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# Altered composition of triglyceride-rich lipoproteins and coronary artery disease in a large case–control study

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ABSTRACT

*Background:* Traditional beta-quantification of plasma lipoproteins by ultracentrifugation separates triglyceride-rich lipoproteins (TGRL) from higher density lipoproteins. The cholesterol in the TGRL fraction is referred to as measured very low-density lipoprotein cholesterol (VLDL-C) recognizing that other TGRL may be present. The measured VLDL-C to total plasma triglyceride (VLDL-C/TG) has long been considered an index of average TGRL composition with abnormally high VLDL-C/TG ratios (≥0.30 with TG > 150 mg/dL) indicative of atherogenic remnant accumulation (type III hyperlipidemia). However, virtually no reports are available which examine potential associations between CAD and VLDL-C/TG at the lower end of the spectrum.

*Methods and results:* We performed ultracentrifugation in 1170 cases with premature-onset, familial CAD and 1759 population-based controls and examined the VLDL-C/TG ratio as an index of TGRL composition. As expected, we found very high CAD risk associated with severe type III hyperlipidemia (OR 10.5, $p = 0.02$). Unexpectedly, however, we found a robust, graded, and independent association between CAD risk and *lower* than average VLDL-C/TG ratios ($p < 0.0001$ as ordered categories or as a continuous variable). Among those in the lowest VLDL-C/TG category (a ratio <0.12), CAD risk was clearly increased (OR 4.5, 95% CI 2.9–6.9) and remained significantly elevated in various subgroups including those with triglycerides below 200 mg/dl, in males and females separately, as well as among those with no traditional CAD risk factors (OR 5.8, 95% CI 1.5–22). Significant compositional differences by case status were confirmed in a subset whose samples were re-spun with measurement of lipids and apolipoprotein B (apo B) in each subfraction.

*Conclusions:* We found a strong, graded, independent, and robust association between CAD and lower VLDL-C/TG ratios. We consider this a novel, hypothesis-generating observation which will hopefully generate additional future studies to provide confirmation and further insight into potential mechanisms.



## 1. Introduction

Triglyceride-rich lipoproteins (TGRL) include a complex array of particles with potentially different cardiovascular risk associations. Ultracentrifugation at a solvent density (D) of plasma (being 1.006 g/ml) yields a $D < 1.006$ supernatant fraction containing essentially all TGRL (chylomicrons, very low density lipoprotein (VLDL), and β-VLDL) having diameters ranging from approximately 30–80 nm for the VLDL particles to considerably larger than this for chylomicrons [1]. In the $D > 1.006$ infranatant fraction, there are intermediate density (IDL), low density (LDL) and high density lipoproteins (HDL). In traditional "beta-quantification," cholesterol in the top fraction is measured (referred to as VLDL-C, although cholesterol from all TGRL is included) and the ratio of VLDL-C to total plasma triglycerides (TG) is considered an index of the composition of TGRL. The average VLDL-C/TG ratio in normolipemic individuals ranges from approximately 0.18 to 0.22 and is the basis of the Friedewald equation used for estimation of LDL cholesterol (LDL-C) in clinical laboratories worldwide [2–5]. The VLDL-C/TG ratio varies substantially from this range in the presence of either TGRL remnants (which are enriched in cholesterol) [5–8] or chylomicrons or very large VLDL (which are relatively enriched in triglyceride) [6].

Cholesterol-enriched TGRL remnants are considered to be highly atherogenic [1,6,7,9–21]. Such TGRL remnants include the highly

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cholesterol-enriched and relatively large "β-VLDL" seen in type III hyperlipidemia, as well as the smaller, more common "slow pre-β" particles which are reported to be less cholesterol-enriched than β-VLDL [22,23]. Type III hyperlipidemia has been traditionally defined as a VLDL-C/TG ≥0.30 with total triglycerides >150 mg/dl [13,16,24–26]. We have previously reported the only population-based estimate of coronary artery disease (CAD) risk associated with this traditional definition of type III hyperlipidemia [27], but there has been virtually no published consideration of the possible associations between CAD and TGRL with VLDL-C/TG values below the normal range.

In this study we examine the entire range of observed VLDL-C/TG for association with CAD utilizing the largest series of CAD cases and controls of which we are aware with ultracentrifuged lipids measured in all.

## 2. Methods

### 2.1. Study participants

Cases included 1104 persons aged 30–75 when examined who had experienced premature CAD defined as myocardial infarction, percutaneous transluminal coronary angioplasty, or coronary artery bypass grafting by age 60 for men or age 70 for women. All cases were recruited from families in which two or more first-degree relatives (parent, sibling or child) had similarly early onset of clinical CAD as previously described [27]. To minimize possible artifactual effects of acute coronary syndrome on lipid levels, patients were seen at least 6 months after their acute event. Controls were also aged 30–75 when screened and were representative of the general Utah population as previously described for the original group 1104 [27]. For this report, we added 655 newly recruited controls with the same age range who were identified through randomly ascertained driver's license records, and thus also considered to represent the general population. This study was approved by the Institutional Review Board of the University of Utah Medical Center and LDS Hospital. All subjects signed informed consent prior to participating.

A participant was considered to have hypertension if taking antihypertensive medication for a prior physician diagnosis of hypertension, or if at exam the mean of two sitting blood pressures (taken with a Critikon Dinamap automated blood pressure machine) was greater than or equal to 140 mmHg systolic or 90 mmHg diastolic. Diabetes was considered present if a prior physician diagnosis had been made or if the fasting serum glucose from their exam was greater than or equal to 126 mg/dl. Cigarette smoking was dichotomized into "ever" or "never" with ever smoking defined as having smoked daily for one year or more. Many patients had quit after onset of their CAD, hence the designation as ever smoking rather than current and former.

### 2.2. Laboratory methods

Generally, lipids were measured as previously described [28]. EDTA-anticoagulated blood samples were collected in the morning after 10–12 h of fasting and prepared according to guidelines of the Lipid Research Clinic's program *Manual of Laboratory Operations* [29]. Lipid and lipoprotein concentrations were measured by a micro-scale ultracentrifugation procedure developed in our laboratory [30]. HDL-C was measured in the supernatant after removal of non-HDL using a magnetized solid-phase dextran sulfate/$MgCl_2$ precipitation reagent [31]. Cholesterol and triglycerides in total plasma and ultracentrifugal subfractions were measured with automated analyzers (FARA II (Roche Diagnostics) or PolyChem (Polymedco)). TGRL were separated from IDL, LDL and HDL by ultracentrifugation (4 h, 157 000 × *g*, 20 °C) in a Beckman TL100 ultracentrifuge and tube slicing. The value for VLDL-C was taken as the measured cholesterol in the $d < 1.006$ g/mL fraction. LDL-C was taken as the cholesterol measured in the bottom fraction minus HDL-C. Type III hyperlipidemia was defined as present if the ratio of VLDL-C/TG was ≥0.30 and plasma total TG were > 150 mg/dl [24]. Our laboratory participates in the CDC Standardization Program and consistently achieves excellent agreement with CDC standards (coefficients of variation in the range of 0.5–3.3% for total cholesterol, triglyceride and HDL-C, with <3% difference in means). Concentrations of apolipoprotein B (using a polyclonal antibody recognizing both B-100 and B-48) was measured in both total plasma and plasma in the $D < 1.006$ g/ml fraction were quantified by liquid-phase, double-antibody radioimmunoassays with the difference taken as LDL-apo B [32]. Estimated β-VLDL-C levels were calculated by an algebraic method using VLDL-C, total triglycerides, LDL-C, and HDL-C as previously described [33–35].

### 2.3. Statistical analysis

The SAS Statistical Software Package (version 9.2 for the PC) was used for data analysis (SAS Institute, Inc. Cary, NC). Statistical analyses on triglycerides were done after logarithmic transformation. Statistical tests included Student's *t*-test, $\chi^2$, Pearson's correlation, and stepwise multiple logistic regression using SAS PROC LOGISTIC. We used the Cochran–Armitage trend test with the exact option (two-sided) to compare the difference in TGRL composition distribution in selected cases and controls (using SAS PROC FREQ). Analysis of covariance was performed using PROC GLM. Because some CAD cases came from the same family, effects of subject relatedness in logistic regression analyses were tested using generalized estimating equations with an exchangeable correlation matrix in PROC GENMOD in SAS. As results were nearly identical with the two methods, *p*-values obtained with PROC LOGISTIC are reported.

## 3. Results

Clinical characteristics of familial CAD cases are compared with controls in Table 1. Highly significant differences by univariate analysis were apparent for standard CAD risk factors as shown.

Our aim in this study was to examine the association of CAD risk with differences in the composition of TGRL. As expected (see Fig. 1), increasing levels of estimated β-VLDL-C were associated with a graded increase in CAD risk. Results are given for logistic regression using a minimally adjusted model (adjusted for age and gender only) and a full model (age, gender, smoking history, hypertension, diabetes, measured LDL-C, log of plasma triglycerides, and HDL-C). In several prior studies, we had found that over 90% of persons meeting traditional cut-points for type III hyperlipidemia (VLDL-C/TG ≥0.30 and triglycerides >150 mg/dl) had estimated β-VLDL-C levels over 35–40 mg/dl [33–35]. Similar results were seen in the current study (all 44 subjects meeting traditional type III criteria had estimated β-VLDL-C above 32 mg/dl, with 36 of the 44 having levels above 40 mg/dl). While the gradient of risk was steep, confidence intervals were wide and only those with the highest estimated β-VLDL-C levels had risk entirely independent of standard risk factors including total plasma triglycerides and HDL cholesterol with no elevation of risk at modest elevations in VLDL-C/TG ratio (i.e., in the range of 0.22 to <0.30) (see Fig. 2). In all subsequent multiple logistic regression models we adjusted for the presence of type III hyperlipidemia as a risk factor unless otherwise specified.

We then examined CAD risk associated with TGRL having lower than usual cholesterol content as shown in Fig. 2. Surprisingly, we found a marked, graded, and statistically highly significant increase in risk associated with *lower* ratios of VLDL-C/TG ($p < 0.0001$ for trend using categories shown in Fig. 2; *p*-values were <0.0001 for