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Positive Association Between Small Dense Low-Density Lipoprotein Cholesterol Concentration and Biomarkers of Inflammation, Thrombosis, and Prediabetes in Non-Diabetic Adults

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Aims: Recent studies suggest elevated levels of small dense low-density lipoprotein cholesterol (sdLDL-C) can predict the risk of incident coronary heart disease (CHD), even in individuals considered to be at low risk for cardiovascular disease(CVD) based on their LDL-C levels. This study aims to prospectively investigate the association between sdLDL-C concentration and traditional and nontraditional CHD risk markers to explore the underlying roles of sdLDL-C in atherogenic processes.

Methods: Between 2009 and 2011, 594 healthy volunteers aged 35−65 years were recruited as control subjects in a study of work-related risk factors and acute CHD. All participants fasted for 12−14 h, and venous blood samples were collected in the morning to measure serum lipid profiles and other CHD-related markers. A standard oral glucose tolerance test was performed on all participants to assess their subclinical diabetes and prediabetes status.

Results: There were significantly positive associations between sdLDL-C concentration and traditional (age, smoking and alcohol drinking habit, blood pressure, body mass index (BMI), serum lipid profiles, and diabetes status) and nontraditional risk factors (complete blood counts, (CBC), fibrinogen, high-sensitivity C-reactive protein, and subclinical diabetes status) for CVD. After adjusting for confounding variables which include age, gender, BMI, hypertension, household income, and smoking and alcohol drinking habits, all atherosclerotic risk markers except D-dimer were significantly and positively associated with sdLDL-C.

Conclusions: Our data indicated sdLDL-C is strongly associated with atherosclerotic risk markers, such as inflammation, thrombosis, hematological markers, and prediabetes. This study supports the hypothesis that sdLDL-C is a promising CVD risk biomarker.

Key words: Small dense LDL-C, CHD, OGTT, Inflammation, Thrombosis

Introduction

Coronary heart disease (CHD) is the most common cause of death among men and women worldwide. Hyperlipidemia is considered one of the most important risk factors for CHD, and low-density lipoprotein cholesterol (LDL-C) remains the primary target of lipid-lowering therapy[1, 2]. However, many individuals with normal levels of LDL-C still develop cardiovascular disease (CVD)[3, 4]. Recent studies suggested that elevated levels of small dense LDL-C (sdLDL-C) can predict the risk for incident CHD even in indi-

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viduals considered to be at a low risk for CVD based on their LDL-C levels[2, 5]. In a community-based cohort study, Hoogeveen *et al.* suggested that sdLDL-C levels highly correlate with an atherogenic lipid profile and are associated with incident CHD[2]. Hirayama and Miida also suggested that sdLDL-C is a better risk marker for CHD than LDL-C[6]. Moriyama *et al.* presented non-HDL-C as the useful surrogate lipid marker for atherogenic sdLDL-C in Japanese subjects with triglyceride (TG) < 400 mg/dL[7]. Although the association between sdLDL-C and CHD has been well documented, the underlying mechanisms of this association have not yet been sufficiently established.

Previous studies have proposed that CHD is usually caused by plaque buildup on the wall of coronary arteries that supply blood to the heart[8, 9]. Plaque is composed of cholesterol deposits, and its formation in the arteries causes the walls inside the arteries to narrow over time, which can partially or completely block blood flow. Fatty plaque may stimulate inflammation in the blood vessels through the production of foam cells. If the plaque ruptures, leukocytes and platelets stick to the site of injury and may clump together to form blood clots which narrow the coronary arteries further and facilitate acute coronary events[10]. Furthermore, blood viscosity was found to correlate with cerebral blood flow and cardiac output, and increased viscosity may increase the risk of thrombosis or thromboembolic events[11]. Therefore, blood viscosity, as well as inflammation and thrombotic factors, may play key roles in atherosclerosis and CHD. However, few studies have focused on the relationships between atherogenic dyslipidemia markers and hematological, inflammatory, and thrombotic biomarkers to explore the better surrogate lipid marker for CHD risk identification.

In addition, the presence of diabetes mellitus (DM) has been considered a CHD "risk equivalent" in the past decade[12], and the latest 2013 ACC/AHA assessment of risk guidelines has considered DM as only one of many variables in its risk assessment equation for CHD[13]. Younis *et al.* reported that sdLDL-C may be more atherogenic in patients with type 2 DM and in non-diabetic individuals because it is more likely to be glycated than more buoyant LDLs[14]. Another community-based cohort study has indicated that while DM was an independent predictor of CVD, sdLDL-C might not be[15]. These studies appear to contradict one another, and thus, the relationship between DM and sdLDL-C needs to be clarified.

In general, clinical decision-making for the detection, management, and prevention of CHD relies on accurate risk evaluation[16]. The pathophysiological processes of atherothrombosis are complicated, whether sdLDL-C is the best risk predictor remains unknown. Furthermore, although the association between atherosclerotic risk markers and sdLDL-C may explain a possible link between sdLDL-C and CHD, the relationship between sdLDL-C and other atherosclerotic risk markers has not yet been explored. In the present study, we comprehensively examined the association between sdLDL-C concentration and traditional and nontraditional CHD risk markers. Thus, the aim of this investigation was to examine whether sdLDL-C is a good marker of CHD risk factors and to explore the underlying roles of sdLDL-C in atherosclerotic processes.

Material and Methods

Study Subjects

From 2009 to 2011, we recruited 594 middle-aged (age range, 40−60 years old) adults without a known history of CHD, stroke, or clinical DM (fasting glucose < 7.0 mmol/L) to participate as a control group of"Work-related factors and cardiovascular events in patients with CHD" study conducted at the National Taiwan University Hospital, Taipei, Taiwan[17, 18]. Medical history, demographic data, and anthropometric data were obtained and used for this analysis. All participants fasted for 12−14 h, and venous blood samples were collected in the morning for analysis of serum lipid profiles, oral glucose tolerance test (OGTT) and CHD-related risk markers. Blood pressure (BP) measurements were performed using a mercury sphygmomanometer in a standardized fashion. Two measurements were taken after 5 min of rest in the sitting position. Body mass index (BMI) was calculated by dividing the weight in kilograms by the square of the height in meters. Hypertensive subjects were defined as subjects with a hypertension history, systolic BP ≥ 140 mmHg or diastolic BP ≥ 90 mmHg. Participants who had never smoked cigarettes during their lifetime or who had smoked during their lifetime but had stopped were classified as nonsmokers, whereas those who smoked daily were classified as current smokers. Additionally, in this study, we defined drinking two or more alcoholic beverages per week as habitual drinking. This study was approved by the Institutional Review Board at the National Taiwan University Hospital. Informed consent was obtained prior to participation.

Lipids, Lipoprotein and OGTT Measurements

Lipid concentrations, including total cholesterol (T-CHO), triglyceride, high-density lipoprotein cholesterol (HDL-C), and LDL-C, were analyzed using a homogeneous enzymatic method on a Toshiba FR-200 automatic chemistry analyzer (Toshiba, Tokyo, Japan)

Table 1. Baseline characteristics of participants stratified by quartiles of sdLDL-C level

Characteristics	sdLDL-C, mg/dL				*p*1-value	*p*2-value
	<21.0 *n*=150	21.0−29.7 *n*=149	29.8−42.4 *n*=147	>42.4 *n*=148		
Age, years	45.17±7.90	45.28±7.58	45.37±7.52	46.48±8.00	0.1529	0.1585
Male gender, %	79.33	86.58	87.07	93.92	0.0030	0.0002
Hypertension, %	19.33	22.82	23.13	35.81	0.0020	0.0015
Systolic blood pressure, mmHg	117.86±12.74	120.06±12.28	122.50±12.92	127.12±14.75	<0.0001	<0.0001
Diastolic blood pressure, mmHg	71.41±9.43	73.23±8.81	75.50±9.12	78.39±10.05	<0.0001	<0.0001
Pulse pressure, mmHg	46.45±6.17	46.82±6.52	47.00±6.93	48.73±7.66	0.0123	0.0049
Body mass index, kg/m^2	24.21±3.35	25.07±3.61	24.99±2.92	26.05±3.03	<0.0001	<0.0001
Waist circumference, cm	81.82±9.10	84.86±10.32	85.14±8.43	88.14±8.01	<0.0001	<0.0001
Current Smoker, %	15.86	12.08	16.67	20.55	0.1746	0.3004
Alcohol Drinking, %	19.86	16.33	22.92	24.31	0.3956	0.3617
Cholesterol, mg/dL	190.31±36.77	220.15±37.86	224.39±44.51	264.44±56.93	<0.0001	<0.0001
Triglyceride, mg/dL	97.83±43.16	118.97±61.30	162.66±64.48	268.77±179.41	<0.0001	<0.0001
HDL-C, mg/dL	57.37±14.91	57.85±16.23	53.04±16.73	54.20±15.39	<0.0001	0.0717
LDL-C, mg/dL	111.29±29.57	139.07±31.35	144.77±37.46	168.87±52.20	<0.0001	<0.0001
Non-HDL-C, mg/dL	132.93±29.70	162.30±28.48	171.35±33.07	210.24±46.97	<0.0001	<0.0001

*p*1-value is from trend test comparisons. *p*2-value is comparing sdLDL-C quartile 4 with quartile 1.
Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Non-HDL-C, non high-density lipoprotein cholesterol.

as previously described[1]. LDL-C was measured by a direct homogenous method (LDL-EX (N) "Seiken", Denka Seiken, Tokyo, Japan). Coefficients of variation (CVs) on T-CHO, TG, HDL-C, and LDL-C were under 3%. The concentration of non-HDL-C was estimated by subtracting HDL-C concentration from the T-CHO level.

A fully automated homogeneous direct method was performed for the determination of sdLDL-C concentration in plasma (sd-LDL-EX "Seiken", Denka Seiken, Tokyo, Japan) on a Toshiba FR-200 automatic chemistry analyzer (Toshiba, Tokyo, Japan). In this direct quantitative method, non-sdLDLs were dissociated by a surfactant and sphingomyelinase, and the cholesterol released from sdLDL was then subjected to an enzymatic reaction. Within-run CVs were below 3%.

After fasting blood sample collection, all subjects underwent an OGTT with a glucose load of 75 g in accordance with the World Health Organization standards. Then, venous blood samples were collected every 30 min from the beginning until two hours following the OGTT, and based on the results of the 2-hour blood glucose levels, individuals were classified according to the American Diabetes Association criteria: individuals with <140 mg/dL (7.78 mmol/L) glucose were classified as normal; 140−199 mg/dL (7.78−11.06 mmol/L) were classified as having impaired glucose tolerance (IGT); and >200 mg/dL (11.11 mmol/L) were classified as diabetic. Plasma glucose concentration was determined using hexokinase assay kits and analyzed on a Toshiba FR-120 automatic chemistry analyzer (Toshiba). The CV for plasma glucose is under 3%.

Inflammatory Marker Measurements

We assessed high-sensitivity C-reactive protein (hs-CRP) concentration using latex agglutination technology (Denka Seiken, Tokyo, Japan) on a Toshiba FR-200 automatic chemistry analyzer. The CBC which includes red blood cells (RBCs), hemoglobin level, hematocrit, platelets, white blood cells (WBCs), and WBC differential (lymphocytes, monocytes, neutrophils, eosinophils, and basophils) were analyzed by flow cytometry using an XE-2100 hematology analyzer (Sysmex Co., Kobe, Japan).

Thrombotic Markers Determinations

Fasting plasma fibrinogen level was measured by the Clauss method on a Sysmex® CA-1500 System (Sysmex Corporation, Kobe, Japan), which is a fully automated analyzer for clotting, chromogenic, and immunologic assays. For D-dimer analysis, we used Innovance™ D-Dimer reagent (Dade-Behring, Marburg, a Siemens Company, Germany) on a Sysmex® CA-1500 Coagulation Analyzer (Sysmex Corporation, Kobe, Japan).

Table 2. Inflammatory and coagulation markers stratified by quartiles of sdLDL-C levels

	sdLDL-C, mg/dL				*p*1-value	*p*2-value
	<21.0 *n*=150	21.0–29.7 *n*=149	29.8–42.4 *n*=147	>42.4 *n*=148		
hs-CRP, mg/dL	0.14±0.36	0.15±0.24	0.15±0.17	0.26±0.70	0.0152	0.0747
White blood cells; 10^3/μL	5.63±1.47	5.93±1.59	5.90±1.29	6.53±1.74	<0.0001	<0.0001
Neutrophils, 10^3/μL	3.18±1.08	3.36±1.21	3.33±1.02	3.78±1.37	0.0004	<0.0001
Lymphocytes, 10^3/μL	1.94±0.60	2.07±0.57	2.08±0.51	2.19±0.59	0.0004	0.0003
Monocytes, 10^3/μL	0.28±0.10	0.31±0.12	0.29±0.10	0.32±0.11	0.0495	0.0013
Eosinophils, 10^3/μL	0.03±0.02	0.03±0.02	0.03±0.02	0.03±0.02	0.1462	0.3579
Basophils, 10^3/μL	0.16±0.12	0.17±0.13	0.18±0.12	0.18±0.12	0.6724	0.1352
Hemoglobin, g/dL	14.70±1.41	15.10±1.28	15.21±1.21	15.57±1.14	<0.0001	<0.0001
Hematocrit, %	44.49±3.70	45.51±3.37	45.87±3.13	46.49±2.96	<0.0001	<0.0001
Red blood cells, 10^6/μL	5.03±0.53	5.24±0.85	5.18±0.45	5.25±0.45	0.0067	0.0002
Platelets, 10^3/μL	238.19±52.52	250.04±58.54	253.25±51.10	257.18±57.87	0.0026	0.0033
Fibrinogen, mg/dL	257.27±58.67	264.97±53.64	262.98±52.83	277.16±55.23	0.0047	0.0031
D-dimer, mg/L	0.33±0.27	0.31±0.24	0.35±0.58	0.31±0.24	0.9968	0.7726

*p*1-value is from trend test comparisons. *p*2-value is comparing sdLDL-C quartile 4 with quartile 1.
Abbreviations: hs-CRP, high-sensitivity C-reactive protein

Diabetogenic Factor Measurements

We measured hemoglobin A1c (HbA1c) using high-performance liquid chromatography technology. To quantitatively determine adiponectin concentration, we used quantitative sandwich enzyme immunoassay technique (Quanti'kine® ELISA).

Statistical Analyses

Continuous variables are expressed as the means ± standard deviations, and categorical data are expressed as percentages. In this study, participants were divided into four groups according to sdLDL-C quartiles (Q1=<21.0 mg/dL, Q2=21.0–29.7 mg/dL, Q3=29.8–42.4 mg/dL, Q4=>42.4 mg/dL). Statistical methods used to test the significance of continuous variables (**Tables 1**, **2**, and **3**) were performed with the Cochran-Armitage trend test. The trend test was used to assess the associations and trends between sdLDL-C and novel biomarkers, such as inflammatory and thrombotic markers and hematological indices. Significant variables were then selected for further analysis using multiple linear regression analysis. All analyses were performed using SAS 9.1 (SAS Institute Inc., Cary, NC). A *p*-value of <0.05 was considered statistically significant.

Results

Patient Characteristics

In this study, we included 594 participants with no known history of clinical CHD. Approximately 85% of patients were male, 25% were hypertensive, 15% were smokers, and 20% had a drinking habit. Participants were divided into four groups according to the quartile distribution of sdLDL-C levels. The clinical characteristics of the participants stratified by quartiles of sdLDL-C levels are shown in **Table 1**. As outlined in **Table 1**, there were no differences in age, smoking or drinking habit between participants with low and high sdLDL-C levels.

Association between sdLDL-C and Cardiovascular Risk Factors

Mean values of traditional risk factors and other characteristics classified by sdLDL-C quartiles are also listed in **Table 1**. The data summary in the table indicates that there were significant differences in conventional risk factors for atherosclerosis, such as BP, pulse pressure, BMI, waist circumference and lipid profiles (including T-CHO, TG, HDL-C, LDL-C, and non-HDL-C), between the four sdLDL-C groups. We observed that individuals with sdLDL-C levels in the highest quartile might have proatherogenic lipid profiles, were more likely to have metabolic syndrome and hypertension, and had higher BMI and waist circumference. Higher BP and pulse pressure were also noted in individuals with higher sdLDL-C levels. Furthermore, the results also showed that the highest lipid levels (including non-HDL-C), except for HDL-C levels, were observed in subjects in the highest sdLDL-C quartile. Interestingly, HDL-C levels were significantly lower than sdLDL-C levels in these subjects; however,

Table 3. OGTT results stratified by quartiles of sdLDL-C levels

Characteristics	sdLDL-C, mg/dL				*p*1-value	*p*2-value
	<21.0 *n*=150	21.0–29.8 *n*=149	29.8–42.4 *n*=147	≥42.4 *n*=148		
OGTT diabetes mellitus, %	2.67	4.03	5.44	12.84	0.0003	0.0010
Impaired glucose tolerance, %	6.00	10.07	11.58	14.19	0.0199	0.0188
Normal glucose tolerance, %	88.00	79.19	75.51	63.51	<0.0001	<0.0001
Impaired fasting glucose, %	7.33	12.08	12.93	25.00	0.0002	<0.0001
Fasting glucose , mg/dL	88.65±7.47	90.84±8.46	92.74±11.72	97.08±15.30	<0.0001	<0.0001
60 min	127.96±37.48	139.01±46.37	151.97±49.82	169.78±58.66	<0.0001	<0.0001
120 min	97.54±23.84	107.30±37.34	113.47±37.90	129.18±58.04	<0.0001	<0.0001
Glu AUC	221.03±47.91	238.11±65.10	255.40±68.99	284.06±93.00	<0.0001	<0.0001
Hemoglobin A1c, %	5.50±0.51	5.56±0.50	5.55±0.47	5.95±1.14	<0.0001	<0.0001
Adiponectin, ng/ml	2540.34±2168.47	2043.80±1827.12	1930.78±1813.36	1481.57±1037.50	<0.0001	<0.0001

*p*1-value is from trend test comparisons. *p*3-value is comparing sdLDL-C quartile 4 with quartile 1.
Abbreviations: OGTT, oral glucose tolerance test; Glu AUC, glucose levels area under curve after OGTT

subjects with the lowest HDL-C levels did not exhibit the highest sdLDL-C levels. Additionally, to clarify how the gender difference affects lipid profiles, we performed subgroups analysis to evaluate the association. The data are shown in **Supplemental Table 1** and show the same trend between sdLDL-C and lipid profiles for both genders.

Circulating Inflammatory Markers are Associated with sdLDL-C Levels

We determined further the strength of the relationship between multiple circulating inflammatory markers and sdLDL-C. As shown in **Table 2**, there was a strong positive correlation in different inflammatory indices, such as leukocyte ($p<0.001$), neutrophil ($p=0.0004$), and lymphocyte counts ($p=0.0004$), between sdLDL-C quartiles. However, of all quartiles, the Q4 sdLDL-C group had the highest hs-CRP level (0.14 ± 0.36, 0.15 ± 0.24, 0.15 ± 0.17 versus 0.26 ± 0.70, $p=0.0152$). In addition, there was a weakly positive correlation between sdLDL-C levels and monocyte counts ($p=0.0495$) in the current study. However, there were no statistically significant differences between sdLDL-C and basophil or eosinophil counts in this study.

Association between Thrombotic Markers and sdLDL-C Levels

Table 2 exhibits the association between sdLDL-C and coagulation markers, including platelet counts, and fibrinogen and D-dimer levels, stratified by quartiles of sdLDL-C levels. As shown in **Table 2**, there was a positive association between sdLDL-C and platelet counts ($p=0.0026$). A significant correlation between sdLDL-C and fibrinogen was noted. However, D-dimer results were not significantly linked with sdLDL-C levels in the current study ($p>0.05$).

Hematological Indices are Associated with the Highest sdLDL-C Levels

All blood cell components affect whole blood viscosity, especially hemoglobin, hematocrit, and RBCs[11] In this study, we identified positive relationships between sdLDL-C and RBC, hemoglobin, and hematocrit measurements. As presented in **Table 2**, hemoglobin, hematocrit, and RBC values were significantly different between four sdLDL-C groups ($p<0.001$, $p<0.001$ and $p=0.0067$, respectively). A higher RBC count, hemoglobin, and hematocrit levels were also noted in the groups with higher sdLDL-C levels.

Diabetogenic Factors are Associated with the Highest sdLDL-C Levels

Table 3 highlights results showing a significant correlation between sdLDL-C quartiles and markers for glucose metabolism. **Table 3** also indicates that higher levels of sdLDL-C are associated with higher glucose levels, DM and prediabetes (IGT and impaired fasting glucose). However, in this study, we also found that sdLDL-C levels are significantly associated with HbA1c and adiponectin levels.

Multivariate Regression Analyses for Estimating the Association between CHD Risk Biomarkers and Quartiles of sdLDL-C Levels

To estimate the specific association between multiple risk biomarkers and sdLDL-C, multiple linear regression models were conducted in this study after

Table 4. Estimate mean values (95% CI) for the associations between sdLDL-C and cardiovascular biomarkers in participants without CAD ($n=594$)[a]

	sdLDL-C, mg/dL				p-value[b]
	<21.0 $n=150$	21.0–29.7 $n=149$	29.8–42.4 $n=147$	>42.4 $n=148$	
hs-CRP, mg/dL	0.13 (0.11, 0.14)	0.15 (0.13, 0.16)	0.15 (0.14, 0.16)	0.27 (0.23, 0.30)	0.0152
White blood cell, 10^3/μL	5.62 (5.52, 5.72)	5.92 (5.78, 6.06)	5.89 (5.83, 5.96)	6.58 (6.46, 6.70) ‡	<0.0001
Neutrophils, 10^3/uL	3.18 (3.12, 3.24)	3.38 (3.29, 3.47)	3.31 (3.26, 3.36)	3.80 (3.71, 3.89) †	0.0004
Lymphocytes, 10^3/uL	1.94 (1.90, 1.97)	2.06 (2.01, 2.10)	2.07 (2.04, 2.10)	2.21 (2.17, 2.25) †	0.0004
Monocytes, 10^3/uL	0.276 (0.273, 0.279)	0.306 (0.299, 0.314)	0.285 (0.279, 0.290)	0.316 (0.308, 0.323)	0.0495
Fibrinogen, mg/dL	256.9 (254.6, 259.1)	264.0 (261.0, 267.1)	262.1 (260.2, 264.1)	276.1 (272.9, 279.3) *	0.0047
D-dimer, mg/L	0.324 (0.316, 0.332)	0.309 (0.306, 0.313)	0.359 (0.329, 0.389)	0.30 (0.284, 0.316)	0.9968
Adiponectin, ng/mL	2573.0 (2354, 2792)	1993.5 (1861, 2126)	1888.1 (1714, 2063) *	1467.2 (1394, 1540) ‡	<0.0001
Glu AUC	218.8 (214.7, 223.0)	234.8 (231.8, 237.8)	254.1 (250.4, 257.8) ‡	282.0 (277.6, 286.5) ‡	<0.0001
Hemoglobin A1c, %	5.48 (5.45, 5.52)	5.53 (5.51, 5.55)	5.54 (5.52, 5.56)	5.95 (5.90, 6.00) ‡	<0.0001
Red blood cells, 10^6/μL	5.03 (4.99, 5.07)	5.24 (5.18, 5.30) *	5.18 (5.15, 5.21)	5.26 (5.23, 5.29)	0.0067
Hematocrit, %	44.54 (44.20, 44.88)	45.59 (45.23, 45.95) *	45.92 (45.69, 46.15) †	46.53 (46.29, 46.78) ‡	<0.0001

[a] Data in tables were derived from the least square mean (95% CI) after adjusting for age, gender, BMI, hypertension, household income, and of smoking and drinking habits

Significance in asterisk of quartiles 2, 3, 4 indicate p-value compared with quartile 1 after multiple linear regression analyses.

[b] p-value for trend were modeled according to median value of each quartile. * $p<0.05$, † $p<0.01$, ‡ $p<0.001$

Abbreviations: hs-CRP, high-sensitivity C-reactive protein; Glu AUC, glucose levels area under curve after OGTT

controlling for potential confounding variables. The data on each biomarker in **Table 4** were derived from the least squares mean (95% CI) according to the sdLDL-C quartiles after adjusting for age, gender, BMI, hypertension, household income, and smoking and drinking habits. Significantly positive correlations were found between sdLDL-C and inflammatory, thrombotic, and hematological, and diabetogenic risk biomarkers, such as RBC, WBC count, WBC differential count, fibrinogen, hs-CRP, hemoglobin, hematocrit, HbA1c, and adiponectin levels and glucose area under the curve (AUC). However, we found that D-dimer results were not significantly associated with sdLDL-C levels ($p>0.05$) and that there was a moderate but significant correlation between sdLDL-C and monocyte counts ($p=0.0495$). Furthermore, to examine the association between the proportion of sdLDL-C out of the total LDL-C (sdLDL-C/LDL-C) and multiple cardiovascular biomarkers, we performed multiple regression analyses, as shown in **Table 5**. Interestingly, the same relationship for sdLDL-C/LDL-C as the absolute amount in this study. Since smoking has an effect on inflammation, we excluded the current smokers to examine the associations between sdLDL-C and cardiovascular biomarkers as shown in **Table 6**. Significantly positive correlations were also found between sdLDL-C and inflammatory, thrombotic, and hematological and diabetogenic risk biomarkers, except for D-dimer and monocytes.

Discussion

This study showed a significant and positive association between atherogenic lipoprotein particle (sdLDL-C) levels and CHD-related risk biomarkers after controlling for confounding variables. To the best of our knowledge, the present study is the first to comprehensively demonstrate the association between sdLDL-C and multiple CHD risk factors, such as inflammatory and thrombotic markers, diabetogenic factors, and hematological indices in a non-CHD population. To address the advantage of measuring sdLDL-C over the more readily levels of TG-rich lipoproteins, we performed analyses to compare sdLDL-C to other lipid profiles, such as LDL-C, non-HDL-C and TG among quartiles of fibrinogen, adiponectin, CRP, WBC, RBC, and platelet data showed in **Supplemental Table 2**. It shows that sdLDL-C is superior to LDL-C, non-HDL-C, or TG for its association with inflammatory and thrombotic markers, diabetogenic factors, and hematological indices. The novel finding of a strong correlation between sdLDL-C and different CHD risk factors indicated the proatherogenic and prothrombotic effects of sdLDL-C, which may subsequently lead to predisposition to CVD. In addition, sdLDL-C might play an important role as a hematological and early thromboembolic marker.

Multiple studies indicate inflammation as a factor involved in the initiation, progression, and insta-

Table 5. Estimate mean value (95% CI) for the association between sdLDL-C/LDL-C and cardiovascular biomarkers in participants without CAD ($n=594$)[a]

	sdLDL-C/LDL-C				*p*-value
	<0.162 $n=149$	0.162−0.211 $n=148$	0.211−0.283 $n=147$	>0.283 $n=150$	
hs-CRP, mg/dL	0.13 (0.1, 0.17)	0.14 (0.08, 0.20)	0.18 (0.14, 0.22)	0.25 (0.18, 0.37)	0.0134
White blood cells, $10^3/\mu L$	5.79 (5.54, 6.05)	5.62 (5.39, 5.85)	6.05 (5.82, 6.28)	6.52 (6.24, 6.80)†	<0.0001
Neutrophils,$10^3/\mu L$	3.29 (3.10, 3.49)	3.17 (2.99, 3.35)	3.48 (3.29, 3.66)	3.72 (3.49, 3.96)*	0.0003
Lymphocytes,$10^3/\mu L$	2.00 (1.90, 2.10)	1.95 (1.87, 2.03)	2.11 (2.01, 2.21)	2.22 (2.12, 2.33)*	0.0001
Monocytes,$10^3/\mu L$	0.30 (0.28, 0.32)	0.27 (0.26, 0.29)	0.30 (0.29, 0.32)	0.31 (0.29, 0.33)	0.1336
Fibrinogen, mg/dL	259.50 (249.61, 269.38)	262.66 (254.65, 270.66)	268.53 (259.60, 277.45)	271.62 (262.25, 280.98)	0.0308
D-dimer, mg/L	0.34 (0.29, 0.39)	0.29 (0.26, 0.33)	0.36 (0.26, 0.46)	0.31 (0.27, 0.34)	0.804
Adiponectin, ng/mL	2415.43 (2085.55, 2745.32)	2110.10 (1827.94, 2392.26)†	1976.89 (1675.18, 2278.60)*	1505.02 (1276.64, 1733.41)‡	<0.0001
Glu AUC	220.41 (212.09, 228.73)	235.98 (226.32, 245.64)*	262.23 (249.97, 274.79)‡	279.89 (264.86, 294.91)‡	<0.0001
Hemoglobin A1c, %	5.51 (5.43, 5.59)	5.53 (5.47, 5.59)	5.64 (5.55, 5.73)	5.88 (5.69, 6.06)‡	<0.0001
Red blood cells, $10^6/\mu L$	5.12 (5.04, 5.20)	5.05 (4.97, 5.14)	5.23 (5.11, 5.36)	5.29 (5.20, 5.38)*	0.0056
Hematocrit,%	45.37 (44.81, 45.94)	44.50 (43.93, 45.07)	46.06 (45.57, 46.54)*	46.40 (45.88, 46.92)*	0.0002

[a] Data in tables were derived from the least square mean (95% CI) after adjusting for age, gender, BMI, hypertension, household income, smoking and drinking habits.
*$p<0.05$, †$p<0.01$, ‡$p<0.001$
Significance in asterisk of quartiles 2, 3, 4 indicate *p*-value compared with quartile 1 after multiple linear regression analyses.
[b] *p*-value for trend were modeled according to median value of each quartile.
Abbreviations: hs-CRP, high-sensitivity C-reactive protein; Glu AUC, glucose levels area under curve after OGTT

bility of atherosclerotic plaques[19, 20]. Several lines of evidence suggested that sdLDL-C rather than LDL-C exhibits enhanced atherogenic potential and could be an independent risk factor for CHD[2, 14]. In our study, sdLDL-C levels significantly correlated with hs-CRP levels, total counts of leukocytes, and differential counts. The significant correlations between sdLDL-C, hs-CRP, and elevated WBC counts (mainly neutrophils) suggest that sdLDL-C may be strongly associated with inflammatory processes involved in atherosclerosis[10]. Our investigation also showed a significant association between sdLDL-C and fibrinogen levels. Several studies have reported that fibrinogen is associated with a high risk of CHD[21, 22]. The positive association between sdLDL-C and plasma fibrinogen in our finding indicated sdLDL-C a risk marker for CHD in this non-CHD population.

In the present study, we also demonstrated that sdLDL-C levels exhibit a strong association with hematological indices, namely, RBC, hemoglobin, and hematocrit levels. Previous studies proposed that all blood cell components are well-known to affect whole blood viscosity[23]. Furthermore, blood viscosity is known to have a close relationship with blood flow. Increased blood viscosity may increase the risk of thrombosis or thromboembolic events[11]. Increased sdLDL-C might alter the exchange between circulating and cell membrane lipids, or induce oxidative stress, thereby decreasing RBC deformability and increasing blood viscosity[24, 25]. Therefore, we propose that sdLDL-C might influence blood viscosity by modifying hemoconcentration and RBC deformability. The present study demonstrated that the strong association between sdLDL-C and hematological indices could at least partially explain why sdLDL-C is the most powerful cardiovascular risk indicator among all fractions of lipoprotein cholesterol, which may suggest some benefit of measuring sdLDL-C as a prognostic marker for CHD.

Previous studies demonstrated that post-challenge hyperglycemia status predisposes individuals to a higher risk of atherosclerosis[26] or CVD[27] that can be attributed to a rapid increase in oxidative stress after meals or post-glucose load[28]. Therefore, post-challenge hyperglycemia may trigger the interaction between oxidative stress, remnant-like lipoproteins, LDL-C, and sdLDL-C and subsequently elevate the risk of cardiovascular complications[26-28]. In the present study, we observed that post-challenge hyperglycemia at different time-courses (60 and 120 min) and Glucose AUC were strongly related to sdLDL-C; therefore, it is reasonable to postulate that sdLDL-C is a good biomarker of diabetogenic potential and CVDs. Additionally, our previous study also provides direct evidence that the occurrence of post-challenge hyperglycemia—which was independent of fasting status or glycated hemoglobin may potentiate the effect of sdLDL-C on the risk of subclinical atherosclerosis in terms of aortic stiffness by brachial-ankle pulse wave velocity[26]. Interestingly,

Table 6. Estimate mean value (95% CI) for the association between sdLDL-C and cardiovascular biomarkers in participants without CAD excluded current smokers ($n=489$)

	sdLDL-C, mg/dL				p-value[b]
	<21.0 $n=150$	21.0–29.7 $n=149$	29.8–42.4 $n=147$	>42.4 $n=148$	
hs-CRP, mg/dL	0.13 (0.11, 0.14)	0.15 (0.13, 0.16)	0.15 (0.14, 0.16)	0.27 (0.23, 0.30)	0.0142
White blood cells, 10^3/μL	5.62 (5.52, 5.72)	5.92 (5.78, 6.06)	5.89 (5.83, 5.96)	6.58 (6.46, 6.70) ‡	<0.0001
Neutrophils,10^3/μL	3.18 (3.12, 3.24)	3.38 (3.29, 3.47)	3.31 (3.26, 3.36)	3.80 (3.71, 3.89) †	0.0003
Lymphocytes,10^3/μL	1.94 (1.90, 1.97)	2.06 (2.01, 2.10)	2.07 (2.04, 2.10)	2.21 (2.17, 2.25) *	0.0004
Monocytes,10^3/μL	0.276 (0.273, 0.279)	0.306 (0.299, 0.314)	0.285 (0.279, 0.290)	0.316 (0.308, 0.323)	0.0509
Fibrinogen, mg/dL	256.9 (254.6, 259.1)	264.0 (261.0, 267.1)	262.1 (260.2, 264.1)	276.1 (272.9, 279.3) *	0.004
D-dimer, mg/L	0.324 (0.316, 0.332)	0.309 (0.306, 0.313)	0.359 (0.329, 0.389)	0.30 (0.284, 0.316)	0.9891
Adiponectin, ng/mL	2573.0 (2354, 2792)	1993.5 (1861, 2126)	1888.1 (1714, 2063) *	1467.2 (1394, 1540) †	<0.0001
Glu AUC ,mg/dL	218.8 (214.7, 223.0)	234.8 (231.8, 237.8)	254.1 (250.4, 257.8) ‡	282.0 (277.6, 286.5) ‡	<0.0001
Hemoglobin A1c, %	5.48 (5.45, 5.52)	5.53 (5.51, 5.55)	5.54 (5.52, 5.56)	5.95 (5.90, 6.00) ‡	<0.0001
Red blood cells, 10^6/μL	5.03 (4.99, 5.07)	5.24 (5.18, 5.30) *	5.18 (5.15, 5.21)	5.26 (5.23, 5.29)	0.0086
Hematocrit,%	44.54 (44.20, 44.88)	45.59 (45.23, 45.95)	45.92 (45.69, 46.15) *	46.53 (46.29, 46.78) ‡	<0.0001

[a] Data in tables were derived from the least square mean (95% CI) after adjusting for age, gender, BMI, hypertension, household income, smoking and drinking habits.
Significance in asterisk of quartiles 2, 3, 4 indicate p-value compared with quartile 1 after multiple linear regression analyses.
[b] p-value for trend were modeled according to median value of each quartile. * $p<0.05$, † $p<0.01$, ‡ $p<0.001$
Abbreviations: hs-CRP, high-sensitivity C-reactive protein; Glu AUC, glucose levels area under curve after OGTT

this study also provided important evidence of an inverse relationship between the levels of adiponectin and sdLDL-C, which is consistent with previous studies[29] and provides further support for sdLDL-C as a diabetogenic marker. The present study further revealed the utility of quantitative sdLDL-C measurements for the risk assessment of atherosclerotic disease.

The amount of sdLDL-C is considered to be a useful marker of atherogenic dyslipidemia. The sdLDL-C, contains a higher percentage of glycated apolipoprotein B (apoB), which can enhance the affinity for proteoglycans in the arterial wall and subsequently predispose to glycation[28]. Our previous study and other studies have shown that apoB may be a better predictor of cardiovascular disease than LDL-C[30, 31]. As previous studies have shown higher sdLDL-C predicted higher risk of future CHD or cardiovascular events[5, 32], further studies to assess both apoB and sdLDL-C in combination may provide a better risk prediction for CHD.

The strength of this study was to comprehensively examine the association between sdLDL-C and traditional as well as novel risk factors for atherosclerosis to reveal the underlying roles of sdLDL-C in atherosclerotic processes in the relatively healthy population. Second, this study was to propose the potential utility of sdLDL-C levels in preventive medicine for CHD and their specific roles in atherogenesis.

Although this study had the undeniable merit of offering valuable insights into the association between sdLDL-C and multifaceted atherosclerotic processes, it had several limitations. First, the cross-sectional design of this study and lack of causal analysis design limited the causal interpretation of these findings. Further investigation is needed to explore the effects and potential mechanisms of sdLDL-C and their relationships with risk factors of CHD. Second, the methodology we used for measuring monocytes could not distinguish between the monocyte subset distributions, thus, we could not obtain insights into the function of sdLDL-C in the atherosclerotic processes. Further studies with larger sample sizes are necessary to clarify the findings.

In this study we used a direct, homogeneous method for the quantification of sdLDL-C, a modified photometric test procedure which is commercially available, fully applicable to an automated chemistry analyzer without manual pretreatment of the sample, reducing the assay time, and suitable for use in the general clinical practice. Thus, the clinical implications of our study may contribute and broaden the usage of sdLDL-C in preventive healthcare medicine.

In conclusion, this study provides comprehensive evidences for the association between sdLDL-C and CHD-related traditional and novel risk factors. These results support sdLDL-C as a potential novel biomarker of CVD risk factors.

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Conflict of Interest

No competing interests.

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Supplemental Table 1. Association between levels of sd-LDL-C and lipid profiles stratified by gender

Gender Number	sdLDL-C, mg/dL				*p*-value
	<21.0 Male=119 Female=31	21.0–29.7 Male=129 Female=20	29.8–42.4 Male=128 Female=19	>42.4 Male=139 Female=19	
Cholesterol, mg/L					
Male	189.17±36.97	218.53±39.19	222.21±44.97	264.36±56.83	<0.0001
Female	194.68±36.24	230.55±26.23	239.11±39.16	265.67±61.89	<0.0001
Triglyceride, mg/dL					
Male	100.29±46.10	124.39±63.47	167.56±64.08	269.88±178.05	<0.0001
Female	88.42±27.88	84.05±25.57	129.63±58.56	251.56±210.32	<0.0001
HDL-C, mg/dL					
Male	54.96±13.11	55.04±14.17	51.17±15.84	53.52±14.37	0.1840
Female	66.64±17.79	75.91±17.36	65.65±17.53	64.69±25.71	0.8299
LDL-C, mg/dL					
Male	112.50±31.11	140.84±32.72	144.09±38.31	169.58±52.53	<0.0001
Female	106.68±22.55	127.70±16.94	149.31±31.64	157.89±48.25	<0.0001
Non-HDL-C, mg/dL					
Male	134.21±30.84	163.49±29.63	171.04±33.59	210.84±47.04	<0.0001
Female	128.04±24.69	154.64±18.23	173.46±30.07	200.98±47.62	<0.0001

Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Non-HDL-C, non high-density lipoprotein cholesterol.

Supplemental Table 2. Association between levels of sdLDL-C as well as lipid profiles and quartiles of CHD-related markers: fibrinogen, adiponectin, hs-CRP, WBC, RBC and platelets

	Ⅰ	Ⅱ	Ⅲ	Ⅳ	*P*
Fibrinogen					
sdLDL-C	29.69 ± 14.24	32.96 ± 17.38	32.81 ± 15.17	35.77 ± 17.28	0.0036
LDL-C	128.94 ± 38.84	144.47 ± 45.36	140.77 ± 41.49	148.69 ± 45.49	0.0011
Non_HDL-C	155.68 ± 37.65	171.33 ± 48.37	170.10 ± 43.44	177.40 ± 44.27	0.0003
TG	148.99 ± 95.80	156.84 ± 119.44	159.21 ± 117.83	174.86 ± 123.85	0.0704
Adiponectin					
sdLDL-C	37.11 ± 16.99	33.67 ± 14.68	33.02 ± 16.41	28.09 ± 15.50	< 0.0001
LDL-C	142.45 ± 42.39	141.78 ± 41.80	139.69 ± 45.16	139.99 ± 45.55	0.5534
Non HDL-C	174.81 ± 43.79	170.25 ± 43.85	166.08 ± 46.42	165.50 ± 44.84	0.0528
Triglycerides	199.91 ± 146.49	174.78 ± 132.86	149.52 ± 99.30	123.82 ± 84.90	< 0.0001
hs-CRP					
sdLDL-C	27.64 ± 13.29	31.58 ± 15.17	36.24 ± 17.84	35.90 ± 15.83	< 0.0001
LDL-C	131.32 ± 38.90	140.80 ± 43.00	149.66 ± 47.50	142.59 ± 42.73	0.0075
Non HDL-C	156.19 ± 38.90	169.69 ± 46.01	178.25 ± 47.86	171.12 ± 42.74	0.0012
TG	125.84 ± 82.76	159.59 ± 125.20	174.34 ± 120.95	171.03 ± 97.20	0.0004
WBC					
sdLDL-C	28.47 ± 13.83	31.12 ± 14.23	35.18 ± 15.52	36.92 ± 19.42	< 0.0001
LDL-C	136.77 ± 44.12	140.16 ± 38.76	141.81 ± 41.98	144.81 ± 49.23	0.0895
Non HDL-C	160.75 ± 46.23	168.50 ± 39.30	170.89 ± 42.10	176.09 ± 49.89	0.0018
Triglycerides	123.16 ± 79.79	154.81 ± 134.68	174.85 ± 136.60	194.09 ± 115.69	< 0.0001
RBC					
sdLDL-C	28.27 ± 14.35	34.84 ± 16.73	34.29 ± 16.26	34.21 ± 16.64	0.0052
LDL-C	130.68 ± 40.89	147.36 ± 47.12	139.47 ± 39.88	145.93 ± 44.93	0.0192
Non HDL-C	156.22 ± 42.29	176.79 ± 49.35	169.34 ± 40.92	173.76 ± 43.98	0.0072
Triglycerides	128.24 ± 116.19	174.75 ± 136.66	175.01 ± 131.75	168.40 ± 91.72	0.0117
Platelets					
sdLDL-C	30.58 ± 15.95	30.50 ± 12.67	33.76 ± 14.60	36.75 ± 20.01	< 0.0001
LDL-C	129.49 ± 43.34	141.49 ± 43.39	142.23 ± 37.61	150.19 ± 47.83	< 0.0001
Non HDL-C	159.17 ± 46.65	169.59 ± 43.82	169.22 ± 38.36	178.18 ± 48.29	0.0003
Triglycerides	157.28 ± 146.09	154.59 ± 108.81	158.94 ± 104.73	176.09 ± 122.55	0.1276

P value is the trend test.