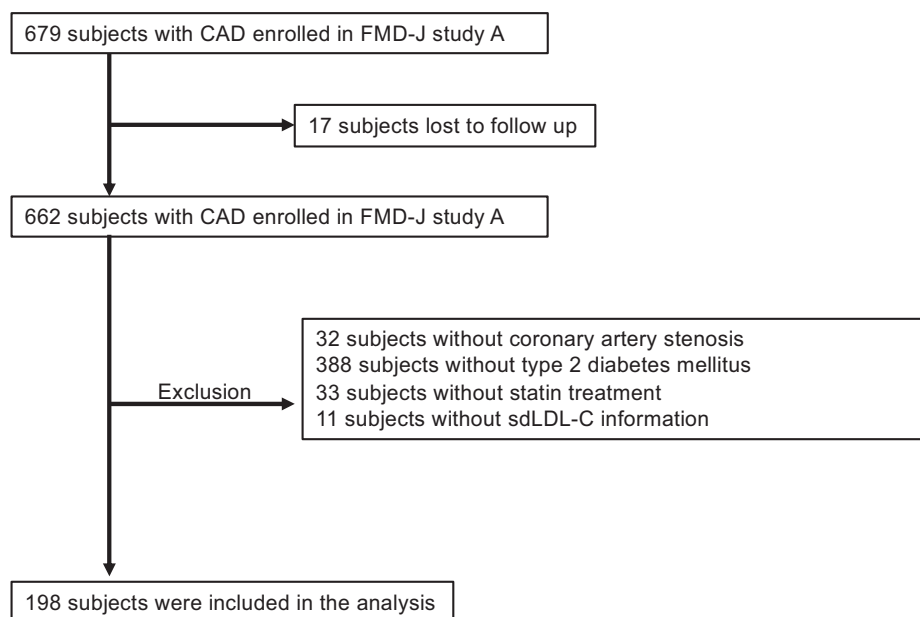
The protocol was registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN000012950).

### References

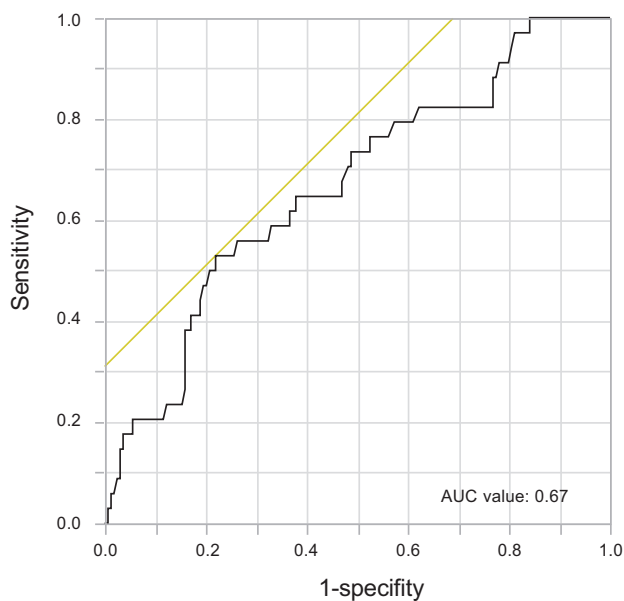
- 1) Haffner SM, Lehto S, Rönnemaa T, Pyörälä K and Laakso M: Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *The New England journal of medicine*, 1998; 339: 229-234
- 2) 10. Cardiovascular Disease and Risk Management: Standards of Medical Care in Diabetes-2019. *Diabetes Care*, 2019; 42: S103-s123
- 3) Cannon CP, Braunwald E, McCabe CH, Rader DJ, Rouleau JL, Belder R, Joyal SV, Hill KA, Pfeffer MA and Skene AM: Intensive versus moderate lipid lowering with statins after acute coronary syndromes. *The New England journal of medicine*, 2004; 350: 1495-1504
- 4) Pitt B, Waters D, Brown WV, van Boven AJ, Schwartz L, Title LM, Eisenberg D, Shurzinske L and McCormick LS: Aggressive lipid-lowering therapy compared with angioplasty in stable coronary artery disease. *Atorvastatin versus Revascularization Treatment Investigators*. *The New England journal of medicine*, 1999; 341: 70-76
- 5) Kajikawa M, Maruhashi T, Kishimoto S, Matsui S, Hashimoto H, Takaeko Y, Yusoff FM, Kihara Y, Chayama K, Goto C, Noma K, Nakashima A, Tomiyama H, Takase B, Kohro T, Suzuki T, Ishizu T, Ueda S, Yamazaki T, Furumoto T, Kario K, Inoue T, Koba S, Watanabe K, Takemoto Y, Hano T, Sata M, Ishibashi Y, Node K, Maemura K, Ohya Y, Furukawa T, Ito H, Ikeda H, Yamashina A and Higashi Y: Target of Triglycerides as Residual Risk for Cardiovascular Events in Patients With Coronary Artery Disease - Post Hoc Analysis of the FMD-J Study A. *Circulation journal : official journal of the Japanese Circulation Society*, 2019; 83: 1064-1071
- 6) Miller M, Cannon CP, Murphy SA, Qin J, Ray KK and Braunwald E: Impact of triglyceride levels beyond low-density lipoprotein cholesterol after acute coronary syndrome in the PROVE IT-TIMI 22 trial. *Journal of the American College of Cardiology*, 2008; 51: 724-730
- 7) Hokanson JE and Austin MA: Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. *J Cardiovasc*

- Risk, 1996; 3: 213-219
- 8) Wang J, Shen X, He S, An Y, Gong Q, Li H, Zhang B, Shuai Y, Chen Y, Hu Y and Li G: Hypertriglyceridaemia predicts subsequent long-term risk of cardiovascular events in Chinese adults: 23-year follow-up of the Daqing Diabetes Study. *Diabetes Metab Res Rev*, 2019; 35: e3163
  - 9) Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *Jama*, 2001; 285: 2486-2497
  - 10) Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, Chapman MJ, De Backer GG, Delgado V, Ference BA, Graham IM, Halliday A, Landmesser U, Mihaylova B, Pedersen TR, Riccardi G, Richter DJ, Sabatine MS, Taskinen MR, Tokgozoglu L and Wiklund O: 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. *Eur Heart J*, 2020; 41: 111-188
  - 11) Kinoshita M, Yokote K, Arai H, Iida M, Ishigaki Y, Ishibashi S, Umemoto S, Egusa G, Ohmura H, Okamura T, Kihara S, Koba S, Saito I, Shoji T, Daida H, Tsukamoto K, Deguchi J, Dohi S, Dobashi K, Hamaguchi H, Hara M, Hiro T, Biro S, Fujioka Y, Maruyama C, Miyamoto Y, Murakami Y, Yokode M, Yoshida H, Rakugi H, Wakatsuki A and Yamashita S: Japan Atherosclerosis Society (JAS) Guidelines for Prevention of Atherosclerotic Cardiovascular Diseases 2017. *J Atheroscler Thromb*, 2018; 25: 846-984
  - 12) Madsen CM, Varbo A and Nordestgaard BG: Unmet need for primary prevention in individuals with hypertriglyceridaemia not eligible for statin therapy according to European Society of Cardiology/European Atherosclerosis Society guidelines: a contemporary population-based study. *Eur Heart J*, 2018; 39: 610-619
  - 13) Sarwar N, Sandhu MS, Ricketts SL, Butterworth AS, Di Angelantonio E, Boekholdt SM, Ouwehand W, Watkins H, Samani NJ, Saleheen D, Lawlor D, Reilly MP, Hingorani AD, Talmud PJ and Danesh J: Triglyceride-mediated pathways and coronary disease: collaborative analysis of 101 studies. *Lancet (London, England)*, 2010; 375: 1634-1639
  - 14) Nordestgaard BG and Varbo A: Triglycerides and cardiovascular disease. *Lancet (London, England)*, 2014; 384: 626-635
  - 15) Hoogeveen RC, Gaubatz JW, Sun W, Dodge RC, Crosby JR, Jiang J, Couper D, Virani SS, Kathiresan S, Boerwinkle E and Ballantyne CM: Small dense low-density lipoprotein-cholesterol concentrations predict risk for coronary heart disease: the Atherosclerosis Risk In Communities (ARIC) study. *Arterioscler Thromb Vasc Biol*, 2014; 34: 1069-1077
  - 16) Austin MA, King MC, Vranizan KM and Krauss RM: Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk. *Circulation*, 1990; 82: 495-506
  - 17) Feingold KR, Grunfeld C, Pang M, Doerrler W and Krauss RM: LDL subclass phenotypes and triglyceride metabolism in non-insulin-dependent diabetes. *Arterioscler Thromb*, 1992; 12: 1496-1502
  - 18) Hirano T, Oi K, Sakai S, Kashiwazaki K, Adachi M and Yoshino G: High prevalence of small dense LDL in diabetic nephropathy is not directly associated with kidney damage: a possible role of postprandial lipemia. *Atherosclerosis*, 1998; 141: 77-85
  - 19) Hirano T, Kodera R, Hirashima T, Suzuki N, Aoki E, Hosoya M, Oshima T, Hayashi T, Koba S, Ohta M, Satoh N and Ito Y: Metabolic Properties of Low-density Lipoprotein (LDL) Triglycerides in Patients with Type 2 Diabetes, Comparison with Small Dense LDL-Cholesterol. *J Atheroscler Thromb*, 2022; 29: 762-774
  - 20) Tomiyama H, Kohro T, Higashi Y, Takase B, Suzuki T, Ishizu T, Ueda S, Yamazaki T, Furumoto T, Kario K, Inoue T, Koba S, Watanabe K, Takemoto Y, Hano T, Sata M, Ishibashi Y, Node K, Maemura K, Ohya Y, Furukawa T, Ito H and Yamashina A: A multicenter study design to assess the clinical usefulness of semi-automatic measurement of flow-mediated vasodilatation of the brachial artery. *Int Heart J*, 2012; 53: 170-175
  - 21) Maruhashi T, Soga J, Fujimura N, Idei N, Mikami S, Iwamoto Y, Iwamoto A, Kajikawa M, Matsumoto T, Oda N, Kishimoto S, Matsui S, Hashimoto H, Takaeko Y, Yamaji T, Harada T, Han Y, Aibara Y, Mohamad Yusoff F, Hidaka T, Kihara Y, Chayama K, Noma K, Nakashima A, Goto C, Tomiyama H, Takase B, Kohro T, Suzuki T, Ishizu T, Ueda S, Yamazaki T, Furumoto T, Kario K, Inoue T, Koba S, Watanabe K, Takemoto Y, Hano T, Sata M, Ishibashi Y, Node K, Maemura K, Ohya Y, Furukawa T, Ito H, Ikeda H, Yamashina A and Higashi Y: Increased arterial stiffness and cardiovascular risk prediction in controlled hypertensive patients with coronary artery disease: post hoc analysis of FMD-J (Flow-mediated Dilation Japan) Study A. *Hypertens Res*, 2020;
  - 22) Hirano T and Ito Y: Accuracy of Small Dense Low-density Lipoprotein-cholesterol Concentration Estimated via Sampson's Equation in Healthy Subjects and Patients with Diabetes. *J Atheroscler Thromb*, 2023; 30: 979-989
  - 23) Ito Y, Fujimura M, Ohta M and Hirano T: Development of a homogeneous assay for measurement of small dense LDL cholesterol. *Clin Chem*, 2011; 57: 57-65
  - 24) 2. Classification and Diagnosis of Diabetes. *Diabetes Care*, 2017; 40: S11-s24
  - 25) Shimamoto K, Ando K, Fujita T, Hasebe N, Higaki J, Horiuchi M, Imai Y, Imaizumi T, Ishimitsu T, Ito M, Ito S, Itoh H, Iwao H, Kai H, Kario K, Kashiwara N, Kawano Y, Kim-Mitsuyama S, Kimura G, Kohara K, Komuro I, Kumagai H, Matsuura H, Miura K, Morishita R, Naruse M, Node K, Ohya Y, Rakugi H, Saito I, Saitoh S, Shimada K, Shimosawa T, Suzuki H, Tamura K, Tanahashi N, Tsuchihashi T, Uchiyama M, Ueda S and Umemura S: The Japanese Society of Hypertension Guidelines for the Management of Hypertension (JSH 2014). *Hypertens Res*, 2014; 37: 253-390
  - 26) Klose G, Beil FU, Dieplinger H, von Eckardstein A, Foger B, Gouni-Berthold I, Heigl F, Koenig W, Kostner GM, Landmesser U, Laufs U, Leistikow F, Marz W, Noll G, Parhofer KG, Paulweber B, Riesen WF, Schaefer JR, Steinhagen-Thiessen E, Steinmetz A, Toplak H, Wanner C and Windler E: New AHA and ACC guidelines on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk. *Wien Klin Wochenschr*, 2014; 126: 169-175

- 27) Wilson PW, Castelli WP and Kannel WB: Coronary risk prediction in adults (the Framingham Heart Study). *Am J Cardiol*, 1987; 59: 91g-94g
- 28) Maruhashi T, Soga J, Fujimura N, Idei N, Mikami S, Iwamoto Y, Iwamoto A, Kajikawa M, Matsumoto T, Oda N, Kishimoto S, Matsui S, Hashimoto H, Aibara Y, Mohamad Yusoff F, Hidaka T, Kihara Y, Chayama K, Noma K, Nakashima A, Goto C, Tomiyama H, Takase B, Kohro T, Suzuki T, Ishizu T, Ueda S, Yamazaki T, Furumoto T, Kario K, Inoue T, Koba S, Watanabe K, Takemoto Y, Hano T, Sata M, Ishibashi Y, Node K, Maemura K, Ohya Y, Furukawa T, Ito H, Ikeda H, Yamashina A and Higashi Y: Endothelial Dysfunction, Increased Arterial Stiffness, and Cardiovascular Risk Prediction in Patients With Coronary Artery Disease: FMD-J (Flow-Mediated Dilation Japan) Study A. *J Am Heart Assoc*, 2018; 7:
- 29) Sekimoto T, Koba S, Mori H, Sakai R, Arai T, Yokota Y, Sato S, Tanaka H, Masaki R, Oishi Y, Ogura K, Arai K, Nomura K, Kosaki R, Sakai K, Tsujita H, Kondo S, Tsukamoto S, Tsunoda F, Shoji M, Matsumoto H, Hamazaki Y and Shinke T: Small Dense Low-Density Lipoprotein Cholesterol: A Residual Risk for Rapid Progression of Non-Culprit Coronary Lesion in Patients with Acute Coronary Syndrome. *J Atheroscler Thromb*, 2021; 28: 1161-1174
- 30) Higashioka M, Sakata S, Honda T, Hata J, Shibata M, Yoshida D, Goto K, Kitazono T, Osawa H and Ninomiya T: The Association of Small Dense Low-Density Lipoprotein Cholesterol and Coronary Heart Disease in Subjects at High Cardiovascular Risk. *J Atheroscler Thromb*, 2021; 28: 79-89
- 31) Inukai T, Yamamoto R, Suetsugu M, Matsumoto S, Wakabayashi S, Inukai Y, Matsutomo R, Takebayashi K and Aso Y: Small low-density lipoprotein and small low-density lipoprotein/total low-density lipoprotein are closely associated with intima-media thickness of the carotid artery in Type 2 diabetic patients. *J Diabetes Complications*, 2005; 19: 269-275
- 32) Sakai K, Koba S, Nakamura Y, Yokota Y, Tsunoda F, Shoji M, Itoh Y, Hamazaki Y and Kobayashi Y: Small dense low-density lipoprotein cholesterol is a promising biomarker for secondary prevention in older men with stable coronary artery disease. *Geriatr Gerontol Int*, 2018; 18: 965-972
- 33) Jin JL, Zhang HW, Cao YX, Liu HH, Hua Q, Li YF, Zhang Y, Wu NQ, Zhu CG, Xu RX, Gao Y, Li XL, Cui CJ, Liu G, Sun J, Dong Q, Guo YL and Li JJ: Association of small dense low-density lipoprotein with cardiovascular outcome in patients with coronary artery disease and diabetes: a prospective, observational cohort study. *Cardiovasc Diabetol*, 2020; 19: 45
- 34) Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, Kirby A, Sourjina T, Peto R, Collins R and Simes R: Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet* (London, England), 2005; 366: 1267-1278
- 35) Nishikura T, Koba S, Yokota Y, Hirano T, Tsunoda F, Shoji M, Hamazaki Y, Suzuki H, Itoh Y, Katagiri T and Kobayashi Y: Elevated small dense low-density lipoprotein cholesterol as a predictor for future cardiovascular events in patients with stable coronary artery disease. *J Atheroscler Thromb*, 2014; 21: 755-767
- 36) Wang L, Bordi PL, Fleming JA, Hill AM and Kris-Etherton PM: Effect of a moderate fat diet with and without avocados on lipoprotein particle number, size and subclasses in overweight and obese adults: a randomized, controlled trial. *J Am Heart Assoc*, 2015; 4: e001355
- 37) Kotani K, Tsuzaki K, Sakane N and Taniguchi N: The Correlation Between Small Dense LDL and Reactive Oxygen Metabolites in a Physical Activity Intervention in Hyperlipidemic Subjects. *J Clin Med Res*, 2012; 4: 161-166
- 38) Ishii J, Kashiwabara K, Ozaki Y, Takahashi H, Kitagawa F, Nishimura H, Ishii H, Iimuro S, Kawai H, Muramatsu T, Naruse H, Iwata H, Tanizawa-Motoyama S, Ito H, Watanabe E, Matsuyama Y, Fukumoto Y, Sakuma I, Nakagawa Y, Hibi K, Hiro T, Hokimoto S, Miyauchi K, Ohtsu H, Izawa H, Ogawa H, Daida H, Shimokawa H, Saito Y, Kimura T, Matsuzaki M and Nagai R: Small Dense Low-Density Lipoprotein Cholesterol and Cardiovascular Risk in Statin-Treated Patients with Coronary Artery Disease. *J Atheroscler Thromb*, 2022; 29: 1458-1474
- 39) Nishikido T, Oyama J, Keida T, Ohira H and Node K: High-dose statin therapy with rosuvastatin reduces small dense LDL and MDA-LDL: The Standard versus high-dose therapy with Rosuvastatin for lipiD lowering (SARD) trial. *J Cardiol*, 2016; 67: 340-346
- 40) Matsuzaki M, Kita T, Mabuchi H, Matsuzawa Y, Nakaya N, Oikawa S, Saito Y, Sasaki J, Shimamoto K and Itakura H: Large scale cohort study of the relationship between serum cholesterol concentration and coronary events with low-dose simvastatin therapy in Japanese patients with hypercholesterolemia. *Circulation journal: official journal of the Japanese Circulation Society*, 2002; 66: 1087-1095



**Supplementary Fig. 1.** Flow chart of the study design



**Supplementary Fig. 2.** Receiver-operator characteristic (ROC) curve of serum small dense low-density lipoprotein cholesterol levels for predicting primary cardiovascular events

**Supplementary Table 1.** Univariate Analysis of Relationships of sdLDL-C with Variables

Variables	sdLDL-C	
	r	P value
Age, yr	-0.20	0.005
Body mass index, kg/m <sup>2</sup>	0.06	0.42
Systolic blood pressure, mmHg	0.11	0.11
Diastolic blood pressure, mmHg	0.21	0.003
Heart rate, bpm	0.20	0.005
Total cholesterol, mg/dL	0.56	<0.001
TG, mg/dL	0.61	<0.001
HDL-C, mg/dL	-0.19	0.01
LDL-C, mg/dL	0.36	<0.001
Glucose, mg/dL	0.24	<0.001
Hemoglobin A1c, %	0.32	<0.001
Creatinine, mg/dL	-0.11	0.13
eGFR, mL/min per 1.73 m <sup>2</sup>	0.16	0.02
Framingham risk score, %	0.34	<0.001

sdLDL-C indicates small dense low-density lipoprotein cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; Univariate analysis of the relations between sdLDL-C, TG levels and variables (Pearson's correlation analysis).

**Supplementary Table 2.** Clinical Outcomes of Subjects in Groups According to sdLDL-C Levels

Variables, n (%)	Total (n=198)	sdLDL-C <40.0 mg/dL (n=136)	sdLDL-C ≥ 40.0 mg/dL (n=62)	P value
Primary cardiovascular events	34 (17.2)	16 (11.1)	18 (33.3)	<0.001
Death from cardiovascular disease	3 (1.5)	2 (1.4)	1 (1.9)	0.81
Non-fatal stroke	3 (1.5)	0 (0)	3 (5.6)	0.004
Angina pectoris	22 (11.1)	12 (8.3)	10 (18.5)	0.04
Non-fatal acute coronary syndrome	6 (3.0)	2 (1.4)	4 (7.4)	0.03

sdLDL-C indicates small dense low-density lipoprotein cholesterol

Primary cardiovascular events include death from cardiovascular disease, non-fatal acute coronary syndrome, non-fatal stroke, and angina pectoris.

**Supplementary Table 3.** Association of sdLDL-C Levels with Primary Cardiovascular Events During Follow-up

Variable sdLDL-C, mg/dL	Unadjusted HR (95% CI) P value	Adjusted* HR (95% CI) P value	Adjusted† HR (95% CI) P value	Adjusted‡ HR (95% CI) P value	Adjusted§ HR (95% CI) P value
<40.0	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
≥ 40.0	3.18 (1.62-6.23) <0.001	3.25 (1.63-6.47) <0.001	3.54 (1.77-7.09) <0.001	3.16 (1.48-6.79) 0.003	2.89 (1.11-7.48) 0.03

sdLDL-C indicates small dense low-density lipoprotein cholesterol; CI, confidence interval; HR, hazard ratio.

\*adjusted for age, gender.

†adjusted for age, gender, body mass index, hypertension and current smoking.

‡adjusted for age, gender, body mass index, triglyceride.

§adjusted for age, gender, body mass index, systolic blood pressure, triglyceride, low dense lipoprotein cholesterol, glucose and current smoking.

**Supplementary Table 4.** Clinical Characteristics of Subjects in Groups According to TG Levels

Variables	Total ( <i>n</i> =198)	TG < 150 mg/dL ( <i>n</i> =127)	TG ≥ 150 mg/dL ( <i>n</i> =71)	<i>P</i> value
Age, yr	64 ± 8	65 ± 8	62 ± 9	0.01
Gender, men/women	161/37	99/28	62/9	0.10
Body mass index, kg/m <sup>2</sup>	25.9 ± 4.2	25.6 ± 4.1	26.6 ± 4.2	0.10
Systolic blood pressure, mmHg	133 ± 18	134 ± 18	131 ± 16	0.43
Diastolic blood pressure, mmHg	74 ± 10	74 ± 11	75 ± 10	0.75
Heart rate, bpm	66 ± 12	66 ± 11	67 ± 11	0.88
Total cholesterol, mg/dL	166 ± 30	161 ± 28	175 ± 31	0.002
TG, mg/dL	147 ± 111	93 ± 28	243 ± 137	N/A
HDL-C, mg/dL	50 ± 14	53 ± 14	44 ± 12	<0.001
LDL-C, mg/dL	88 ± 25	89 ± 22	85 ± 29	0.30
sdLDL-C, mg/dL	32.4 ± 14.8	26.2 ± 11.0	43.4 ± 14.2	<0.001
Glucose, mg/dL	141 ± 45	134 ± 36	152 ± 56	0.01
Hemoglobin A1c, %	7.2 ± 1.0	7.0 ± 0.9	7.4 ± 1.1	0.01
Creatinine, mg/dL	0.86 ± 0.24	0.86 ± 0.24	0.86 ± 0.25	0.92
eGFR, mL/min per 1.73 m <sup>2</sup>	71 ± 18	70 ± 19	72 ± 17	0.41
Current Smoking, <i>n</i> (%)	26 (13.4)	13 (10.3)	13 (19.1)	0.09
Exercise habit, <i>n</i> (%)	116 (60.4)	77 (61.6)	39 (58.2)	0.65
Alcohol drink, <i>n</i> (%)	66 (34.2)	44 (34.9)	22 (32.8)	0.77
Medical history, <i>n</i> (%)				
Hypertension	187 (94.4)	122 (96.1)	65 (91.6)	0.18
Previous myocardial infarction	92 (46.5)	56 (44.1)	36 (50.7)	0.37
Previous angina pectoris	111 (56.1)	74 (58.3)	37 (52.1)	0.40
Medications, <i>n</i> (%)				
Antiplatelet drugs	193 (97.5)	124 (97.6)	69 (97.2)	0.84
Statins	198 (100)	127 (100)	71 (100)	
Fibrates	1 (0.5)	1 (0.79)	0 (0)	0.45
Eicosapentaenoic acids	14 (7.1)	10 (7.9)	4 (5.6)	0.56
Ezetimibe	18 (9.1)	10 (7.9)	8 (11.3)	0.43
ACEIs/ARBs	144 (72.7)	90 (70.9)	54 (76.1)	0.43
β-blockers	89 (44.9)	61 (48.0)	28 (39.4)	0.24
Calcium-channel blockers	100 (50.5)	68 (53.5)	32 (45.1)	0.25
Diuretics	31 (15.7)	22 (17.3)	9 (12.7)	0.39
Any-antidiabetic drugs	165 (83.3)	109 (85.8)	56 (78.9)	0.21
Oral antidiabetic drugs	149 (75.3)	96 (75.6)	53 (74.7)	0.88
Insulin	33 (16.7)	27 (21.3)	6 (8.5)	0.02
Framingham risk score, %	16 ± 8	15 ± 7	19 ± 9	<0.001

TG indicates triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; sdLDL-C, small dense low-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; ACEIs, angiotensin-converting enzyme inhibitors; ARBs, angiotensin receptor blockers.



**Supplementary Table 5.** Univariate Analysis of Relationships of TG Levels with Variables

Variables	TG	
	r	P value
Age, yr	-0.25	<0.001
Body mass index, kg/m <sup>2</sup>	0.15	0.04
Systolic blood pressure, mmHg	-0.01	0.85
Diastolic blood pressure, mmHg	0.11	0.14
Heart rate, bpm	0.06	0.43
Total cholesterol, mg/dL	0.30	<0.001
HDL-C, mg/dL	-0.37	<0.001
LDL-C, mg/dL	-0.11	0.15
sdLDL-C, mg/dL	0.61	<0.001
Glucose, mg/dL	0.26	<0.001
Hemoglobin A1c, %	0.22	0.003
Creatinine, mg/dL	0.007	0.92
eGFR, mL/min per 1.73 m <sup>2</sup>	0.07	0.31
Framingham risk score, %	0.21	0.004

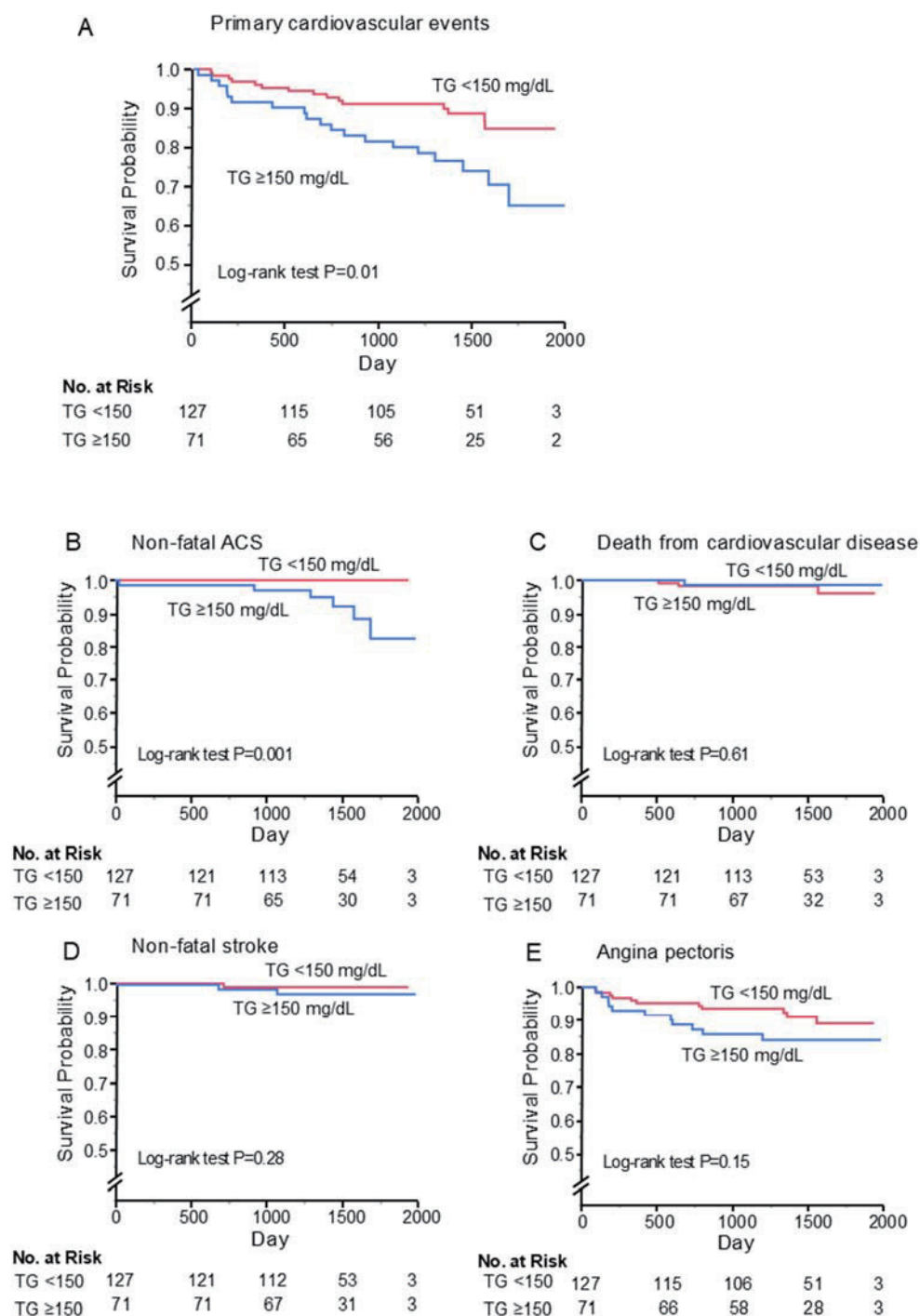
TG indicates triglycerides; sdLDL-C, small dense low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; Univariate analysis of the relations between sdLDL-C, TG levels and variables (Pearson's correlation analysis).

**Supplementary Table 6.** Clinical Outcomes of Subjects in Groups According to TG Levels

Variables, n (%)	Total (n=198)	TG <150 mg/dL (n=127)	TG ≥150 mg/dL (n=71)	P value
Primary cardiovascular events	34 (17.2)	15 (11.8)	19 (26.8)	0.008
Death from cardiovascular disease	3 (1.5)	3 (2.4)	0 (0)	0.19
Non-fatal stroke	3 (1.5)	1 (0.8)	2 (2.8)	0.26
Angina pectoris	22 (11.11)	11 (8.7)	11 (15.5)	0.14
Non-fatal acute coronary syndrome	6 (3.0)	0 (0)	6 (8.5)	<0.001

TG indicates triglycerides.

Primary cardiovascular events include death from cardiovascular disease, non-fatal acute coronary syndrome, non-fatal stroke, and angina pectoris.



**Supplementary Fig. 3.** Kaplan-Meier curves of cumulative event-free survival of first major cardiovascular events (A), non-fatal acute coronary artery syndrome (B), death from cardiovascular disease (C), non-fatal stroke (D), and angina pectoris (E) according to serum triglycerides (TG) levels

**Supplementary Table 7.** Association of TG Levels with Primary Cardiovascular Events During Follow-up

Variable TG, mg/dL	Unadjusted HR (95% CI) <i>P</i> value	Adjusted* HR (95% CI) <i>P</i> value	Adjusted† HR (95% CI) <i>P</i> value	Adjusted‡ HR (95% CI) <i>P</i> value	Adjusted§ HR (95% CI) <i>P</i> value
< 150	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
≥ 150	2.38 (1.21-4.68) 0.01	2.42 (1.21-4.84) 0.01	2.56 (1.29-5.13) 0.008	1.64 (0.73-3.68) 0.23	1.62 (0.68-3.87) 0.28

TG indicates triglycerides; CI, confidence interval; HR, hazard ratio.

\* adjusted for age, gender.

† adjusted for age, gender, body mass index, hypertension and current smoking.

‡ adjusted for age, gender, body mass index, small dense low lipoprotein cholesterol.

§ adjusted for age, gender, body mass index, systolic blood pressure, small dense low lipoprotein cholesterol, low dense lipoprotein cholesterol, glucose and current smoking.

**Supplementary Table 8.** Associations of sdLDL-C Levels and TG Levels with Primary Cardiovascular Events During Follow-up

Variables, <i>n</i> (%)	Group 1	Group 2	Group 3	Group 4	<i>P</i> value
	TG < 150 mg/dL		TG ≥ 150 mg/dL		
	sdLDL-C < 40.0 mg/dL ( <i>n</i> =112)	sdLDL-C ≥ 40.0 mg/dL ( <i>n</i> =15)	sdLDL-C < 40.0 mg/dL ( <i>n</i> =32)	sdLDL-C ≥ 40.0 mg/dL ( <i>n</i> =39)	
Primary cardiovascular events	10 (8.9)	5 (33.3)	6 (18.8)	13 (33.3)	0.002
Death from cardiovascular disease	2 (1.8)	1 (6.7)	0 (0)	0 (0)	0.28
Non-fatal stroke	0 (0)	1 (6.7)	0 (0)	2 (5.1)	0.04
Angina pectoris	8 (7.1)	3 (20.0)	4 (12.5)	7 (18.0)	0.18
Non-fatal acute coronary syndrome	0 (0)	0 (0)	2 (6.3)	4 (10.3)	0.01

TG indicates triglycerides; sdLDL-C, small dense low-density lipoprotein cholesterol.

Primary cardiovascular events include death from cardiovascular disease, non-fatal acute coronary syndrome, non-fatal stroke, and angina pectoris.

Group 1 indicates TG of < 150 mg/dL and sdLDL-C of < 40.0 mg/dL; group 2, TG of < 150 mg/dL and sdLDL-C of ≥ 40.0 mg/dL; group 3, TG of ≥ 150 mg/dL and sdLDL-C of < 40.0 mg/dL; group 4, TG of ≥ 150 mg/dL and sdLDL-C of ≥ 40.0 mg/dL.

**Supplementary Table 9.** Clinical Characteristics of Subjects in Groups According to LDL-C

Variables	Total (n=191)	LDL-C < 70 mg/dL (n=44)	LDL-C ≥ 70 mg/dL (n=147)	P value
Age, yr	64 ± 8	64 ± 8	64 ± 9	0.92
Gender, men/women	161/37	38/6	116/31	0.27
Body mass index, kg/m <sup>2</sup>	25.9 ± 4.2	25.8 ± 4.2	26.0 ± 4.1	0.85
Systolic blood pressure, mmHg	133 ± 18	128 ± 18	134 ± 17	0.06
Diastolic blood pressure, mmHg	74 ± 10	73 ± 9	75 ± 10	0.25
Heart rate, bpm	66 ± 12	68 ± 12	66 ± 11	0.27
Total cholesterol, mg/dL	166 ± 30	139 ± 20	173 ± 27	< 0.001
TG, mg/dL	147 ± 111	150 ± 90	131 ± 63	0.12
HDL-C, mg/dL	50 ± 14	50 ± 18	50 ± 13	0.99
LDL-C, mg/dL	88 ± 25	58 ± 13	97 ± 20	N/A
sdLDL-C, mg/dL	32.4 ± 14.8	25.9 ± 12.0	33.3 ± 13.6	0.002
Glucose, mg/dL	141 ± 45	144 ± 61	137 ± 38	0.38
Hemoglobin A1c, %	7.2 ± 1.0	7.0 ± 1.0	7.2 ± 1.0	0.30
Creatinine, mg/dL	0.86 ± 0.24	0.88 ± 0.28	0.85 ± 0.23	0.44
eGFR, mL/min per 1.73 m <sup>2</sup>	71 ± 18	70 ± 17	71 ± 18	0.79
Current Smoking, n (%)	26 (13.4)	9 (20.5)	17 (11.8)	0.15
Exercise habit, n (%)	111 (59.7)	29 (65.9)	82 (57.8)	0.33
Alcohol drink, n (%)	63 (33.7)	14 (31.8)	49 (34.3)	0.76
Medical history, n (%)				
Hypertension	181 (94.8)	40 (90.9)	141 (95.9)	0.19
Dyslipidemia	191 (100)	44 (100)	147 (100)	
Previous myocardial infarction	87 (45.6)	21 (47.7)	66 (44.9)	0.74
Previous angina pectoris	109 (57.1)	23 (52.3)	86 (58.5)	0.46
Medications, n (%)				
Antiplatelet drugs	186 (97.4)	43 (97.7)	143 (97.4)	0.87
Statin	191 (100)	44 (100)	147 (100)	
Fibrates	1 (0.5)	0 (0)	1 (0.7)	0.58
Eicosapentaenoic acids	14 (7.3)	3 (6.8)	11 (7.5)	0.88
Ezetimibe	18 (9.4)	4 (9.1)	14 (9.5)	0.93
ACEIs/ARBs	140 (73.3)	32 (72.7)	108 (73.5)	0.92
β-blockers	84 (44.0)	18 (40.9)	66 (44.9)	0.64
Calcium-channel blockers	99 (51.8)	22 (50.0)	77 (52.4)	0.78
Diuretics	30 (15.7)	6 (13.6)	24 (16.3)	0.67
Any-antidiabetic drugs	159 (83.3)	39 (88.7)	120 (81.6)	0.28
Oral antidiabetic drugs	143 (74.9)	33 (75.0)	110 (74.8)	0.98
Insulin	32 (16.8)	10 (22.7)	22 (15.0)	0.23
Framingham risk score, %	16 ± 8	13 ± 7	17 ± 8	0.01

LDL-C indicates low density lipoprotein cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; sdLDL-C, small dense low-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; ACEIs, angiotensin-converting enzyme inhibitors; ARBs, angiotensin receptor blockers.

**Supplementary Table 10.** Univariate Analysis of Relationships of LDL-C Levels with Variables

Variables	LDL-C	
	r	P value
Age, yr	-0.07	0.35
Body mass index, kg/m <sup>2</sup>	0.06	0.44
Systolic blood pressure, mmHg	0.15	0.04
Diastolic blood pressure, mmHg	0.11	0.14
Heart rate, bpm	0.06	0.39
Total cholesterol, mg/dL	0.86	<0.001
TG, mg/dL	-0.11	0.15
HDL-C, mg/dL	0.13	0.08
sdLDL-C, mg/dL	0.36	<0.001
Glucose, mg/dL	-0.19	0.01
Hemoglobin A1c, %	0.07	0.32
Creatinine, mg/dL	-0.07	0.36
eGFR, mL/min per 1.73 m <sup>2</sup>	-0.01	0.89
Framingham risk score, %	0.27	<0.001

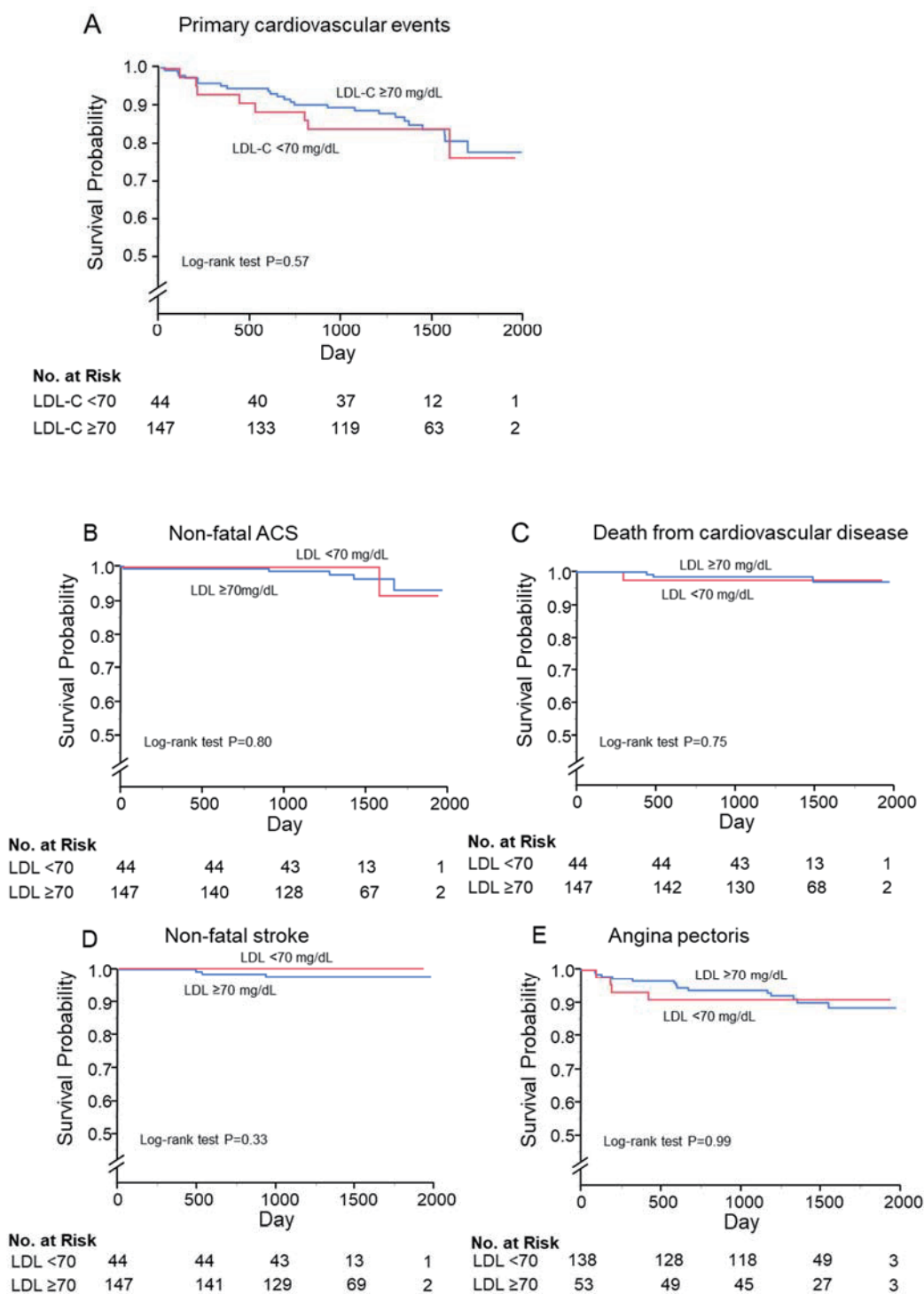
LDL-C indicate low-density lipoprotein cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; sdLDL-C, small dense low-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; Univariate analysis of the relations between LDL-C, levels and variables (Pearson's correlation analysis).

**Supplementary Table 11.** Clinical Outcomes of Subjects in Groups According to LDL-C Levels

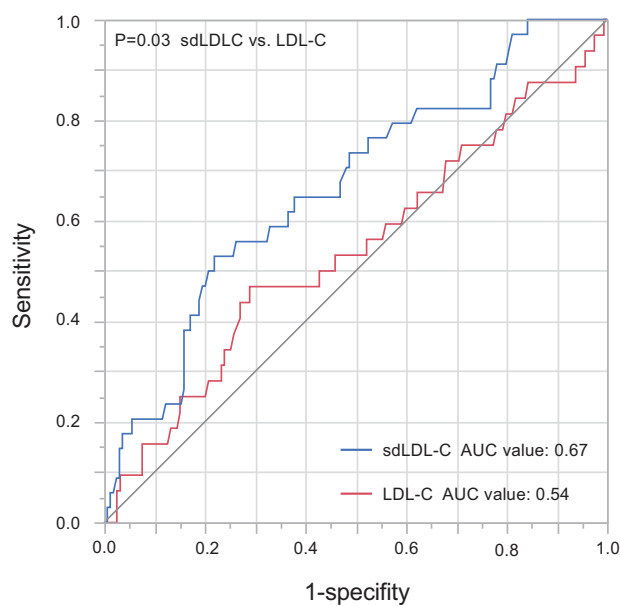
Variables, n (%)	Total (n=191)	LDL-C <70 mg/dL (n=44)	LDL-C ≥70 mg/dL (n=147)	P value
Primary cardiovascular events	32 (16.8)	8 (18.2)	24 (16.3)	0.77
Death from cardiovascular disease	4 (2.1)	1 (2.3)	3 (2.0)	0.92
Non-fatal stroke	3 (1.6)	0 (1.5)	3 (2.0)	0.34
Angina pectoris	18 (9.4)	4 (9.1)	14 (9.5)	0.93
Non-fatal acute coronary syndrome	6 (3.1)	1 (2.2)	5 (3.4)	0.71

LDL-C indicates low-density lipoprotein cholesterol.

Primary cardiovascular events include death from cardiovascular disease, non-fatal acute coronary syndrome, non-fatal stroke, and angina pectoris.



**Supplementary Fig. 4.** Kaplan-Meier curves of cumulative event-free survival of first major cardiovascular events (A), non-fatal acute coronary syndrome (B), death from cardiovascular disease (C), non-fatal stroke (D), and angina pectoris (E) according to serum lipoprotein cholesterol (LDL-C) levels



**Supplementary Fig. 5.** Receiver operating characteristic (ROC) curves of serum small dense low-density lipoprotein cholesterol (sdLDL-C) levels and serum lipoprotein cholesterol (LDL-C) levels for predicting primary cardiovascular event