The official journal of the Japan Atherosclerosis Society and
the Asian Pacific Society of Atherosclerosis and Vascular Diseases

Original Article

J Atheroscler Thromb, 2022; 29: 762-774. http://doi.org/10.5551/jat.62789

Metabolic Properties of Lowdensity Lipoprotein (LDL) Triglycerides in Patients with Type 2 Diabetes, Comparison with Small Dense LDL-Cholesterol

Tsutomu Hirano[1], Rieko Kodera[1], Takeshi Hirashima[1], Natsuko Suzuki[1], Ema Aoki[1], Mitsuru Hosoya[1], Taito Oshima[1], Toshiyuki Hayashi[2], Shinji Koba[3], Motoko Ohta[4], Noriyuki Satoh[4] and Yasuki Ito[4]

[1]Diabetes Center, Ebina General Hospital, Kanagawa, Japan
[2]Yurakubashi Clinic, Tokyo, Japan
[3]Division of Comprehensive Internal Medicine, Department of Perioperative Medicine, Showa University School of Dentistry, Tokyo, Japan
[4]R&D Department, Denka Co., Ltd., Niigata, Japan

Aims: Abnormal compositional changes in low-density lipoprotein (LDL) particles, such as triglyceride (TG) enrichment and size reduction, are common in patients with diabetes. Several cohort studies have demonstrated that LDL-TG and sdLDL-cholesterol (C) are sensitive biomarkers for predicting atherosclerotic cardiovascular diseases beyond LDL-C. Although sdLDL has been extensively studied, little is known about the properties of LDL-TG. We investigated similarities or differences between LDL-TG and sdLDL-C.

Methods: Fasting plasma was obtained from 1,085 patients with type 2 diabetes who were enrolled in the diabetes regional cohort study (ViNA Cohort). LDL-TG and sdLDL-C concentrations were measured using a homogeneous assay established by us. In a subset of subjects, LDL-TG and sdLDL-C levels were measured postprandially or after treatment with lipid-lowering drugs.

Results: In a quartile analysis, higher LDL-TG quartiles were associated with higher frequency of female and fibrate users, whereas sdLDL-C quartiles were associated with frequency of men, drinking, and metabolic syndrome-related measurements. Higher quartiles of LDL-TG/LDL-C were associated with smoking, drinking, fibrate users, and statin users. LDL-TG was significantly correlated with TG, LDL-C, sdLDL-C, and apolipoprotein (apo) B, with apoB being the primary determinant. LDL-TG correlated to high sensitive C-reactive protein (CRP) independently of other lipids. Mean LDL-TG did not change with fasting/non-fasting. Statin treatment reduced LDL-TG, whereas fibrates increased it, but these drugs reduced sdLDL-C equally.

Conclusions: LDL-TG levels were more tightly regulated by the number of LDL particles than plasma TG levels were. SdLDL-C was closely associated with metabolic syndrome-related factors, whereas LDL-TG was associated with low-grade systemic inflammation.

Key words: LDL triglycerides, Small dense LDL-cholesterol, Diabetes, Metabolic syndrome, Apolipoprotein B

Abbreviations: TG: triglycerides, C: cholesterol, LDL: low-density lipoprotein, sdLDL: small dense low-density lipoprotein, lbLDL: Large buoyant LDL, VLDL: very-low-density lipoprotein, HDL: high-density lipoprotein, TRL: triglyceride-rich lipoprotein, Apo: Apolipoprotein, HsCRP: high sensitive C-reactive protein, HL: hepatic lipase, LPL: lipoprotein lipase, Lp(a): Lipoprotein (a), CHD: coronary heart disease, CVD: cerebrovascular disease, ASCVD: atherosclerotic cardiovascular disease, UAR: urinary albumin ratio, CETP: cholesterylester transfer protein

Introduction

Diabetes is a major cause of atherosclerotic cardiovascular disease (ASCVD), and diabetes-related dyslipidemia is closely associated with high prevalence of ASCVD, independent of hyperglycemia[1]. Diabetic dyslipidemia is characterized by elevated triglycerides (TGs), lowered high-density lipoprotein (HDL)-cholesterol (C), and the preponderance of small dense low-density lipoproteins (sdLDL)[2]. In addition,

patients with diabetes preferentially have TG-enriched lipoproteins across all lipoprotein spectra, including LDL[3, 4]. It is well recognized that sdLDL particles are more atherogenic than large buoyant (lb) LDL particles[3-6]. Our group has established a fully automated assay kit for quantifying sdLDL-C levels[7], and this assay system was adopted in famous cohort studies such as in the community atherosclerosis risk (ARIC) study and Hisayama study[8, 9]. All studies have consistently demonstrated that sdLDL-C is superior to LDL-C in predicting ASCVD. Recently, our group has established a fully automated LDL-TG assay kit[10]. Using this kit, Saeed *et al.*[11] reported that LDL-TG was a sensitive marker for predicting coronary heart disease (CHD) and cerebrovascular disease (CVD) in the ARIC study. They also found that the predictive power of LDL-TG was superior to that of remnant-like particles (RLP)-C, which represent atherosclerotic TRL remnants[11]. There are several other studies that have already indicated that LDL-TG levels can predict ASCVD beyond LDL-C[12-14]. Atherosclerosis is considered to be chronic inflammation of blood vessels, and high sensitive C-reactive protein (hsCRP) is a sensitive biomarker of systemic inflammation. Interestingly, some studies have pointed out that LDL-TG levels are more closely associated with the inflammatory marker C-reactive protein (CRP) than with LDL-C[11, 12].

SdLDL is produced from TG-rich LDL by the action of hepatic lipase (HL)[15]. Therefore, LDL-TG may have a precursor–product relationship with sdLDL[16]. Although SdLDL-C has been extensively studied, properties of LDL-TG are not well understood.

In this study, we investigated the correlation between LDL-TG and various metabolic and lipid parameters in a large number of patients with type 2 diabetes to clarify the similarities or differences between LDL-TG and sdLDL-C.

Methods

Subjects

Subjects of this study (n=1,085) were participants in the "ViNA" cohort study. The ViNA cohort study began on October 1, 2019 and will carry out regular tests, assessment of diabetic complications, and prognostic surveys within the next 2, 4, and 6 years. The subjects were diabetic patients aged 30–89 who had been treated at the Ebina General Hospital Diabetes Center for over a year. Exclusion criteria were patients with malignancies currently being treated, patients with severe liver, endocrine, and respiratory disorders, and patients undergoing hemodialysis. In this study, we used baseline blood and urine samples collected from October 1, 2019 to September 30, 2020. Subject characteristics were listed in a part of **Table 1**. Two hundred thirty-two (21%) patients were insulin users. Most subjects with type 2 diabetes (n=884) were treated with the following hypoglycemic agents alone or in combination: a sulfonylurea, metformin, pioglitazone, dipeptidyl peptidase-4 inhibitor, sodium-glucose cotransporter-2 inhibitor, α-glucosidase inhibitor, and glucagon-like peptide-1 receptor agonist. The majority of hypertensive patients (n=645) used antihypertensive drugs such as calcium channel blockers, angiotensin II receptor blockers, diuretics, and beta blockers alone or in combination. Subjects with hyperlipidemia were treated with statins (n=598), ezetimibe (n=74), fibrates (n=75), or omega-3 fatty acids (n=39) alone or in combination. All patients are taught an appropriate diet proposed by the Japan Diabetes Foundation by a dietitian.

A subset of subjects (n=44) was retrospectively investigated for lipid levels in non-fasting plasma on another day when fasting blood samples were collected. Breakfast was taken between 6:00 am and 8:00 am, and blood was drawn at the hospital from 8:00 am to 10: 00 am. Therefore, postprandial samples were collected 1–4 hours after breakfast. The interval between the two blood draws was 1 to 4 months. Another subset of subjects retrospectively investigated fasting lipid levels before and 2–4 months after treatment with either statins (n=12) or fibrates (n=11). The statins were pitavastatin (1 mg / d, n=9) and rosuvastatin (2.5 mg / d, n=3), and the fibrate was pemafibrate (0.2 mg / d) only. In a preliminary study, we measured TG and cholesterol concentrations in LDL subfractions obtained from 10 healthy volunteers.

Measurements

Plasma samples were taken in the morning after overnight fasting. LDL-TG and sdLDL-C concentrations were measured directly in plasma by the homogeneous method established by our group (Denka Co.,Ltd., Tokyo). All blood samples, including

Address for correspondence: Tsutomu Hirano, Diabetes Center, Ebina General Hospital, Kawaharaguchi 1320 Ebina City, Kanagawa, Japan
E-mail: hirano@med.showa-u.ac.jp

Received: January 7, 2021 Accepted for publication: March 15, 2021

Table 1. Measurements stratifiedby LDL-TG quartile in subjectswith diabetes

Quartile	Total	Q1	Q2	Q3	Q4	*p* trend*1
LDL-TG (mg/dl)	14.5 (6.7-59.7)[a]	10.2 (6.7-11.8)	13.3 (11.9-14.4)	15.8 (14.5-17.3)	20.4 (17.4-59.7)	
Number	1085	266	272	276	271	
Male	680 (63%)[b]	192 (72%)	172 (63%)	169 (61%)	147 (54%)	<0.0001
Age (y)	67.0 (11.1)[c]	67.5 (10.2)	67.0 (10.7)	66.5 (12.0)	66.9 (11.4)	ns
Current smoker	183 (17%)	35 (13%)	46 (17%)	53 (19%)	49 (18%)	ns
Alcohol drinke	382 (35%)	94 (35%)	89 (33%)	96 (35%)	103 (38%)	ns
Coronary heart disease	117 (11%)	32 (12%)	33 (12%)	29 (11%)	23 (8%)	ns
Cerebelovascular disease	91 (8%)	22 (8%)	20 (7%)	26 (9%)	23 (8%)	ns
Type 1 diabetes	27 (3%)	8 (3%)	5 (2%)	6 (2%)	8 (3%)	ns
Insuilin	232 (21%)	54 (20%)	54 (20%)	65 (24%)	59 (22%)	ns
insulin dose (U)	23 [15-33][d]	22 [14-34]	23 [16-32]	24 [16-32]	22 [16-34]	ns
Hypotensive drugs	645 (59%)	157 (59%)	164 (60%)	154 (56%)	170 (63%)	ns
Hypolipidemic drugs	681 (63%)	158 (59%)	189 (69%)	161 (58%)	173 (64%)	ns
Statins	597 (55%)	149 (56%)	176 (65%)	140 (51%)	132 (49%)	0.0076
Fibrates	75 (7%)	5 (2%)	10 (4%)	19 (7%)	41 (15%)	<0.0001
SBP (mmHg)	132 (14)	130 (14)	131 (13)	133 (15)	134 (14)	0.0426
BMI (Kg/m^2)	25.2 (4.2)	24.4 (3.7)	25.2 (4.0)	25.5 (4.3)	25.7 (4.7)	0.0051
Fatty liver*2	130 (49%)	21 (37%)	33 (49%)	37 (52%)	39 (55%)	0.0439
VFA (cm^2)*3	159.2 (81.2)	148.1 (63.6)	156.1 (80.6)	157.5 (82.0)	174.4 (94.8)	ns
ALT (U)	20 [15-30]	18 [15-25]	22 [15-32]	20 [15-29]	21 [15-34]	ns
γGTP (U)	25 [17-42]	21 [16-32]	25 [18-40]	25 [17-42]	30 [18-58]	<0.0001
HbA1c (%)	7.4 (0.9)	7.3 (0.7)	7.4 (0.9)	7.5 (0.9)	7.4 (1.0)	ns
Glucose (mg/dL)	149 (39)	145 (33)	150 (39)	148 (38)	153 (47)	ns
C-peptide (mg/dL)	1.3 [0.8-1.9]	1.2 [0.8-1.6]	1.4 [0.9-2.0]	1.3 [0.8-1.8]	1.4 [0.9-2.1]	0.0416
TC (mg/dL)	182 (31)	172 (27)	174 (28)	185 (29)	195 (34)	<0.0001
TG (mg/dL)	104 [76-149]	87 [66-121]	104 [78-151]	106 [74-153]	117 [93-177]	<0.0001
%LDL-TG/TG	13.9 [9.5-19.6]	11.6 [8.2-15.3]	12.6 [8.9-16.8]	14.9 [10.5-21.2]	18.2 [12.1-22.7]	<0.0001
LDL-C (mg/dL)	102 (24)	93 (22)	98 (21)	105 (23)	112 (26)	<0.0001
%LDL-TG/LDL-C	14.4 (11.9-18.0)	11.0 (9.2-12.9)	13.5 (11.7-15.7)	15.1 (13.1-17.7)	19.0 (16.2-23.2)	<0.0001
HDL-C (mg/dL)	54 (14)	56 (14)	53 (14)	55 (15)	53 (14)	ns
NonHDL-C (mg/dL)	127 (28)	115 (23)	121 (24)	130 (26)	142 (31)	<0.0001
lbLDL-C (mg/dL)	71 (21)	67 (19)	69 (18)	74 (21)	73 (23)	0.0001
sdLDL-C (mg/dL)	28.0 [21.3-38.1]	23.5 [18.2-30.8]	27.7 [20.7-34.5]	28.9 [22.2-37.0]	35.5 [25.0-50.1]	<0.0001
TRL-C (mg/dL)	23 [18-29]	21 [17-25]	22 [17-28]	23 [18-30]	27 [20-35]	<0.0001
apoB (mg/dL)	87 (18)	78 (15)	84 (15)	89 (16)	99 (21)	<0.0001
apoCIII (mg/dL)	10.4 (8.3-12.9)	10.0 (8.0-12.0)	10.1 (8.4-12.5)	10.4 (8.5-12.7)	11.2 (8.7-14.1)	0.0002
apoE (mg/dL)	4.1 (1.3)	3.8 (1.2)	3.9 (1.1)	4.1 (1.2)	4.6 (1.4)	<0.0001
Lp (a) (mg/dL)	13.1 [6.2-24.8]	11.7 [5.7-22.6]	15.0 [6.6-25.5]	12.7 [6.2-23.1]	12.8 [6.1-31.6]	ns
eGFR (ml/min/1.73m^2)	70.9 (20.0)	71.0 (18.3)	70.1 (20.8)	72.1 (19.5)	70.2 (21.3)	ns
UAR (mg/g)	16.2 [6.8-51.7]	13.8 [5.4-49.9]	16.5 [7.2-54.0]	14.0 [6.1-36.4]	19.9 [8.2-74.4]	ns
WBC	6583 (1714)	6131 (1567)	6590 (1513)	6654 (1785)	6950 (1871)	<0.0001
hsCRP (ng/L)	0.06 [0.03-0.13]	0.03 [0.02-0.07]	0.05 [0.03-0.12]	0.07 [0.03-0.15]	0.09 [0.04-0.21]	<0.0001

a): median (total range) b): *n* (%) c): mean (SD) d): median [IQR]
*1 The *p* trend was estimated by Cochran-Armitagetrend test for categoricalvariablesor Jonckheere-Terpstratrend test for continuous variables.
*2 Total 267 subjects were measured Fatty liver.
*3 Total 195 subjects were measuredVFA.

LDL-TG and sdLDL-C, were immediately measured using an automated analyzer without storage in the refrigerator. The principles of these assays were fully explained previously[7, 17]. Of note is that the direct LDL-TG and LDL-C assays measure LDL, densities (d)=1.019–1.063 g/ml, and the direct sdLDL-C assays measure LDL, d=1.044–1.063 g/ml. LbLDL-C was calculated by subtracting sdLDL-C from LDL-C;

nonHDL-C was calculated by subtracting HDL-C from TC; and TG-rich lipoprotein (TRL)-C was calculated by subtracting LDL-C and HDL-C from total-C (TC). The distribution of TG and cholesterol in the lbLDL (d=1.019–1.043) and sdLDL (d=1.044–1.063) fractions was preliminarily examined separately in the plasma by ultracentrifugation (n=10). Fatty liver was diagnosed based on the CT scan image by the radiologists, and the visceral fat area (VFA) was measured from the same image using the fat scan program (Fujifilm, Tokyo). Apolipoproteins, Lipoprotein (Lp) (a), hsCRP, C-peptide, and albumin were measured using commercially available test kits. Albumin in urine was corrected by urinary creatinine and represented as the urinary albumin ratio (UAR).

Details of the present study were provided to all subjects who consented to participate, and written informed consent form was obtained from all participants prior to the study. This study was approved by the ethics committee of Ebina General Hospital.

Statistics

All continuous variables were expressed as mean ± SD or median (interquartile range (IQR)). Differences between groups were examined using either Student's t-test or Mann–Whitney U test. Significance was evaluated using Wilcoxon signed-rank test. The chi-squared test of association was used to discover whether there was a relationship between two categorical variables. The p trend was estimated using the Cochran–Armitage trend test for categorical variables or the Jonckheere–Terpstra trend test for continuous variables. Correlations between variables were evaluated using Spearman's correlation analysis. Multivariate linear regression was employed to analyze the effects of LDL-TG, sdLDL-C or hsCRP on other related factors. A p-value less than 0.05 was considered statistically significant. Analyses were performed using JMP software version 15 (SAS Institute, Cary, NC, USA).

Results

Table 1 illustrates characteristics of the subjects and measured values of the subjects as a whole and values stratified by the LDL-TG quartile (Q). The higher quartiles were more frequent in females (less males). In fact, LDL-TG levels were higher in females than in males in total subjects (15.9 ± 0.2 vs. 14.7 ± 0.2 mg/dl, $p<0.0001$, data not presented). CHD and CVD prevalence did not change in different quartiles. There was no significant difference in the use of hypoglycemic agents among the four groups (data not presented). Two hundred thirty-two (21%) patients were insulin users. The number of insulin users, insulin dose, and rate of type 1 diabetes did not change at LDL-TG quartiles. Fibrate use increased, but statin use decreased with higher quartiles. Systolic blood pressure (SBP), body mass index (BMI), and the presence of fatty liver increased slightly at higher quartiles. Gamma-GTP and C-peptide increased, but glucose and hemoglobin A1c did not change at higher quartiles. The higher quartiles of LDL-TG were significantly associated with higher levels of TC, TG, LDL-C, nonHDL-C, sdLDL-C, TRL-C, apo B, apo CIII, apo E, and lbLDL-C. The LDL-TG/LDL-C and LDL-TG/plasma TG percentages also increased with the higher quartiles of LDL-TG. The estimated glomerular filtration rate (eGFR) or UAR did not change with higher quartiles. White blood cell count (WBC) and hsCRP increased significantly with higher LDL-TG quartiles.

Table 2 illustrates the various measurements layered by the quartile of the LDL-TG/LDL-C (%). The higher LDL-TG/LDL-C quartiles were significantly associated with the frequency of females, smokers, and CHD. The higher the quartile, the higher the number of statin, fibrate, and insulin users. Metabolic syndrome-related factors such as BMI and VFA did not change between quartiles. The LDL-TG/LDL-C quartile was significantly associated with high concentrations of TC, TG, LDL-C, NonHDL-C, lbLDL-C, sdLDL-C, and TRL-C. ApoB was inversely correlated with the higher quartiles of LDL-TG/LDL-C. UAR, WBC, and hsCRP increased with higher LDL-TG/LDL-C quartiles.

Table 3 lists the various measurements stratified by the sdLDL-C quartile. Higher sdLDL-C quartiles were significantly associated with younger, more frequent males, more drinkers, CHD, higher insulin dose, and less frequent type 1 diabetes and insulin users. The higher the quartile, the larger the number of fibrate users and the smaller the number of statin users. Metabolic syndrome-related factors such as SBP, fatty liver, VFA, C-peptide, and blood glucose levels increased significantly with higher quartiles. The higher the quartile of sdLDL-C, the higher are TC, TG, LDL-C, and non-HDL-C and the lower are HDL-C, TRL-C, apoB, apo CIII, and apo E; this is in contrast with lbLDL-C or Lp(a). WBC and hsCRP mildly increased as the sdLDL-C quartile increased.

Table 4 presents the univariate and multivariate correlations between LDL-TG or sdLDL-C and plasma lipid parameters. Similarly to quartile analysis, LDL-TG was significantly correlated with many lipid parameters in the linear regression analysis. In particular, apoB was highly correlated with LDL-TG

Table 2. Measurements stratifiedby quartile of %LDL-TG/LDL-C in subjects withdiabetes

Quartile	Q1	Q2	Q3	Q4	*p* trend*1
%LDL-TG/LDL-C	10.3 (5.0-11.8)	13.1 (11.8-14.4)	16.0 (14.4-18.0)	21.3 (18.0-48.1)	
Number	270	276	267	272	
Male	187 (69%)	177 (64%)	155 (58%)	161 (59%)	0.0059
Age	67 (11)	66 (11)	68 (11)	67 (11)	ns
Current smoker	33 (12%)	45 (16%)	37 (14%)	68 (25%)	0.0004
Alcohol drinker	86 (32%)	97 (35%)	97 (36%)	102 (38%)	ns
Coronary heart disease	12 (4%)	22 (8%)	32 (12%)	51 (19%)	<0.0001
Cerebelovascular disease	18 (7%)	17 (6%)	33 (12%)	23 (8%)	ns
Type 1 DM	6 (2%)	7 (3%)	8 (3%)	6 (2%)	ns
Insuilin	48 (18%)	55 (20%)	62 (23%)	67 (25%)	0.0322
insulin dose	23 [11-34]	20 [14-34]	22 [16-32]	24 [18-34]	ns
Statins	117 (43%)	147 (53%)	165 (62%)	168 (62%)	<0.0001
Fibrates	7 (3%)	14 (5%)	23 (9%)	31 (11%)	<0.0001
SBP (mmHg)	131 (14)	132 (13)	132 (14)	132 (15)	ns
BMI (Kg/m^2)	24.8 (3.9)	25.2 (4.0)	25.3 (4.3)	25.5 (4.6)	ns
Fattyliver*2	35 (51%)	25 (42%)	27 (46%)	43 (54%)	ns
VFA (cm^2)*3	169 (67)	156 (87)	132 (74)	177 (90)	ns
ALT (U)	20 [15-27]	20 [14-30]	20 [14-29]	21 [16-34]	ns
γGTP (U)	22 [16-33]	24 [18-41]	26 [17-43]	27 [18-53]	0.0013
HbA1c (%)	7.4 (0.9)	7.4 (0.8)	7.4 (0.9)	7.4 (1.0)	ns
Glucose (mg/dL)	146 (37)	147 (33)	152 (42)	152 (45)	ns
C-peptide (mg/dL)	1.2 [0.8-1.7]	1.3 [0.8-1.8]	1.3 [0.9-1.9]	1.4 [0.8-2.2]	0.0058
TC (mg/dL)	193 (26)	187 (29)	179 (30)	167 (31)	<0.0001
TG (mg/dL)	93 [70-130]	104 [78-143]	108 [80-156]	108 [78-176]	<0.0001
LDL-C (mg/dL)	115 (22)	108 (22)	99 (21)	87 (23)	<0.0001
LDL-TG (mg/dL)	11.1 [9.6-13.1]	14.1 [12.1-15.9]	15.5 [13.6-18.1]	18.2 [15.4-22.3]	<0.0001
HDL-C (mg/dL)	56 (13)	55 (14)	54 (14)	52 (16)	0.0011
NonHDL-C (mg/dL)	137 (26)	132 (25)	125 (27)	115 (29)	<0.0001
lbLDL-C (mg/dL)	83 (18)	76 (18)	67 (18)	57 (18)	<0.0001
sdLDL-C (mg/dL)	28.7 [22.8-38.6]	29.0 [22.7-37.3]	28.3 [21.3-38.1]	24.8 [17.7-38.5]	0.0024
TRL-C (mg/dL)	22 [17-26]	23 [18-28]	24 [18-31]	24 [18-33]	0.0011
apoB (mg/dL)	92 (17)	90 (16)	86 (17)	82 (21)	<0.0001
apoCIII (mg/dL)	10.1 [8.2-12.4]	10.6 [8.6-12.5]	10.3 [8.7-13.1]	10.4 [7.9-13.7]	ns
apoE (mg/dL)	4.1 (1.1)	4.1 (1.1)	4.1 (1.3)	4.2 (1.5)	ns
Lp (a) (mg/dL)	12.6 [6.6-22.1]	14.3 [6.8-25.8]	13.9 [5.7-25.9]	12.0 [5.4-27.2]	ns
eGFR (ml/min/1.73m^2)	72 (19)	71 (19)	70 (19)	70 (23)	ns
UAR (mg/g)	11.5 [5.3-43.5]	14.0 [5.8-45.1]	18.6 [7.2-47.3]	22.4 [9.6-75.2]	<0.0001
WBC	6232 (1416)	6431 (1597)	6736 (1927)	6938 (1797)	<0.0001
hsCRP (ng/L)	0.05 [0.02-0.10]	0.06 [0.03-0.11]	0.06 (0.03-0.16]	0.07 [0.03-0.17]	<0.0001

(r=0.449). In multivariate analysis, LDL-C, log-sdLDL-C, or log-TG lost their association with LDL-TG when apo B was entered as the independent variable. When apoCIII and E were added to this multivariate analysis, apoB and apoE were independently positively correlated with LDL-TG.

SdLDL-C was significantly correlated with all lipid parameters. ApoB (r=0.76), apo CIII (r=0.61), nonHDL-C (r=0.69), and TG (r=0.62) were highly correlated with sdLDL-C. The multivariate analysis revealed that apoB, log-TG, and apo CIII were independently associated with sdLDL-C.

Table 5 presents the relationship between hsCRP levels and various measurements. HsCRP levels were high in CVD subjects and low in type 1 diabetes. HsCRP was associated with metabolic syndrome-related factors such as SBP, BMI, VFA, C-peptide, hyperglycemia, and dyslipidemia, and kidney

Table 3. Measurements stratified by sdLDL-cholesterol quartile in subjects with diabetes

Quartile	Q1	Q2	Q3	Q4	*p* trend*1
sdLDL-C (mg/dl)	17.5 (8.5-21.3)	24.6 (21.4-28.0)	32.4 (28.1-38.0)	48.7 (38.1-117.6)	
Number	276	270	268	271	
Male	166 (60%)	151 (56%)	174 (65%)	189 (70%)	0.0041
Age	68 (11)	67 (11)	68 (11)	65 (12)	0.0114
Current smoker	46 (17%)	38 (14%)	42 (16%)	57 (21%)	ns
Alcohol drinker	86 (31%)	82 (30%)	91 (34%)	123 (45%)	0.0004
Coronary heart disease	43 (16%)	30 (11%)	23 (9%)	21 (8%)	0.0019
Cerebelovascular disease	26 (9%)	24 (9%)	22 (8%)	19 (7%)	ns
Type 1 diabetes	13 (5%)	7 (3%)	5 (2%)	2 (1%)	0.0027
Insuilin	71 (26%)	61 (23%)	57 (21%)	43 (16%)	0.0054
insulin dose	20 [14-30]	24 [16-33]	21 [11-31]	28 [20-40]	ns
Hypelipidemic agents	177 (64%)	171 (63%)	170 (63%)	163 (60%)	ns
Statins	162 (59%)	150 (56%)	153 (57%)	132 (49%)	0.0347
Fibrates	13 (5%)	18 (7%)	14 (5%)	30 (11%)	0.0102
Hypotensive agents	166 (60%)	160 (59%)	154 (57%)	165 (61%)	ns
SBP (mmHg)	130 (15)	131 (13)	132 (14)	133 (14)	0.0452
BMI (Kg/m^2)	24.3 (4.3)	25.2 (4.3)	25.1 (3.7)	26.2 (4.3)	<0.0001
Fatty liver*2	17 (25%)	27 (50%)	39 (58%)	47 (61%)	<0.0001
VFA (cm^2)*3	105 (59)	157 (71)	170 (77)	203 (82)	<0.0001
ALT (U)	16 [13-23]	20 [15-29]	21 [16-30]	26 [17-39]	<0.0001
γGTP (U)	19 [14-28]	24 [16-37]	25 (18-38)	37 (22-66)	<0.0001
HbA1c (%)	7.3 (0.8)	7.3 (0.8)	7.4 (0.9)	7.5 (1.0)	ns
Glucose (mg/dL)	141 (40)	149 (35)	150 (37)	157 (44)	<0.0001
C-peptide (mg/dL)	1.0 [0.7-1.6]	1.2 [0.8-1.8]	1.3 [0.9-1.9]	1.6 [1.1-2.2]	<0.0001
TC (mg/dL)	159 (24)	180 (26)	186 (27)	202 (30)	<0.0001
TG (mg/dL)	73 [57-96]	93 [71-121]	115 [91-151]	166 [120-227]	<0.0001
LDL-C (mg/dL)	83 (17)	101 (19)	107 (21)	118 (24)	<0.0001
LDL-TG (mg/dL)	13.2 [10.6-16.0]	14.2 [11.6-16.5]	14.6 [12.3-17.0]	16.6 [13.8-20.9]	<0.0001
HDL-C (mg/dL)	57 (15)	57 (15)	54 (14)	50 (12)	<0.0001
NonHDL-C (mg/dL)	102 (18)	123 (20)	132 (21)	152 (26)	<0.0001
lbLDL-C (mg/dL)	66 (16)	76 (19)	74 (21)	66 (24)	ns
TRL-C (mg/dL)	19 [15-23]	22 [17-27]	24 [19-30]	32 [24-41]	<0.0001
apoB (mg/dL)	70 (10)	83 (11)	91 (12)	106 (17)	<0.0001
apoCIII (mg/dL)	8.2 [6.9-9.9]	9.6 [8.2-11.2]	11.1 [9.3-13.0]	13.8 [11.2-16.6]	<0.0001
apoE (mg/dL)	3.6 (1.1)	3.9 (1.0)	4.0 (1.1)	4.9 (1.4)	<0.0001
Lp (a) (mg/dL)	14.3 [7.1-28.7]	14.4 [6.6-26.4]	11.8 [5.9-20.9]	11.9 [5.6-24.0]	0.0119
eGFR (ml/min/1.73m^2)	70 (21)	70 (18)	72 (20)	72 (21)	ns
UAR (mg/g)	18.3 [6.6-54.3]	13.7 [5.7-40.8]	16.5 [7.3-45.9]	19.3 [7.5-73.8]	ns
WBC	6301 (1762)	6419 (1682)	6701 (993)	6919 (1046)	<0.0001
hsCRP (ng/L)	0.05 [0.02-0.11]	0.06 [0.03-0.14]	0.05 [0.03-0.11]	0.08 [0.04-0.17]	<0.0001

dysfunction. LDL-TG had the strongest association with hsCRP among lipid parameters. The multivariate analysis revealed that log_LDL-TG was highly associated with log_hsCRP independently of other lipid parameters when adjusted for CVD, type 1 diabetes, hypotensive drugs, statins, fibrates, SBP, BMI, HbA1c, log_ALT, log_γGTP, glucose, log_C-peptide, log_UAR, and WBC. Its correlation was found to be the strongest among other lipid parameters. By contrast, sdLDL-C lost its correlation with hsCRP in the multivariate analysis.

Fig. 1 illustrates the difference in lipid levels between fasting and postprandial conditions on different days of the same individual. Mean levels of TG increased in postprandial samples, but mean levels of non-fasting LDL-C, non-HDL-C, LDL-TG, and sdLDL-C were similar to those at fasting.

Fig. 2 illustrates the differences (d) in TG, LDL-

Table 4. Correlations between LDL-TG or sdLDL-C and lipoprotein parameters

Univariate	LDL-TG		Univariate	sdLDL-C	
	Spearman's rs	*p*		Spearman's rs	*p*
TC	0.2930	<0.0001	TC	0.5315	<0.0001
TG	0.2305	<0.0001	TG	0.6222	<0.0001
LDL-C	0.3040	<0.0001	LDL-C	0.5495	<0.0001
HDL-C	-0.0749	0.0136	HDL-C	-0.1902	<0.0001
Non-HDL-C	0.3739	<0.0001	Non-HDL-C	0.6901	<0.0001
lbLDL-C	0.1165	0.0001	lbLDL-C	0.0085	ns
sdLDL-C	0.3245	<0.0001	LDL-TG	0.3245	<0.0001
TRL-C	0.2407	<0.0001	TRL-C	0.5050	<0.0001
apoB	0.4493	<0.0001	apoB	0.7612	<0.0001
apoCIII	0.1382	<0.0001	apoCIII	0.6197	<0.0001
apoE	0.2504	<0.0001	apoE	0.4030	<0.0001
Multivariate	**log_LDL-TG**		**Multivariate**	**log_sdLDL-C**	
	β	*p*		*β*	*p*
R2=0.263			R2=0.695		
apoB	0.8978	<0.0001	apoB	0.7112	<0.0001
LDL-C	-0.4480	<0.0001	LDL-C	-0.0898	0.0288
log_TG	-0.0795	0.0243	log_TG	0.3211	<0.0001
Multivariate	**log_LDL-TG**		**Multivariate**	**log_sdLDL-C**	
	β	*p*		*β*	*p*
R2=0.301			R2=0.727		
log_TG	0.0495	ns	log_TG	0.2017	<0.0001
LDL-C	-0.5507	<0.0001	LDL-C	0.0183	ns
log_sdLDL-C	-0.0243	ns	log_LDL-TG	-0.0009	ns
apoB	1.0325	<0.0001	apoB	0.5699	<0.0001
log_apoCIII	-0.2858	<0.0001	log_apoCIII	0.2658	<0.0001
apoE	0.0974	0.0016	apoE	-0.0300	ns

C, LDL-TG, and sdLDL-C concentrations before and after treatment with statins or fibrates. As expected, TG was reduced by fibrate treatment, and LDL-C was reduced by statin treatment. LDL-TG was increased by fibrates but reduced by statins. Both lipid-lowering drugs reduced sdLDL-C to the same extent. D-LDL-TG was significantly correlated with d-lbLDL-C but not with d-TG or d-sdLDL-C. This result suggested that fibrates increased LDL-TG through an increase in lbLDL, but it was not clear how much LDL-TG was distributed in the lbLDL or sdLDL fraction. Then, we preliminarily measured the distribution of TG and cholesterol in the LbLDL and SdLDL fractions of plasma separated by ultracentrifugation. The median concentration of lbLDL-TG was 10 mg/dl, and sdLDL-TG was 4 mg/dl, indicating that lbLDL-TG was more than twice as high as sdLDL-TG **(Supplementary Table 1)**.

Discussion

We examined the correlation between LDL-TG and various parameters in more than 1,000 diabetic patients to find the metabolic properties of this new risk factor for ASCVD. Given that sdLDL is produced from TG-rich LDL[18], it is not surprising that there is a significant correlation between LDL-TG and sdLDL-C. However, despite the proposed precursor–product relationships between them[17], these risk factors were found to have different properties. SdLDL-C was strongly correlated with metabolic syndrome-related factors such as increased VFA, SBP, blood glucose, fatty liver, and hyper-TG/low HDL-C. By contrast, LDL-TG had a limited association with these measurements. SdLDL is preferentially produced from the large TG-rich VLDL (VLDL1), and VLDL1 production is enhanced by the presence of insulin

Table 5. High sensitive C-reactiveprotein (hsCRP) levels and associations with various measurements

	Yes	No	*p*
Male	0.05 (0.03-0.12)*	0.07 (0.03-0.15)	ns**
Current smoker	0.06 (0.03-0.14)	0.06 (0.03-0.13)	ns
Alcohol drinker	0.06 (0.03-0.14)	0.06 (0.03-0.13)	ns
Coronary heart disease	0.05 (0.02-0.11)	0.06 (0.03-0.14)	ns
Cerebelovascular disease	0.08 (0.04-0.24)	0.06 (0.03-0.13)	0.0012
Type 1 diabetes	0.02 (0.02-0.07)	0.06 (0.03-0.13)	0.0089
Insuilin	0.06 (0.03-0.15)	0.06 (0.03-0.13)	ns
Hypotensive drugs	0.06 (0.03-0.14)	0.05 (0.02-0.12)	0.0004
Hypolipidemic drugs	0.05 (0.03-0.13)	0.06 (0.03-0.14)	ns
Statins	0.05 (0.02-0.12)	0.07 (0.03-0.15)	0.0004
Fibrates	0.08 (0.04-0.16)	0.06 (0.03-0.13)	0.0195
Fatty liver	0.11 (0.05-0.22)	0.05 (0.02-0.13)	<0.0001

Univariate	hsCRP	
	Spearman's rs	*p*
Age	0.0045	ns
SBP	0.0696	0.0226
BMI	0.3424	<0.0001
VFA, *n* = 195	0.3779	<0.0001
ALT	0.1298	<0.0001
γGTP	0.3123	<0.0001
HbA1c	0.1431	<0.0001
Glucose	0.0899	0.0032
C-peptide	0.2426	<0.0001
TC	0.0762	0.0124
TG	0.2451	<0.0001
LDL-C	0.1446	<0.0001
LDL-TG	0.2829	<0.0001
%LDL-TG/LDL-C	0.1482	<0.0001
HDL-C	-0.1689	<0.0001
NonHDL-C	0.1831	<0.0001
lbLDL-C	0.0592	ns
sdLDL-C	0.1507	<0.0001
TRL-C	0.1013	0.0009
apoB	0.2056	<0.0001
apoCIII	0.1116	0.0002
apoE	0.1427	<0.0001
Lp (a)	0.0384	ns
eGFR	-0.0515	ns
UAR	0.0698	0.0224
WBC	0.3079	<0.0001

Multivariate (adjusted)	log_hsCRP	
	β	*p*
TC	0.0555	0.0491
log_TG	0.0372	ns
LDL-C	0.0716	0.0112
HDL-C	-0.0358	ns
NonHDL-C	0.0805	0.0042
log_TRL-C	0.0345	ns
apoB	0.0782	0.0053
log_apoCIII	0.0136	ns
apoE	0.0565	0.0402
log_sdLDL-C	-0.0151	ns
log_LDL-TG	0.1724	<0.0001

Multivariate analysis is adjusted for cerebelovascular disease, type 1 diabetes, hypotensive drugs, statins, fibrates, SBP, BMI, HbA1c, log_ALT, log_γGTP, glucose, log_C-peptide, log_UAR, and WBC. *range, **not significant

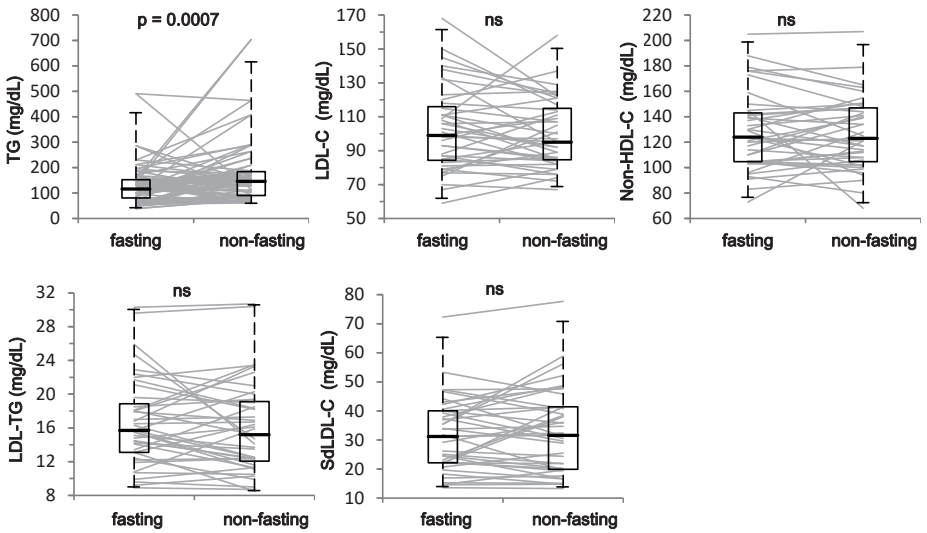

Fig. 1. Changes in lipid concentrations between fasting and non-fating states

TG, LDL-TG, non-HDL-C, LDL-TG, and sdLDL-TG concentrations in fasting and non-fasting plasma (1–4 hours after breakfast) on different days in the same individual (n=44). The interval between the two blood draws was 1 to 4 months. The whiskers of the box plots indicate the central 95% of the distribution. Significance was evaluated using the Wilcoxon signed-rank test. ns=not significant

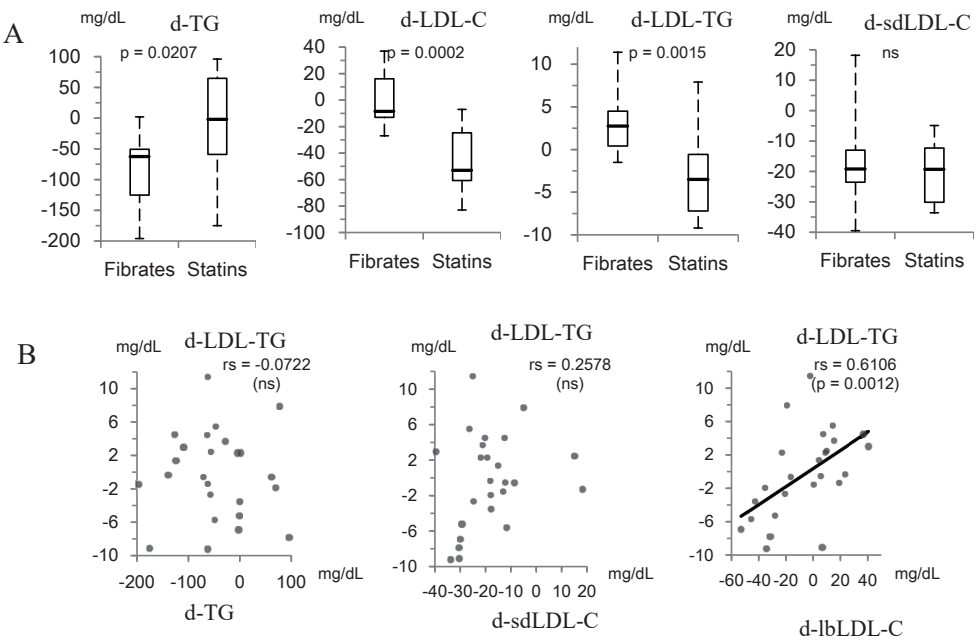

Fig. 2. Difference (d) in lipid concentrations before and after treatments with either fibrates or statins (A) and correlations between d-LDL-TG and d-TG, d-LDL-C, or d-sdLDL-C in all treated subjects (B)

The statins are pitavastatin (1 mg/day, n=9) and rosuvastatin (2.5 mg/d, n=3), and the fibrate is pemafibrate (0.2 mg/d, n=11). A: The whiskers of the box plots indicate the central 95% of the distribution. Significance was evaluated using the Wilcoxon rank sum test. The letters "rs" indicate Spearman's rank correlation coefficient.

resistance in the liver and adipose tissue supplying excess free fatty acids to the liver[19]. VLDL1-TG is a major regulator of LDL particle size[20]. Unlike sdLDL-C, LDL-TG did not strongly correlate with metabolic syndrome-related measurements. This suggests that VLDL1 production does not critically regulate the TG content of LDL. Mechanisms leading to the formation of sdLDL are involved in

cholesterylester transfer protein (CETP) and HL, besides VLDL1 production. CETP promotes the transfer of TG from VLDL to LDL; the resulting TG-rich LDL is the preferred substrate for HL; and increased lipolysis of TG-rich LDL results in the formation of sdLDL[19]. Therefore, an increase in CETP and a decrease in HL produce TG-rich LDL and can increase LDL-TG levels. Silbernagel[14] reported that LDL-TG levels were strongly associated with HL region variation and that LDL-TG-elevating alleles were associated with low HL activity. HL activity has been reported to be enhanced by insulin resistance and increased in type 2 diabetes[21, 22]. Therefore, sdLDL-C, and LDL-TG may have the opposite relationship to HL; that is, low HL activity increases LDL-TG but reduces sdLDL-C. This may in part explain why sdLDL-C is associated with metabolic syndrome-related factors but LDL-TG is not. LDL-TG had higher levels in women than in men. This was already recognized in previous studies[11, 12]. Applebaum[23] reported that administration of ethinyl estradiol significantly suppressed HL activity in post-heparin plasma in healthy women. Estrogen-induced suppression of HL may explain high levels of LDL-TG in women.

We found that both LDL-TG and sdLDL-C were closely associated with apoB, but TG was more closely associated with sdLDL-C than with LDL-TG. It is speculated that enhanced production of VLDL simply leads to increased levels of LDL-TG and sdLDL-C, whereas LDL modeling through VLDL-LDL cascade and lipid transfer with other lipoproteins may be more strongly associated with sdLDL formation than with TG-rich LDL formation. It has been suggested that the LDL-TG/LDL-C ratio is superior to LDL-TG levels for detecting the presence of metabolic disorders[24]. As illustrated in **Table 2**, the higher the ratio of LDL-TG/LDL-C, the higher the prevalence of CHD; this was not observed for LDL-TG levels. This alternative approach has the potential to enhance the predictive power of LDL-TG for ASCVD._In this study, 232 (21%) patients were insulin users, and insulin plays an important role in lipid metabolism[3, 6]. We previously reported that sdLDL-C levels decreased after the introduction of intensive insulin therapy. This was closely associated with the TG-lowering effect of insulin[25]. Consistent with previous studies, sdLDL-C levels were lower in insulin users. By contrast, higher sdLDL-C was positively correlated with C-peptide levels. Because high levels of fasting C-peptide reflect endogenous hyperinsulinemia due to insulin resistance, our result is consistent with the well known notion that insulin resistance is a potent up-regulator of sdLDL levels[2, 3, 6]. Unlike sdLDL-C, no significant effect of insulin use or C-peptide on LDL-TG was found. However, prospective studies are required to elucidate the effects of insulin on LDL-TG levels.

HsCRP is used as a sensitive biomarker for vascular inflammation. Marz *et al.*[12] reported for the first time that LDL-TG was strongly associated with hsCRP and WBC, suggesting that the atherogenicity of LDL-TG was due to its inflammatory properties. We observed that the association between LDL-TG and hsCRP was stronger than the association of LDL-C, apo B, or sdLDL-C with hsCRP. Moreover, the multivariate analysis revealed that the correlation coefficient was more than twice as high for LDL-TG (β=0.1724) as that for LDL-C (β=0.0716). Silbernagel *et al.*[15] speculated that HL was down-regulated by systemic inflammation, leading to elevated LDL-TG levels. Stahlman *et al.*[4] proposed another speculation that palmitic acid in the TG molecule of LDL particles promoted inflammation. Further research will be required to elucidate the possibility that TG in LDL can cause more inflammation than cholesterol in LDL.

Unlike plasma TG levels, mean LDL-TG levels did not change significantly between fasting and postprandial conditions. These two blood samples were taken on different days when the subject visited the hospital. Therefore, in some samples, LDL-TG changed significantly before and after a meal. Nonetheless, this general clinical approach suggests that fasting/non-fasting is not a critical issue for LDL-TG assessment. This is also true for the sdLDL-C measurement.

We unexpectedly observed an increase in LDL-TG in subjects after treatment with fibrates. This supports our new finding that LDL-TG is not definitively regulated by plasma TG levels. We found that changes in lbLDL-C were positively correlated with changes in LDL-TG. It is well known that fibrates increase LDL size[26]; thus, sdLDL-C decreases, and lbLDL-C increases[27]. Our preliminary study using ultracentrifugation revealed that TG in LDL was twice as much in the lbLDL fraction as that in the sdLDL fraction **(Supplementary Table 1)**. Therefore, it was suggested that the increase in lbLDL by fibrates could outweigh the decrease in sdLDL and increase the amount of TG in the total LDL. Fibrates promote TRL lipolysis, which may referentially increase lbLDL production. We have already reported that sdLDL-C decreased to the same extent in fibrates and statins[28] and confirmed this result in the present study. Therefore, it is unlikely that fibrates will increase the overall atherogenicity of LDL.

This study has some limitations. First, laboratory

measurements were performed only once at baseline; therefore, it is not possible to evaluate LDL-TG or sdLDL-C as risk factors for ASCVD. The design of current case-control studies often reverses causality. For example, patients with ASCVD are typically treated with intensive statins. This often results in lower levels of LDL-TG or sdLDL-C than those of its non-ASCVD counterpart. Second, it is not possible to compare LDL-TG levels in non-diabetic and diabetic subjects because this cohort study did not include the non-diabetic population. Third, the data of fasting/non-fasting and the data of lipid-lowering treatment are designed for retrospective observational studies with small sample sizes. In particular, the mechanism of fibrate-induced LDL-TG elevation requires a prospective study design with appropriate control arms.

Conclusions

LDL-TG and sdLDL-C had different metabolic properties. SdLDL-C was closely associated with metabolic syndrome-related factors, whereas LDL-TG was associated with low-grade systemic inflammation.

Acknowledgement

We would like to thank all the staff of the clinical laboratory medicine department of Ebina General Hospital for the sample analysis, and Shoko Ishiwatari for her wonderful support for editing.

Authors' Contributions

TH designed, conducted this study, analyzed the data, and wrote. RK, YH, NS, EA, MH, and TO collected data. MO conducted an additional study. NS and YI analyzed the data. THa and SK contributed to finalizing the paper. All authors critically revised the manuscript for important intellectual content and approved the final version of the manuscript.

Funding

This study was partially supported by Denka Co., Ltd. The source of funding was not involved in designing, conducting surveys, analyzing, or interpreting the data.

COI

Tsutomu Hirano receives advisor free from Denka Co., and lecture free from Kowa Co.

Other authors have no COI.

References

1) Sone H, Tanaka S, Tanaka S, Iimuro S, Oida K, Yamasaki Y, Oikawa S, Ishibashi S, Katayama S, Ohashi Y, Akanuma Y, Yamada N: Japan Diabetes Complications Study Group, Serum level of triglycerides is a potent risk factor comparable to LDL cholesterol for coronary heart disease in Japanese patients with type 2 diabetes: subanalysis of the Japan Diabetes Complications Study (JDCS). J Clin Endocrinol Metab, 2011; 96: 3448-3456
2) Taskinen MR: Diabetic dyslipidaemia: from basic research to clinical practice. Diabetologia, 2003; 46: 733-749
3) Verges B: Pathophysiology of diabetic dyslipidaemia: where are we? Diabetologia, 2015; 58: 886-899
4) Stahlman M, Pham HT, Adiels M, Mitchell TW, Blanksby SJ, Fagerberg B, K Ekroos K, Borén J: Clinical dyslipidaemia is associated with changes in the lipid composition and inflammatory properties of apolipoprotein-B-containing lipoproteins from women with type 2 diabetes. Diabetologia, 2012; 55: 1156-1166
5) Berneis KK, Krauss RM: Metabolic origins and clinical significance of LDL heterogeneity. J Lipid Res, 2002; 43: 1363-1379
6) Hirano T: Pathophysiology of Diabetic Dyslipidemia. J Atheroscler Thromb, 2018; 25: 771-782
7) Ito Y, Fujimura M, Ohta M, Hirano T: Development of a homogeneous assay for measurement of small dense LDL cholesterol. Clin Chem, 2011; 57: 57-65
8) Hoogeveen RC, Gaubatz JW, Sun W, Dodge RC, Crosby JR, Jiang J, Couper D, Virani SS, Kathiresan S, Boerwinkle E, 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
9) Higashioka M, Sakata S, Honda T, Hata J, Yoshida D, Hirakawa Y, Shibata M, Goto K, Kitazono T, Osawa H, Ninomiya T: Small Dense Low-Density Lipoprotein Cholesterol and the Risk of Coronary Heart Disease in a Japanese Community. J Atheroscler Thromb, 2020; 27: 669-682
10) Ito Y, Ohta M, Ikezaki H, Hirao Y, Machida A, Schaefer EJ, Furusyo N: Development and population results of a fully automated homogeneous assay for LDL triglyceride. JALM, 2018; 2: 746-756
11) Saeed A, Feofanova EV, Yu B, Sun W, Virani SS, Nambi V, Coresh J, Guild CS, Boerwinkle E, Ballantyne CM, Hoogeveen RC: Remnant-Like Particle Cholesterol, Low-Density Lipoprotein Triglycerides, and Incident Cardiovascular Disease. J Am Coll Cardiol, 2018; 72: 156-169
12) März W, Scharnagl H, Winkler K, Tiran A, Nauck M, Boehm BO, Winkelmann BR: Low-density lipoprotein triglycerides associated with low-grade systemic inflammation, adhesion molecules, and angiographic coronary artery disease: the Ludwigshafen Risk and Cardiovascular Health study. Circulation, 2004; 110: 3068-3074
13) Ding XH, Ye P, Wang XN, Cao RH, Yang X, Xiao WK,

Zhang Y, Bai YY, Wu HM: The predictive value of baseline LDL-TG level on major adverse cardiovascular events in a followed up cohort population. Eur Rev Med Pharmacol Sci, 2017; 21: 1060-1064
14) Jin JL, Zhang HW, Cao YX, Liu HH, Hua Q, Li YF, Zhang Y, Guo YL, Wu NQ, Zhu CG, Xu RX, Gao Y, Li XL, Cui CJ, Liu G, Sun J, Dong Q, Santos R, Li JJ: Long-term prognostic utility of low-density lipoprotein (LDL) triglyceride in real-world patients with coronary artery disease and diabetes or prediabetes. Cardiovasc Diabetol, 2020; 19: 152
15) Silbernagel G, Scharnagl H, Kleber ME, Delgado G, Stojakovic T, Laaksonen R, Erdmann J, Rankinen T, Bouchard C, Landmesser U, Schunkert H, März W, Grammer TB: LDL triglycerides, hepatic lipase activity, and coronary artery disease: An epidemiologic and Mendelian randomization study. Atherosclerosis, 2019; 282: 37-44
16) Brunzell JD, Zambon A, Deeb SS: The effect of hepatic lipase on coronary artery disease in humans is influenced by the underlying lipoprotein phenotype. Biochim Biophys Acta, 2012 M; 1821: 365-372
17) Miller M: Low-Density Lipoprotein Triglycerides: Widening the Atherogenic Landscape in CVD Risk Assessment. J Am Coll Cardiol, 2018; 72: 170-172
18) Duran EK, Aday AW, Cook NR, Buring JE, Ridker PM, Pradhan AD: Triglyceride-Rich Lipoprotein Cholesterol, Small Dense LDL Cholesterol, and Incident Cardiovascular Disease. J Am Coll Cardiol, 2020; 75: 2122-2135
19) Adiels M, Olofsson SO, Taskinen MR, Borén J: Overproduction of very low-density lipoproteins is the hallmark of the dyslipidemia in the metabolic syndrome. Arterioscler Thromb Vasc Biol, 2008; 28: 1225-1236
20) Packard CJ: Triacylglycerol-rich lipoproteins and the generation of small, dense low-density lipoprotein. Biochem Soc Trans, 2003; 31: 1066-1069
21) Ruotolo G, Parlavecchia M, Taskinen MR: Normalization of lipoprotein composition by intraperitoneal insulin in IDDM. Role of increased hepatic lipase activity. Diabetes Care, 1994; 17: 6-12
22) Laakso, M., H. Sarlund, C. Ehnholm, E. Voutilainen, A. Aro, and K. Pyorala: Relationship between postheparin plasma lipases and high-density lipoprotein cholesterol in different types of diabetes. Diabetologia, 1987; 30: 703-706
23) Applebaum DM, Goldberg AP, Pykälistö OJ, Brunzell JD, Hazzard WR: Effect of estrogen on post-heparin lipolytic activity. Selective decline in hepatic triglyceride lipase. J Clin Invest, 1977; 59: 601-608
24) Fujii Y, Nouso K, Matsushita H, Kariyama K, Sakurai T, Takahashi Y, Chiba H, Hui SP, Ito Y, Ohta M, Okada H: Low-Density Lipoprotein (LDL)-Triglyceride and Its Ratio to LDL-Cholesterol as Diagnostic Biomarkers for Nonalcoholic Steatohepatitis. J Appl Lab Med, 2020; 5: 1206-1215
25) Hayashi T, Hirano T, Yamamoto T, Ito Y, Adachi M: Intensive insulin therapy reduces small dense low-density lipoprotein particles in patients with type 2 diabetes mellitus: relationship to triglyceride-rich lipoprotein subspecies. Metabolism, 2006; 55: 879-884
26) Rizzo M, Berneis K: The clinical significance of the size of low-density-lipoproteins and the modulation of subclasses by fibrates. Curr Med Res Opin, 2007; 23: 1103-1111
27) Ishibashi S, Yamashita S, Arai H, Araki E, Yokote K, Suganami H, Fruchart JC, Kodama T; K-877-04 Study Group: Effects of K-877, a novel selective PPARalpha modulator (SPPARMalpha), in dyslipidaemic patients: A randomized, double blind, active- and placebo-controlled, phase 2 trial. Atherosclerosis, 2016; 249: 36-43
28) Tokuno A, Hirano T, Hayashi T, Mori Y, Yamamoto T, Nagashima M, Shiraishi Y, Ito Y, Adachi M: The effects of statin and fibrate on lowering small dense LDL-cholesterol in hyperlipidemic patients with type 2 diabetes. J Atheroscler Thromb, 2007; 14: 128-132

Supplemental Table 1. Cholesterol and triglyceride concentrations in ultracentrifugally isolated large buoyant (lb) and small dense (sd) LDL fractions

Percentage composition of total-cholesterol and triglycerides in sdLDL and lbLDL fractions from 10 healthy volunteers after obtaining written informed consent. The LbLDL and SdLDL fractions were separated by ultracentrifugation with densities of 1.019 to 1.043 and 1.044 to 1.063 g/ml, respectively.

	lb LDL (mg/dl)		sd LDL (mg/dl)		lb LDL/sd LDL	
	Cho	TG	Cho	TG	Cho	TG
	32.4	10.4	68.2	14.1	0.5	0.7
	61.0	9.2	39.5	4.9	1.5	1.9
	18.0	9.4	48.0	15.8	0.4	0.6
	71.3	10.7	64.0	7.9	1.1	1.4
	82.7	11.5	40.1	4.9	2.1	2.4
	55.3	5.7	24.5	2.6	2.3	2.2
	45.5	18.7	57.8	10.5	0.8	1.8
	62.2	12.0	26.5	4.5	2.3	2.7
	62.4	9.0	34.6	3.5	1.8	2.6
	78.2	12.7	42.8	3.7	1.8	3.5
median [IQR]	61 [42-72]	10 [9-12]	41 [32-59]	4 [3-11]	1.6 [0.7-2.1]	2 [1.2-2.6]

Plasma was obtained from 10 healthy volunteers.
LbLDL fraction: density of 1.019-1.043. SdLDL fraction:density of 1.044-1.063