Very Low Density Lipoprotein Cholesterol Associates with Coronary Artery Calcification in Type 2 Diabetes beyond Circulating Levels of Triglycerides

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Abstract

Objective—While recent genomic studies have focused attention on triglyceride (TG) rich lipoproteins in cardiovascular disease (CVD), little is known of very low-density lipoprotein cholesterol (VLDL-C) relationship with atherosclerosis and CVD. We examined, in a high-risk type-2 diabetic population, the association of plasma VLDL-C with coronary artery calcification (CAC).

Methods—The Penn Diabetes Heart Study (PDHS) is a cross-sectional study of CVD risk factors in type-2 diabetics (n=2118, mean age 59.1 years, 36.5% female, 34.1% Black). Plasma lipids including VLDL-C were calculated (n=1879) after ultracentrifugation.

Results—In Tobit regression, VLDL-C levels were positively associated with increasing CAC after adjusting for age, race, gender, Framingham risk score, body mass index, C-reactive protein, exercise, medication and alcohol use, hemoglobin A1c, and diabetes duration [Tobit ratio (TR) and 95% confidence interval (CI) 0.38 (0.12–0.65), P=0.005] and even after inclusion of apolipoprotein B data [TR 0.31 (0.03–0.58), P=0.030]. Approximately 3-fold stronger effect was observed in women [TR 0.75 (0.16 – 1.34), P=0.013] than men [TR 0.20 (−0.10–0.50), P=0.189; gender interaction P=0.034]. Plasma VLDL-C was related more strongly to CAC scores than TG levels (e.g., Akaike information criteria of 7263.65 v. 7263.94) and had stronger CAC association in individuals with TGs >150mg/dl (TR 0.80, P=0.010) vs. those with TGs <150 mg/dl (TR 0.27, P=0.185).

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Conclusions—In PDHS, VLDL-C is associated with CAC independent of established CVD risk factors, particularly in women, and may have value even beyond apolipoprotein B levels and in patients with elevated TGs.

Keywords

VLDL-C; triglycerides; subclinical atherosclerosis

INTRODUCTION

Serum triglyceride (TG) and TG-rich lipoproteins (TGRL) have emerged as a risk factor for cardiovascular disease (CVD) independent of low-density lipoprotein cholesterol (LDL-C).[1–4] Many prospective epidemiological studies suggest that while elevated serum TGs are associated with coronary heart disease (CHD), this relationship is attenuated after adjusting for high-density lipoprotein cholesterol (HDL-C) and LDL-C.[5] However, such adjustments may actually obscure true causal relationships particularly given our incomplete understanding of the biological pathways in metabolism of TGRL.[6] Recent genomic studies provide additional evidence for a causal and independent association between loci related to TG pathways and CVD. Therefore, there is resurgent interest in TGRL as causal biomarkers of CVD risk and target for novel therapeutic agents.[6–8]

While TGRL may be causal in atherosclerosis and predictors of CVD, fasting serum TG levels, the most commonly measured biomarker of TGRL, may not capture the true extent of this risk. Practically, TG levels are subject to large physiologic and temporal variability resulting in heterogeneous values within the same patient. Indeed, postprandial or non-fasting TGs and lipids are now thought to be better predictors of vascular disease than fasting TGs.[9] Serum TG levels also fail to fully reflect the cholesterol content of very low-density lipoprotein cholesterol (VLDL) and remnant lipoprotein particles (the metabolic products of VLDL and chylomicrons), which may be the most atherogenic TGRL particles. Thus, focus is shifting to alternative measures of TGRL metabolism as potential superior biomarkers of TGRL-related CVD risk.[10] Such measures may be particularly informative in type 2 diabetes and metabolic syndrome where remnant particles may be elevated due to poor post-prandial clearance in insulin resistance. Remnant particles can be measured directly, but methods are not standardized and therefore challenging for reproducibility across large patient populations.[11]

VLDL-C measurement is relatively straightforward and captures a distinct aspect of TGRL than serum TG. For the current analysis, following serum ultracentrifugation, we estimated VLDL-C levels in a large sample (n=1879) of type 2 diabetic patients recruited to the Penn Diabetes Heart Study (PDHS). We determined the association of VLDL-C with coronary artery calcification (CAC), an independent predictor of CVD risk, and assessed whether VLDL-C had value in predicting CAC scores beyond standard risk factors, including levels of plasma apolipoprotein B (apoB) and TGs.

METHODS

Study Participants

Details of PDHS have been reported previously.[12] Briefly, PDHS is a single center cross-sectional study of 2118 subjects with type 2 diabetes mellitus. PDHS was designed to identify novel clinical, biochemical, and genetic risk factors for coronary atherosclerosis in type 2 diabetes mellitus. Participants were recruited between 2001 and 2011 from primary care and endocrinology clinics associated with the Hospital of the University of Pennsylvania. The inclusion criteria were a clinical diagnosis of T2DM (defined as fasting blood glucose >126 mg/dl, 2-h post-prandial glucose >200 mg/dl, or use of oral hypoglycemic agents/insulin in a subject greater than age 40 years), age of 35–75 years, and negative pregnancy test (if female). The University of Pennsylvania (U.Penn) IRB approval was obtained and informed consent was obtained from all study subjects. Exclusion criteria included clinical coronary artery disease (defined as myocardial infarction, coronary revascularization, angiographic coronary disease or positive stress test), insulin use prior to age 35, a serum creatinine >2.5 mg/dl and a weight >300 lb (136.4 kg). Of 2118 subjects originally recruited, 239 were excluded due to incomplete lipid profile data, thus 1879 participants were included. The demographic and clinical characteristics of those excluded did not differ from the overall sample (not shown).

Study Parameters

Participants were evaluated at the Clinical and Translational Research Center at Penn after a 12-hour overnight fast. Clinical parameters, including blood pressure, body mass index, and waist circumference were assessed as previously reported.[12] Serum lipid parameters (total TGs, total cholesterol (TC), HDL-C, LDL-C and VLDL-C) were determined after ultracentrifugation (β-quantification technique) at time of recruitment in Penn's Centers for Disease Control-certified lipid laboratory using enzymatic assays (Hitachi 912, Roche Diagnostic Systems Inc., NJ, USA).[13] Specifically, VLDL-C was estimated as TC minus cholesterol in the bottom fraction i.e., cholesterol in the d>1.006 (bottom) fraction following ultracentrifugation.[14] Plasma apoB and C-Reactive Protein (CRP) levels were batch-assayed using high-sensitivity latex turbidimetric immunoassay (Wako Ltd., Osaka Japan). Laboratory test results were generated by personnel blinded to the clinical characteristics and CAC scores of research subjects. Framingham risk scores were calculated using TC (similar results were obtained using LDL-C).[15] Participants were classified as having the metabolic syndrome using the revised National Cholesterol Education Program definition; all PDHS participants were considered to have met glucose criteria.[16] Cardiac electron beam CT studies were performed using an Imatron C-150 CT scanner (GE-Imatron, South San Francisco, CA). CAC scores were quantified by the Agatston method as previously published.[17]

Statistical Analyses

Data are reported as median (interquartile range, IQR) for continuous variables and as proportions for categorical variables. Our primary analysis was of the association of VLDL-C with CAC data; secondary and subgroup analyses were complementary. Therefore, we did not adjust for multiple testing. For univariate comparisons of cardiovascular risk factors

between individuals in different VLDL-C quartile categories, chi-square tests, Student's t-tests and Wilcox rank sum tests were used. Based on our and others experience, we applied Tobit conditional regression for multivariable analysis of CAC data.[18] Briefly, Tobit regression combines a logistic regression of the presence of CAC (any CAC present vs. CAC zero score) with a linear regression (of log-transformed CAC) when CAC is present to produce a single estimate for the relation of risk factors with CAC data. Because of skewed distribution, VLDL-C was log-transformed for modeling. Multivariable associations of VLDL-C with CAC were assessed in incremental Tobit models: Model 1 was adjusted for age, gender and race; Model 2 was additionally adjusted for medications (statins/zetia, niacin/fibrates, insulin, metformin, thiazolidinediones, sulfonylureas, angiotensin converting enzyme inhibitor, calcium channel blocker, beta blocker, diuretic), alcohol use, exercise, TC-based Framingham risk score; Model 3 was further adjusted for duration of diabetes, hemoglobin A1c (HbA1c), body mass index (BMI) and CRP levels; Model 4 was adjusted in addition for plasma apoB levels. Because plasma TG and VLDL-C values were highly correlated (Spearman R=0.88), these lipid parameters were not included in models together. To explore whether VLDL-C was associated with CAC independent of plasma TGs, we assessed the relation of VLDL-C with CAC in adjusted models where participants were stratified into those with low (<150 mg/dl) and high TG (>150 mg/dl) values. We also compared plasma VLDL-C and TG association with CAC in non-nested models using Akaike information criteria (AIC) and Bayesian information criteria (BIC).[19, 20] A priori we tested whether the association of VLDL-C with CAC was modified (interaction) by gender and race. All analyses were performed using STATA version 12.0 software (Statacorp, College Station, Texas).

RESULTS

Characteristics of the study sample by VLDL-C values

The PDHS study sample has been described previously.[12] Briefly, median age of the full study sample (N= 2118 patients) was 59 (IQR 53–66), 36.5% were female and 60.3% were Caucasian with 34.1% black. Table 1 shows the characteristics of the VLDL-C study sub-sample (n=1879) across VLDL-C quartiles. Subjects with higher VLDL-C were more likely to be male, white, younger, more hypertensive and have longer duration of diabetes, higher HbA1c values and greater use of sulfonylureas. They were also more likely to smoke and drink alcohol but less likely to exercise and less frequently on a statin. Therefore, these potentially confounding variables were included in our multivariable modeling. Spearman correlations revealed modest association between VLDL-C and several lipid and metabolic parameters e.g., plasma TGs (R=0.88, P<0.0001), plasma apoB (R=0.45, P<0.0001), HDL-cholesterol (R=0.42, P<0.0001) and BMI (R=0.14, P<0.0001) in both men and women. In crude analyses, increasing VLDL-C quartiles were associated with higher CAC scores and with the prevalence of CAC scores >0 (Table 1). As expected CAC scores were higher in males than females and higher in whites and Hispanics than in Blacks. (Supplemental Table 1)

Figure 1 shows the distribution of VLDL-C values for PDHS participants with circulating TG values above and below 150 mg/dl, a clinically meaningful cut-point.[16] Notably, there

was substantial overlap in VLDL-C in these two groups with non-overlapping TG values suggesting that VLDL-C provides information distinct from total TGs regarding TGRL metabolism.

Association of VLDL-C with CAC in multivariable models

While there was no significant race interaction for VLDL-C association with CAC, there tended to be a gender difference, so results are presented stratified by gender as well as for the full sample. VLDL-C levels were positively associated with increasing CAC after adjusting for age, gender, and race, Framingham risk scores, exercise, HbA1c, medication and alcohol use, (Tobit Ratio (TR) 0.38 CI 0.12–0.65, P=0.005) and even after further adjusting for duration of diabetes, BMI, and CRP (TR 0.38 CI 0.12–0.65, P=0.005). Furthermore, addition of plasma apoB levels did not attenuate association of VLDL-C with CAC (TR 0.31 CI 0.03–0.58, p=0.030) (Table 2). Although the same trends were evident in both genders, there was approximately 3-fold stronger association of VLDL-C with CAC in women than in men (gender interaction P=0.034 in Model 1 and P=0.048 in Model 4), with statistically significant effects observed in women but not in men (Table 2). These finding persisted in subgroup analysis in patients not on lipid lowering therapy. (Supplemental Table 2)

VLDL-C is associated with CAC in participants with elevated TG levels

VLDL-C may relate to CAC and CVD independent of TG levels because cholesterol-rich VLDL particles may impart greater atherogenic risk than total TGs which are distributed across all lipoprotein particles, not just VLDL. Because TG and VLDL-C values were highly correlated, however, their inclusion in models together can lead to excess colinearity and potential spurious findings. Therefore, we compared plasma VLDL-C and TG association with CAC in non-nested models using AIC and BIC scores and found that both AIC and BIC scores were slightly but consistently lower for VLDL-C (e.g., in fully adjusted Model 4; AIC 7263.65, BIC 7402.11) than TG (AIC 7263.94, BIC 7402.40). This suggests that VLDL-C performs better than TG in predicting CAC scores when all other confounders and covariates are held equal. Furthermore, we stratified PDHS participants into those with TG values below and above 150 mg/dl, a cut point used in clinical practice.[16] In these stratified analyses, VLDL-C had stronger association with CAC in individuals with TGs >150mg/dl (TR 0.80, P=0.01) than those with TGs <150 mg/dl (TR 0.27, P=0.189) (Table 3) and followed the same gender patters as the primary analysis. Sensitivity analysis showed consistent results, TR 0.59 (P=0.013) and TR 0.19 (P=0.424) in individuals with TGs above or below the median value (116mg/dl) respectively.

DISCUSSION

No prior studies have examined the relationship of CAC with circulating levels of VLDL-C, an alternative to serum TGs as a marker of TGRL. In patients with type 2 diabetes, a setting of abnormal TGRL metabolism and increased CVD risk, we found that VLDL-C levels were positively associated with higher CAC scores after controlling for numerous traditional CV risk factors, and even after inclusion of plasma apoB data. Consistent with published gender patterns for TGs, the association of VLDL-C with CAC was significantly stronger in women

than men.[2, 9] VLDL-C levels related to greater CAC scores in individuals with high TGs suggesting possible incremental value for VLDL-C in CVD risk prediction beyond serum TGs.

Renewed interest in TGRL as causal and therapeutic targets in heart disease has been driven by large epidemiological studies,[1–4] trial failures of HDL-C therapies in CVD,[21] and recent genomic work suggesting that TGRL may be causal in atherosclerotic CVD.[6–8] The relationship between TGs and CVD has been the subject of much debate over the past several decades. On the one hand, many epidemiological studies have suggested that the relationship of TGs with CVD is lost after adjusting for both HDL-C and non-HDL-C, and that given knowledge of TC and HDL-C, assessment of TG provided little incremental information for CVD risk prediction.[1, 5] This was particularly thought to be the case in diabetic patients, where high TG levels are often associated with low HDL-C levels and relative insulin resistance.[22] The Framingham study showed than compared to healthy controls, diabetic patients were twice as likely to have low HDL-C levels, and accordingly, the independent effect of TGs on CVD was lost after adjusting for HDL-C.[23] Yet other epidemiological studies and large meta-analyses support an independent association of TGs with CVD beyond LDL-C and HDL-C.[1–4] PROCAM, a prospective study of 4849 middle aged men showed that fasting TGs were associated with future cardiovascular events, independent of LDL-C and HDL-C.[24] In the Copenhagen Male Study of 2906 white men, rates of CVD were 50% higher in those with TGs in the middle tertile and 120% higher in the upper tertile as compared to those in the lowest TG tertile, after adjustment for conventional risk factors including LDL-C and HDL-C.[3] A sub analysis of a post acute coronary syndrome population in PROVE IT-TIMI 22 showed that on treatment TG levels <150 mg/dl, independent of LDL-C, reduced risk of CVD, and that each 10mg/dl reduction in TG level was associated with a 1.6% reduction of primary endpoint.[25] Finally, recent genetic data strongly support a causal role for TG associated loci in CAD.[6–8] In large meta-analysis of genome wide association studies of plasma lipids, many TG-associated loci, unlike those for HDL-C, also relate to CVD. Indeed, Kathiresan and colleagues showed recently that even after accounting for effects on LDL-C or HDL-C, TG-associated loci still had a strong independent relation to CVD.[8]

There are several proposed mechanisms for why TGRL may have causal relations to CVD beyond more traditionally measured lipids, TC, LDL-C and HDL-C. TGRL particles are increased in excess calorie consumption particularly in insulin resistant states because of failure to clear post-prandial lipid excursions and due to increased hepatic VLDL production. VLDL particles from patients with hypertriglyceridemia may promote atherosclerosis. Each TGRL particle transports more cholesterol than each LDL particle, and therefore TGRLs are potentially more atherogenic on a per-particle basis.[26] When TGRLs are elevated, as in subjects with high TG, large VLDL contain more cholesterol relative to these same particles from subjects with normal TG levels.[27] VLDL sized lipoproteins are found in human intima and have been isolated from atherosclerotic lesions.[28, 29] Furthermore VLDL and IDL levels are related to the progression of atherosclerosis in humans.[30] Particular TGRLs are the only native, unmodified lipoproteins that can *in vitro* cause rapid, receptor-mediated macrophage lipid accumulation.[26, 31] Additionally, VLDL from subjects with elevated TGs bind with high affinity both to LDL receptors and to a

unique TGRL/apoB48 receptor expressed specifically by monocytes, macrophages, and endothelial cells.[32] As a secondary consequence, increased TGRL levels also drive (via TG activation of cholesterol ester transfer protein (CETP) and exchange of TG for cholesterol) increased generation of small dense cholesterol-poor LDL particles that are atherogenic but do not result in increased circulating LDL-C levels. Similarly, TGRL drives remodeling of HDL-C to smaller cholesterol-poor particles that may lack atheroprotective functions and do not correlate with HDL-C levels.

Serum TGs are imperfect measures of atherogenic TGRL for several reasons. First, there is considerable inter-individual and intra-individual variation in plasma TG levels.[33] Second, circulating TGRL are extremely heterogeneous particles both in terms of size and composition, and total serum TG levels are not sensitive to different types of particles carrying TG.[34] Partially catabolized remnant particles are felt to be the most atherogenic.[35] In this context, we hypothesized that cholesterol content of VLDL (VLDL-C) could be superior to total plasma TGs as an atherogenic marker because VLDL-C levels increase as VLDL is metabolized to smaller more atherogenic remnants.

TGRL are of particular importance in diabetic populations because insulin resistance increases hepatic VLDL production and decreases clearance of TGRLs. Studies of TGs in diabetic populations have provided mixed results. In a large study of 11,000 type 2 diabetics, TGs >150 were an independent risk factor for incident CVD in women but not men, yet non-HDL-C may be superior to TGs as a CVD risk factor in diabetes.[36, 37] To our knowledge there are no published studies examining the relationship between VLDL-C and CAC, either in patients with diabetes or in the general population. In our type-2 diabetic PDHS sample, the association of VLDL-C with CAC persisted after adjusting for multiple traditional CVD risk factors, and more importantly, even beyond plasma apoB levels. Comparative non-nested models suggested that plasma VLDL-C has modestly stronger relation than TG levels to CAC. Further, the association of VLDL-C with CAC was strongest in patients with elevated TGs suggesting that VLDL-C may provide insight into atherosclerotic CVD beyond routine lipoprotein profiling. Our findings are supported by a prior small study of lipoprotein fractions in 313 diabetics patients followed for 7 years for CVD events in which higher VLDL-C was associated with increased risk of CVD, particularly in patients with low LDL-C and low HDL-C.[38]

In our study, there was a stronger association of VLDL-C with CAC in women than men. This may be explained by greater lipid abnormalities observed in post-menopausal diabetic women than men. In diabetic populations in particular, women have higher TG levels and have an overall more adverse lipid profile than men.[39] Much of the atheroprotection conferred by female gender disappears after menopause.[40] Indeed, postmenopausal state is associated with significantly higher postprandial TG levels despite similar fasting TGs.[41] Of 672 women studied in PDHS, 83% were over 50 year old and 59% were over 55 years old, suggesting that most of the female PDHS sample was post-menopausal. Our finding is consistent with several studies showing a stronger relation of TGRL markers with CHD/CVD events in post-menopausal women, including diabetic patients, than in men.[2, 42] For example, in a large meta-analysis of nearly 60,000 patients, relative risk for CVD with

elevated TGs was 1.32 in men and 1.76 in women, with similar trends persisting after controlling for other risk factors.[2]

While we chose to estimate VLDL-C, alternative measures of TGRL can be performed. ApoB 100 is present on all apoB100-containing lipoproteins (one apoB per particle) including non-TGRL. Similarly, non HDL-C reflects cholesterol on all atherogenic particles including VLDL and TGRL. Several studies have demonstrated that both these measures are strong predictors of CVD and superior to LDL-C across a broad spectrum of risk including patients on statin therapy.[43, 44] However, because plasma apoB and non HDL-C capture information regarding non TGRL, such as lipoprotein (a) and LDL, they are less specific tools when addressing the independent effects of TGRLs. Indeed, our data suggest that VLDL-C may provide utility beyond knowledge of plasma apoB.

Work by Nordestgaard suggested that a simple calculation (TC - LDL-C - HDL-C) could approximate remnant cholesterol, and that an increase of 1 mmol/l (39 mg/dl) in this estimate was associated with a 2.8-fold increased risk of CHD, independent of HDL-C levels.[10] We calculated this estimate of remnant particles (TC - LDL-C - HDL-C) in our PDHS sample and found quite similar, but not identical, findings for the association with CAC compared to that for VLDL-C in our sample. (Supplemental Table 3) Direct measurement of remnant particles is also possible and these particles have been shown to strongly associate with CVD, especially in patients with normal TC levels.[45] Yet accurate measurement of remnant particles is not straightforward. They are difficult to differentiate from their TG-rich precursors, are rapidly catabolized with low concentrations in plasma, and at any given time, are heterogeneous in size, density, and composition because of dynamic metabolism.[11] Genes and their secreted products that regulate TGRL and relate to CVD (e.g., LPL and apoCIII) also provide biomarkers of TGRL and CVD risk that require further study.[6–8, 46]

Our work has several strengths and limitations. As noted, this is the first work to investigate the association of VLDL-C with CAC, a measure of subclinical atherosclerosis and a well-validated predictor of CVD risk including in type-2 diabetes.[47–49] A large sample of almost 1900 type-2 diabetic patients including significant percentages of women and non-Caucasians were specifically recruited to be free of the confounding influence of chronic kidney disease or CVD at entry. Our analyses were robust to adjustment for multiple risk factors and potential confounders and pointed toward value for VLDL-C even beyond serum apoB and TG data. Limitations include cross-sectional design and lack of hard CVD outcomes or incident CVD events. However, CAC scores are strong independent predictors of CHD events in the general population as well as in prospective studies of patients with type 2 diabetes.[47–50] Besides VLDL-C, additional markers of TGRL were not measured preventing us from making inference regarding the optimal measures for application in clinic. Future studies are needed to examine CVD outcomes for multiple measures of TGRL and their genetic predictors in large prospective studies of both diabetic and non-diabetic populations.

In conclusion, we show an association of VLDL-C with CAC, even after adjustment for traditional risk factors and beyond plasma apoB levels, and that such an association is stronger in females and when TG levels are elevated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Association of VLDL-C with CAC after adjustment for traditional risk factors

- Association of VLDL-C with CAC even beyond apoB levels

- Association of VLDL-C with CAC is significantly stronger in women than men

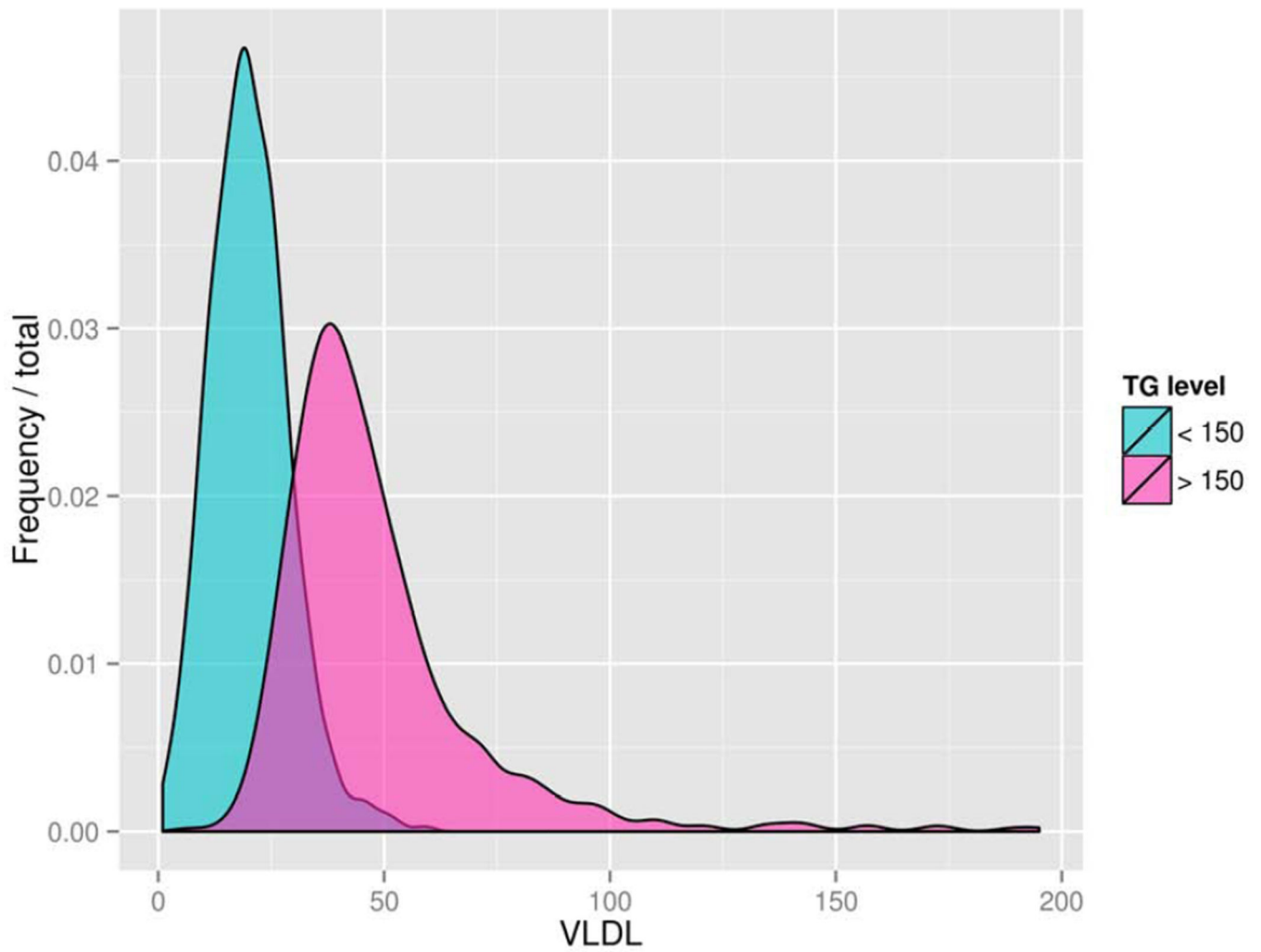- Possible incremental value for VLDL-C in CVD risk prediction beyond serum TGs

Figure 1.
Distribution of plasma VLDL-C across categories of low (<150 mg/dl) and high (>150 mg/dl) plasma triglyceride values.
Abbreviations:
TG=triglyceride
VLDL=very-low density lipoprotein

TABLE 1

Characteristics of the study sample across VLDL-C Quartiles

Variable	VLDL-C Quartile 1 (n=436)	VLDL-C Quartile 2 (n=480)	VLDL-C Quartile 3 (n=483)	VLDL-C Quartile 4 (n=480)	P value*
Age (years)	60 (54–67)	60 (54–66)	60 (53–66)	58 (53–64)	0.012
Female (%)	43.6%	39.8%	32.1%	28.5%	<0.001
Race (%)					<0.001
White	51.6%	54.2%	64.6%	71.9%	
Black	42.7%	40.8%	29.0%	21.6%	
Hispanic	28.6%	21.4%	28.6%	21.4%	
Other	18.8%	21.7%	27.5%	31.9%	
Blood pressure (mmHg)					
Systolic	129 (120–141)	131 (121–141)	131 (122–142)	132 (122–142)	0.008
Diastolic	74 (70–81)	74 (71–82)	77 (71–83)	77 (72–83)	<0.001
Total cholesterol (mg/dl)	156.5 (137–179)	163.5 (144–185)	173 (153–196)	197 (168–224)	<0.001
HDL-cholesterol (mg/dl)	52 (44–64)	47 (41–56)	43 (37–52)	38 (33–46)	<0.001
Triglycerides (mg/dl)	68 (56–83)	96.5 (82–113)	133 (111–164)	217 (179–276)	<0.001
LDL–cholesterol (mg/dl)	88 (74–108)	93 (78–113)	99 (81–119)	102 (77–126)	<0.001
Remnant Cholesterol (TC-LDLC-HDL)	13.6 (11.2–16.6)	19.3 (16.4–22.6)	26.6 (22.2–32.8)	43.4 (35.9–55.3)	<0.001
Body mass index (kg/m^2)	30.8 (27.2–35.0)	32.0 (28.2–36.1)	32.9 (28.9–37.2)	32.4 (29.6–36.7)	<0.001
Waist circumference (cm)	40.3 (36.5–44.0)	41.0 (37.5–45.4)	43.0 (39.9–46.5)	42.5 (39.5–46.5)	<0.001
Metabolic syndrome (%)	64.2%	71.0%	83.0%	94.8%	<0.001
Duration of diabetes (years)	6 (3–12)	6 (2–11)	6 (3–11)	6 (3–10)	0.128
Hemoglobin A1c (%)	6.6% (6.1–7.2)	6.5 (6.0–7.4)	6.7% (6.1–7.8)	7.0% (6.4–8.1)	<0.001
Glucose (mg/dl)	107 (88–129)	109 (90–137)	120 (97–152)	129 (105–164)	<0.001
Current tobacco use (%)	56.4%	59.0%	60.0%	59.9%	0.257
Current alcohol use (%)	52.1%	50.4%	53.4%	49.9%	0.763
10 year Framingham risk (%)‡	11% (7–16)	13% (8–20)	16% (10–25)	20% (13–31)	<0.001
C-reactive protein (mg/L)	1.57 (0.73–3.52)	1.58 (0.86–4.10)	2.08 (0.96–4.56)	2.10 (1.00–4.64)	<0.001
Lipid lowering medications Statins	59.4%	61.9%	55.3%	47.2%	<0.001

Variable	VLDL-C Quartile 1 (n=436)	VLDL-C Quartile 2 (n=480)	VLDL-C Quartile 3 (n=483)	VLDL-C Quartile 4 (n=480)	P value*
Niacin	4.8%	4.0%	6.6%	6.8%	0.060
Fibrates	2.5%	4.4%	6.8%	11.9%	<0.001
Ezetimibe	5.7%	6.5%	5.2%	3.7%	0.111
Fish Oil	11.0%	13.1%	17.8%	12.9%	0.161
Diabetes medications Metformin	62.6%	61.0%	65.6%	61.1%	0.992
Sulfonylureas	28.9%	32.5%	36.4%	40.1%	<0.001
Thiazolidinediones	21.1%	22.1%	23.6%	23.3%	0.349
Insulin	24.3%	20.40%	22.40%	18.9%	0.104
Serum creatinine (mg/dl)	0.90 (0.76–1.00)	0.90 (0.72–1.00)	0.90 (0.80–1.04)	0.90 (0.80–1.10)	<0.001
CAC score					
Median (IQR)	19.5 (0–252.5)	19 (0–282.5)	54 (0–283)	63 (0–311)	0.001
>0 (%)	59.4%	62.3%	67.7%	70.9%	<0.000

Metabolic syndrome was defined as have 3 or more following: waist circumference 102 cm or 40 inches (male), 88 cm or 35 inches(female), dyslipidemia: TG 1.7 mmol/L (150 mg/dl), HDL-C <40 mg/dl (male), < 50 mg/dl (female), blood pressure 130/85 mmHg (or treated for hypertension). Data are presented as median (IQR) or as percentages, except as indicated.

*
P-value is from nonparametric test for trend across ordered groups, an extension of the Wilcoxon rank-sum test. ‡Total cholesterol 10-year Framingham risk percentage.

Abbreviations:
CAC=coronary artery calcium
HDL= high density lipoprotein
LDL = low density lipoprotein
VLDL-C= very-low density lipoprotein cholesterol

TABLE 2

Multivariate models of VLDL-C association with CAC

Model	Full Sample Tobit Ratio (95% CI) P value	Men Tobit Ratio (95% CI) P value	Women Tobit Ratio (95% CI) P value
Model 1	0.40 (0.13–0.64) p=0.001	0.17 (−0.09–0.43) p=0.196	0.98 (0.41–1.54) p=0.001
Model 2	0.38 (0.12–0.65) p=0.005	0.22 (−0.08–0.52) p=0.146	0.62 (0.04–1.22) p=0.035
Model 3	0.38 (0.12–0.65) p=0.005	0.20 (−0.10–0.50) p=0.189	0.75 (0.16–1.34) p=0.013
Model 4	0.31 (0.03–0.58) p=0.030	0.15 (−0.15–0.46) p=0.323	0.72 (0.09–1.36) p=0.026

Model 1: age gender, race.

Model 2: Model 1 + HbA1c, total cholesterol-based Framingham risk score, medications (zetia/statin, niacin/fibrate, ace inhibitor, calcium channel blocker, beta blocker, diuretic, metformin, sulfonylurea, thiazolidinediones, insulin), alcohol use, and exercise.

Model 3: Model 2 + BMI, duration of diabetes, and C-reactive protein.

Model 4: Model 3 + plasma apolipoprotein B.

Abbreviations:
BMI=body mass index
CAC=coronary artery calcium
CI=confidence interval.
HbA1c= hemoglobin A1c
VLDL-C= very-low density lipoprotein cholesterol

TABLE 3

Multivariate association of VLDL-C with CAC stratified by plasma TG levels

Model	Full Sample Tobit Ratio (95% CI) P value	Men Tobit Ratio (95% CI) P value	Women Tobit Ratio (95% CI) P value
TG <150 (mg/dl)	0.27 (−0.13–0.66) p=0.185	0.00 (−0.45–0.45) p=0.988	0.74 (−0.08–1.57) p=0.078
TG >150 (mg/dl)	0.80 (0.19–1.14) p=0.010	0.67 (0.00–1.34) p=0.051	1.31 (−0.13–2.76) p=0.074

Model includes age gender, race, HbA1c, total cholesterol-based Framingham risk score, medications (zetia/statin, niacin/fibrate, ace inhibitor, calcium channel blocker, beta blocker, diuretic, metformin, sulfonylurea, thiazolidinediones, insulin), alcohol use, exercise, BMI, duration of diabetes, and C-reactive protein.

Abbreviations:
CAC=coronary artery calcification
CI=confidence interval.
TG=triglycerides
VLDL-C=very-low density lipoprotein cholesterol