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Calculated Non-HDL Cholesterol Includes Cholesterol in Larger Triglyceride-Rich Lipoproteins in Hypertriglyceridemia

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Context: Calculated non–high-density lipoprotein (HDL) cholesterol (non-HDLC) should selectively include cholesterol from atherogenic lipoproteins to be a reliable risk marker of cardiovascular disease. In hypertriglyceridemia (HTG), there is increased abundance of larger and less atherogenic triglyceride-rich lipoproteins (TRL), namely, larger very-low-density lipoproteins (VLDL), and chylomicrons.

Objective: We aim to demonstrate that serum triglyceride (TG) level has a substantial impact on non-HDLC's ability to represent cholesterol from atherogenic lipoproteins, even though TG is not part of the calculation for non-HDLC.

Design: Analysis of lipid profile data

Settings: Lipid Clinic patient cohort, and Biochemistry Laboratory patient cohort

Patients or Other Participants: 7,492 patients in the Lipid Clinic cohort with baseline lipid profiles documented prior to starting lipid-lowering medications and 156,311 lipid profiles from The Ottawa Hospital Biochemistry Laboratory cohort.

Intervention: None

Main Outcome Measure: Our modeling process includes derivation of TG-interval–specific lipoprotein composition factor (LCF) for TRL, which represents the mass ratio of cholesterol to TG in TRL. A high LCF indicates that the TRLs are mainly the cholesterol-rich atherogenic remnant lipoproteins. A low LCF indicates that the TRLs are mainly the TG-rich larger VLDL and chylomicrons.

Results: As serum TG increases, there is progressive decline in the LCF for TRL, which indicates that the calculated non-HDLC level reflects progressive inclusion of cholesterol from larger TRL. This is shown in both cohorts.

Abbreviations: CM, chylomicron; CVD, cardiovascular disease; HDL, high-density lipoprotein; HDLC, high-density lipoprotein cholesterol; HTG, hypertriglyceridemia; LCF, lipoprotein composition factor; LDL, low-density lipoprotein;LDLC, low-density lipoprotein cholesterol; non-HDLC, non–high-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride; TRL, triglyceride-rich lipoprotein; TRLC, triglyceride-rich lipoprotein cholesterol; VLDL, very-low-density lipoprotein; VLDLC, very-low-density lipoprotein cholesterol.

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Conclusions: Calculated non-HDLC is influenced by TG level. As TG increases, non-HDLC gradually includes more cholesterol from larger TRL, which are less atherogenic than LDL and remnant lipoproteins.

Keywords: non-HDL cholesterol, hypertriglyceridemia, triglyceride-rich lipoproteins

Non–high-density lipoprotein (HDL) cholesterol (non-HDLC) is regarded by several expert lipid management panels as a co-primary target for lipid-lowering therapy, together with low-density lipoprotein cholesterol (LDLC) [1–5]. Non-HDLC is considered by some as superior to LDLC because it encompasses cholesterol in low density lipoproteins (LDL) as well as very-low-density lipoproteins (VLDL) and other triglyceride-rich lipoproteins (TRL), some of which are also atherogenic [2–4, 6–7].

In most clinical settings, LDLC and non-HDLC are calculated from measured values of serum total cholesterol (TC), triglycerides (TG), and HDLC. In the Friedewald equation for LDLC calculation ($LDLC = TC - VLDLC - HDLC$), VLDL cholesterol (VLDLC) is derived with the assumption that the TG:cholesterol mass ratio of VLDL is 5:1, representing the ratio in normal VLDL (5 mg of TG for every 1 mg of cholesterol in normal VLDL) [8]. Hence VLDLC is obtained by dividing TG by 5, in mg/dL, and by 2.2, in mmol/L. The equation works best in normo-triglyceridemic subjects, where the bulk of TRL present are VLDL. In hypertriglyceridemia (HTG), other TRL are present. In mild HTG, VLDL and chylomicron (CM) remnants are present in addition to VLDL. In moderate to more severe HTG, parent VLDL and CM, as well as larger VLDL and CM remnants are present [9–11]. The TG:cholesterol ratio in these TRL cannot be assumed to be 5:1. This led Martin et al to propose a much-improved modified method to calculate LDLC that uses a variable TRL TG:cholesterol ratio in which the ratio increases with increasing TG levels, avoiding the derivation of falsely high triglyceride-rich lipoprotein cholesterol (TRLC) levels and therefore falsely low LDLC levels [12–13].

When the Friedewald equation is used (with a fixed VLDL TG:cholesterol ratio), it is rightly recognized that LDLC should not be calculated in the presence of HTG, set rather arbitrarily as TG > 4.5 mmol/L. There is no such TG restriction placed on the calculation of non-HDLC [1–5, 14–15]. This study explores the question of whether such a restriction should similarly apply.

VLDLC, or more broadly, TRLC, is not part of the equation for the calculation of non-HDLC (non-HDLC = TC − HDLC) but we contend that this has created the false impression that non-HDLC, unlike LDLC, is not influenced by TG status. Since VLDLC or TRLC is not subtracted from TC in the calculation it remains part of non-HDLC. Thus, $non\text{-}HDLC = TRLC + LDLC$. For calculated non-HDLC to be a reliable risk marker of cardiovascular disease (CVD), it should be reflective of atherogenic lipoproteins. Non-HDLC becomes progressively inappropriate for this intended function in the presence of HTG, due to inclusion of cholesterol from larger, less atherogenic TRL (parent VLDL and CM and large CM remnants). Thus, the risk of CVD can be overestimated by non-HDLC in the presence of HTG. This theoretical concept of progressive inclusion of cholesterol from larger, less atherogenic TRL in calculated non-HDLC in the presence of HTG has not been directly demonstrated before. In this study, we have used two large datasets, one from a lipid clinic population and a second from a tertiary care hospital biochemistry laboratory population to demonstrate this concept.

1. Methods

A. Two Patient Cohorts

We received institutional Research Ethics Board (REB) approval (OHSN-REB Protocol ID 20180461-01H) to analyse lipid-profile data from two patient cohorts.

The first cohort is a Lipid Clinic cohort that comprises 7,492 patients who were referred to The Ottawa Hospital's Lipid Clinic and the Chicoutimi Hospital Lipid Clinic. The Chicoutimi Hospital Lipid Clinic has a very large number of people with familial hypercholesterolemia and familial chylomicronemia syndrome due to the historical founder effect in the Saguenay-Lac St Jean (Quebec) region. Lipid profiles from this Lipid Clinic cohort were obtained from fasting patients, prior to starting on lipid-lowering medications. All lipid profiles were performed in accredited clinical laboratories from 1990 to 2017.

The second cohort is a Biochemistry Laboratory (Lab) cohort that comprises 156,311 full lipid profiles (TC, HDLC, and TG) that were performed at The Ottawa Hospital's accredited Biochemistry Lab from March 15, 2014, to January 1, 2018. They were obtained from either inpatients or outpatients, were fasting or nonfasting, and patients could have been on lipid-lowering medications or not. More than 1 profile may belong to a single patient.

B. Derivation of Lipoprotein Composition Factor

We performed a graphical analysis of the association between calculated non-HDLC and TG at intervals of 1 mmol/L, focusing on a TG of 0.01 mmol/L to TG 10 mmol/L. For both cohorts, analysis of data for TG above 10 mmol/L was limited due to the relative sparsity of data. Since *non-HDLC = TRLC + LDLC* is a biological additive relationship, it is best modeled using linear regression [16]. Calculated non-HDLC is equal to lipoprotein composition factor (LCF) × TG + LDLC, where LCF represents an approximation of the ratio of cholesterol to TG in TRL. LCF is the reciprocal/inverse of the Friedewald equation's approximation of VLDLC (ratio of the mass of TG to that of cholesterol) [8]. Thus, when the percentage of TG in TRL increases, LCF decreases.

Conceptually, this is modeled by the equation of a line, Y = slope × X + intercept. For our study, this linear equation takes on the meaning of non-HDLC = LCF × TG + LDLC. Using median quantile regression modeling (nonparametric), the LCF for TRL is the slope of the 0.50 quantile regression line (with 95% confidence interval [CI] from bootstrapping method) for each TG-interval. A decrease of the slope of the 0.50 quantile regression line as TG increases would indicate that non-HDLC is progressively derived from TG-richer TRL. From the quantile regression analysis results for each cohort, we explored the association between the LCF for TRL vs TG by performing linear regression analyses. The coefficient of determination for various TG-intervals were used to detect a TG level above which the correlation weakens in each cohort.

C. Derivation of Core Lipid Composition From the LCF

Being the inverse of Friedewald's approximation of VLDLC, our derived LCF in normotriglyceridemia would be 1/2.2 (0.45) in mmol/L (or 1/5 in mg/dL), denoting the cholesterol:TG ratio in normal VLDL. In terms of lipoprotein composition, the TRL cholesterol:TG ratio of 1:2.2 in mmol/L reflects a lipoprotein composition of ~16.7% cholesterol, and ~83.3% TG. This stems from lipoprotein composition studies that were performed by Frederickson et al [17] and Hatch et al [18], from which Friedewald [8] based the mass ratio in VLDL to be approximately 1 mg cholesterol per 5 mg TG. Similar VLDL composition results were reported by Shen et al [19].

Using proportionality calculations, we are able to determine the lipoprotein composition that is implied by our derived LCF at respective TG-intervals. Thus, an LCF of 0.225 (half of 0.45) would indicate a TRL cholesterol:TG ratio of 1:4.4 in mmol/L, which translates to

a lipoprotein composition of ~9.1% cholesterol and ~90.9% TG, while a LCF of 0.90 (twice 0.45) would indicate a cholesterol:TG ratio of 1:1.1 in mmol/L, which translates to a lipoprotein composition of ~28.6% cholesterol and ~71.4% TG.

D. Derivation of Median LDLC

The median LDLC is the intercept of the 0.50 quantile regression line (with 95% CI from bootstrapping method) for each TG-interval. An increase in the intercept (median LDLC) of the 0.50 quantile regression line as TG increases shows that median LDLC is also influenced by TG-enrichment of TRLs. Following a similar analysis method for the LCF, from the quantile regression analysis results for each cohort, we explored the association between the median LDLC vs TG, by performing linear regression analysis.

E. Statistical Software

All regression modeling and statistical analyses were performed using R software. Quantile regression was done using the quantregGrowth package [20].

2. Results

A. Derived LCF Decreases as TG Increases

The association between calculated non-HDLC and TG is shown in Figure 1, panel A for the Biochemistry Lab cohort, and panel B for the Lipid Clinic cohort, all the way up to TG of 120 mmol/L. The median quantile regression analysis for non-HDLC for each TG increment of 1 mmol/L up to TG 10 mmol/L is shown in Fig. 1C and 1D in the 2 cohorts, respectively. As TG increases, the slope for each median quantile regression line, which represents the LCF for each mmol/L TG increment, decreases. This is seen as a gradual flattening of the line segments. There is a remarkably smooth connection pattern in the regression lines from each 1 mmol/L TG segment to the next, especially for the Biochemistry cohort up to TG ~7 mmol/L. However, beyond ~7 mmol/L TG, the pattern is lost, especially for the Lipid Clinic cohort. This loss of pattern persists into the severe HTG range of TG > 10 mmol/L (data not shown). It should be noted that there are far fewer samples in this range and results are less reliable.

Fig. 2A and 2B show the negative correlation between LCF and TG in the Biochemistry Lab and the Lipid Clinic cohorts, respectively. Analysis of the coefficient of determination over various TG intervals indicate a strong correlation ($R^2 > 0.6$) up to TG of 7 mmol/L as shown in the insets in Fig 2. When TG intervals above 7 mmol/L were included in the analysis, including TG > 10 mmol/L (data not shown), correlation was weaker or lost. It is noteworthy that in both cohorts, the trend of decreasing LCF spans the entire spectrum of TG levels from 0.01 to about 7 mmol/L, including the normotriglyceridemic range of < 2.0 mmol/L.

B. Core Lipid Composition Derived From LCF in HTG

Using proportionality calculation, we have derived the median core lipid composition of lipoproteins represented by the LCF. Fig. 3A shows the theoretical core lipid composition pies based on 3 LCF examples. Fig. 3B shows core lipid composition pies based on 3 actual LCF values from our Biochemistry Lab cohort results. An LCF of 0.43, which denotes a core lipid cholesterol to TG ratio of 1:2.3 in mmol/L and a composition of 15.9% cholesterol and 84.1% TG, is associated with a TG of 2 to 3 mmol/L. This LCF of 0.43 is close to an LCF of 0.45 that Friedewald [8] used to approximate VLDL composition. An LCF of 1.0, denoting a core lipid cholesterol to TG ratio of 1:1 in mmol/L and a composition of 30.6% cholesterol and 69.4% TG, is associated with a TG of 0 to 1 mmol/L. This core lipid composition is consistent

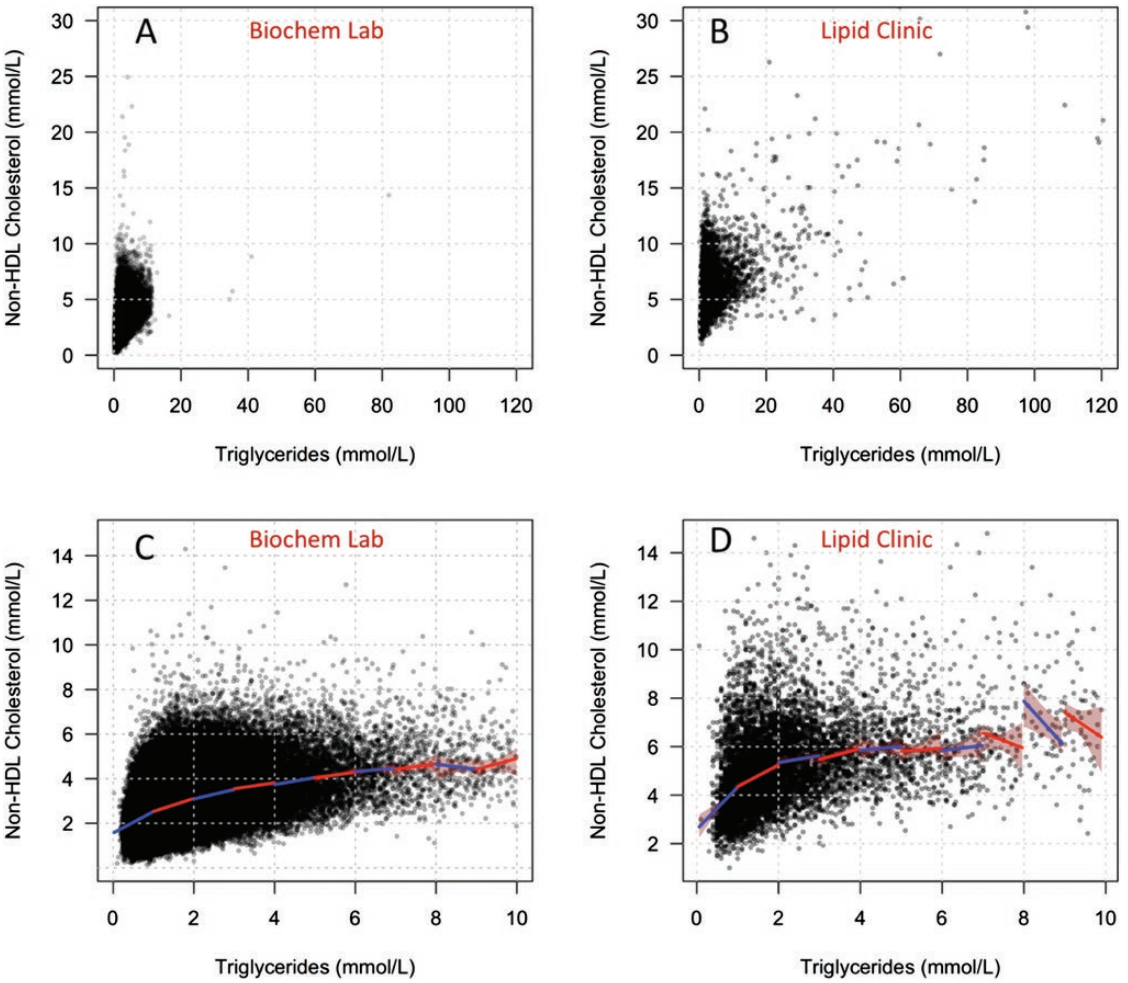

Figure 1. Panel A and C: Relationship between non-HDLC vs TG in the Biochemistry Laboratory cohort. Panel B and D: Relationship between non-HDLC vs TG in the Lipid Clinic cohort. The alternating red and blue lines are the median quantile regression lines for each TG-interval (TG intervals of 1 mmol/L up to TG 10 mmol/L). The slope for each median quantile regression line is our lipoprotein composition factor (LCF) for TRL in the respective TG-interval. Red areas show the 95% CI (obtained via bootstrapping method) for each TG-interval's median quantile regression line. The 95% CIs are very tight for TG 0.01 to 7 mmol/L in panel C and panel D, and are therefore barely visible.

with remnant lipoproteins. Finally, an LCF of 0.15, denoting a core lipid cholesterol to TG ratio of 1:5.5 in mmol/L and a composition of 6.2% cholesterol and 93.8% TG, is associated with a TG of 6 to 7 mmol/L, consistent with an abundance of CM, which contain ~1 mg cholesterol to ~20 mg TG [18–19].

C. Positive Association Between Derived Median LDLC and TG

We next derived the median LDLC, which is the other component of non-HDLC, by quantile regression modeling. As shown in Fig. 4A for the Biochemistry Lab cohort, and Fig. 4B for the Lipid Clinic cohort, there is a positive correlation between our derived median LDLC and TG, again up to a TG of ~7.0 mmol/L. This pattern is similar to that of LCF but in reverse. Within this range, the confidence intervals are progressively greater. Beyond TG ~7.0 mmol/L, the confidence intervals are even greater and there is a loss of association between LDLC and TG.

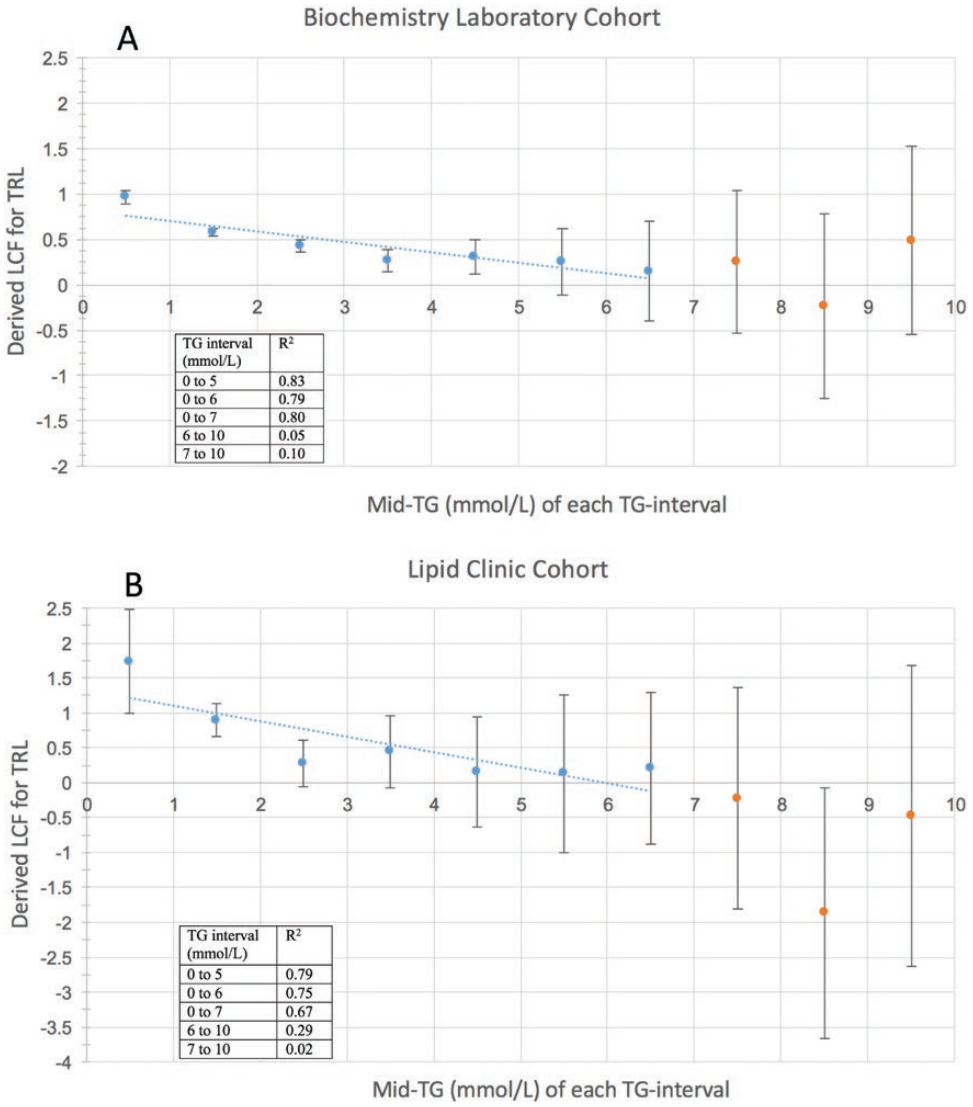

Figure 2. The association between TG-interval-specific LCF (+/- 95% CI calculated using the standard error from the bootstrapping method) and TG in the Biochemistry Laboratory cohort (panel A), and the Lipid Clinic cohort (panel B). The coefficients of determination for the linear regression analysis for various TG intervals is presented in the inset table. Data for each TG-interval is plotted at the mid-TG of the interval.

3. Discussion

We have used 2 large datasets to examine the impact of serum TG concentrations on the calculation and interpretation of non-HDLC. Our main finding in this study is that with increasing serum TG concentrations, there is progressive decline in the LCF of TRL, which indicates that the calculated non-HDLC level reflects progressive inclusion of cholesterol from larger TRL. These larger TRL, while rich in TG, also contain a significant amount of cholesterol. For example, although CM contain a low percentage of cholesterol, their average radius is 6 times that of LDL, so that each CM particle has about 21 to 25 times more cholesterol than a particle of LDL [18–19]. These larger TRL are less atherogenic and therefore calculated non-HDLC becomes less and less reflective of atherogenic TRL in the presence of HTG.

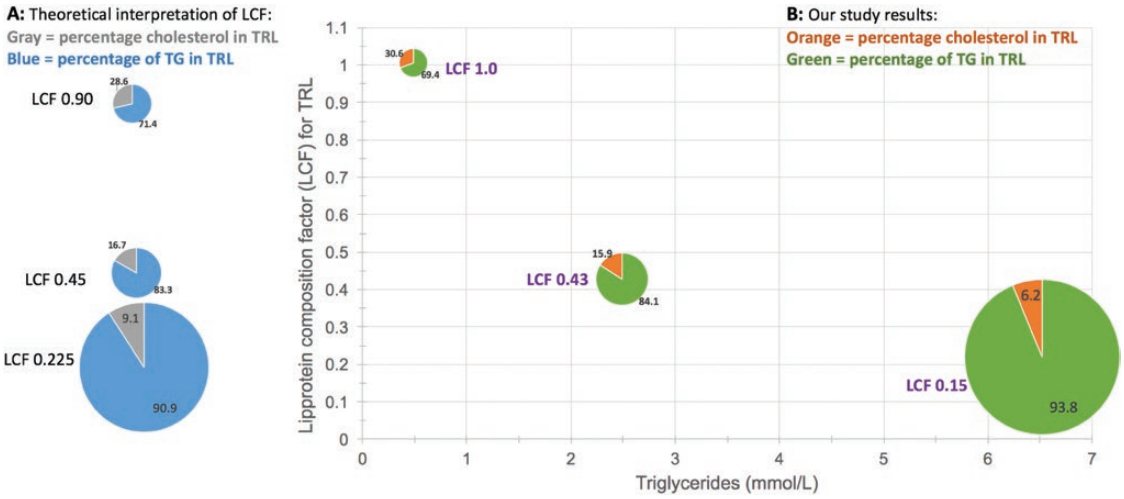

Figure 3. Panel A: Theoretical interpretation of lipoprotein composition based on the lipoprotein composition factor for TRL in terms of percentages of cholesterol and TG in TRL. Panel B: TG-interval-specific lipoprotein composition based on our study's derived lipoprotein composition factors for TRL. We selected 3 TG-interval-specific–derived lipoprotein factors to illustrate the TRL composition. Panel A and Panel B are both drawn to scale according to the relative radius of TRLs as shown by Shen et al [19].

In both datasets, the linear regression model works well with TG up to ~7 mmol/L, yielding a steady and progressive decline in LCF values that have a strong negative correlation with TG. However, above a TG of ~7 mmol/L, the LCF values no longer show a meaningful trend. There are 2 possible reasons for this. The first is that there are far fewer samples with TG > 7 mmol/L, especially in the Biochemistry Lab cohort, making the analysis less reliable. The second is that the linear regression model may not work well with TG levels > 7.0 mmol/L because the model assumes normal or only milder abnormalities in lipoprotein metabolism. LCF values for TG greater than ~7 mmol/L are confounded by more severe abnormalities of lipoprotein metabolism. For example, a severe abnormality that can lead to HTG > 7mmol/L is lipoprotein lipase deficiency, which would be predicted to confound our linear regression model. The work of Brunzell et al, reporting saturation of plasma lipolytic activity above a TG of 5.65 to 7.91 mmol/L [21–23], ties in well with our linear regression model working well only up to a TG of ~7 mmol/L.

It is important to also note that the linear association between LCF values and TG is present not just with TG levels that are considered high (above ~2.0 mmol/L) but also with TG levels within reference range. This indicates that changes in lipoprotein composition in the TRL component is a gradation that begins early, even when serum total TG levels are considered within normal range. In fact, our data in Fig. 3 indicate that the core lipid composition of VLDL assumed in the Friedewald equation (LCF 0.45) is very close to the core lipid composition of TRL that corresponds to a TG of 2 to 3 mmol/L in our data (LCF 0.43). The core lipid composition of TRL associated with TG of 0.01–1 mmol/L is consistent with that of lipoprotein remnants. These TRL are richer in cholesterol in comparison with "regular" VLDL. This is entirely in keeping with current knowledge of lipoprotein metabolism. It is also consistent with data from Martin et al [12], showing that the adjustable factor for the TG:VLDLC ratio varied within the normotriglyceridemic range. Thus, there is no TG inflection point where an exaggerated change in non-HDLC occurs. Similarly, there is no TG inflection point in LDLC calculation by Friedewald equation either, and an arbitrary TG cutoff level of > 4.5 mmol/L was set to avoid gross underestimation of LDLC [8].

Our finding of a positive correlation of LDLC with TG is not unexpected. It reflects conversion of VLDL to LDL. It is noteworthy that this correlation is also significant within a

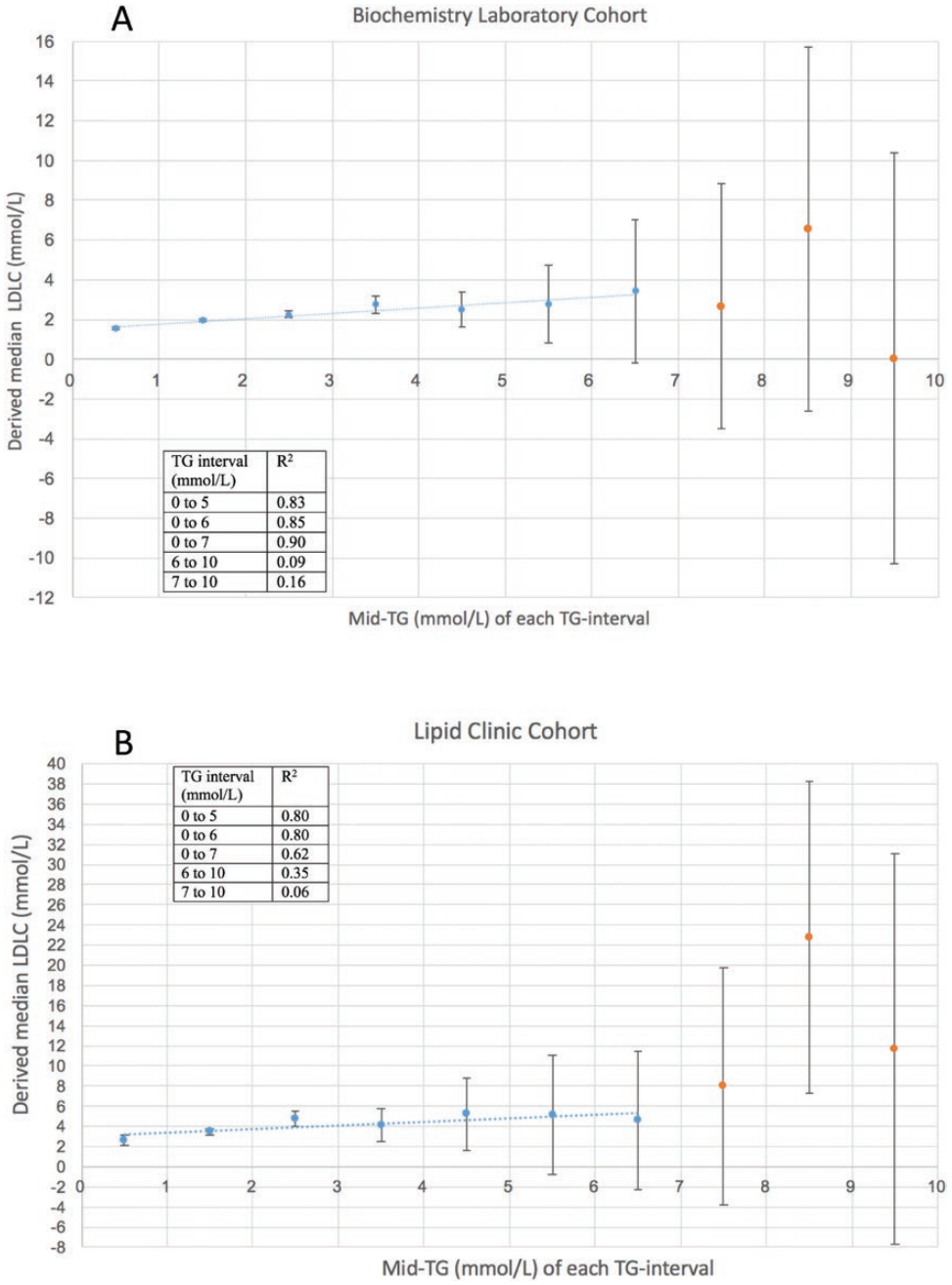

Figure 4. The association between TG-interval-specific intercepts, which represent the median LDLC (+/- 95% CI using the standard error from bootstrapping method), and TG in the Biochemistry Lab cohort (panel A), and the Lipid Clinic cohort (panel B). The coefficient of determination for the linear regression analysis is also presented up to TG 7 mmol/L. Data for each TG-interval is plotted at the mid-TG of the interval. The inset table shows the coefficient of determination for various TG intervals.

TG range of 0.01 to 7 mmol/L, like the negative correlation between LCF and TG, except that the correlation is positive. This finding adds credence to our modeling system.

The main conclusion from our study is that although the actual calculation of non-HDLC as TC minus HDLC does not involve the TG concentration, its level increasingly includes cholesterol in larger TRL with increasing TG. The implication of our findings is that since larger TRL are less atherogenic compared to LDL and remnant lipoproteins, non-HDLC

associated with HTG becomes less and less effective as a CVD risk marker. Guidelines view calculated LDLC as invalid in HTG > 4.5 mmol/L, but view calculated non-HDLC favorably, and even as the risk marker to resort to in HTG [1–5, 15]. Our findings raise caution with this approach. In fact, current knowledge of lipoprotein composition and metabolism would already allow us to draw this conclusion a priori, but our analyses now provide the first empirical evidence to support this conclusion. Another manifestation of the indiscriminate use of non-HDLC as a CVD risk marker is when non-HDLC and apolipoprotein B (apoB) are used almost interchangeably as targets for lipid-lowering therapy without reference to the TG level [1, 5, 15]. We have not compared non-HDLC with apoB in our study but it is likely that since apoB is a marker of lipoprotein particle number while non-HDLC reflects core lipid content, there would be a discordance in the risk reflected by these 2 risk markers in HTG [24–25].

A strength of our study is that we have used 2 large datasets from 2 diverse populations and have demonstrated essentially the same findings, which attest to the validity of the modeling used. A limitation of our study is that our findings are based on statistical modeling of standard lipid profile levels and not on direct measurement of TRLC by ultracentrifugation or direct lipoprotein size assessment by nuclear magnetic resonance spectroscopy. Our study does not include information on age, sex, and other demographic and medical factors. However, the large size of the cohorts has allowed us to see the big picture, and future studies could explore the impact of patient characteristics on our TG-interval–specific LCF model.

The ultimate test of the validity of non-HDLC as a CVD risk marker in the presence of HTG would be a prospective endpoint study. Pending additional data on this matter, we suggest that it would be prudent to use non-HDLC as a CVD risk marker with caution in the presence of HTG. Our data do not allow us to recommend a cutoff level, but noting the similarity between the median LCF vs TG relationship (Fig. 2) and the derived median LDLC vs TG relationship (Fig. 4), but in the converse direction, one might surmise that the same influencers are operating to underestimate LDLC and overestimate the atherogenic risk of non-HDLC. Hence, until a correction similar to the Martin approach to LDLC calculation [12] is made to the non-HDLC calculation, the same arbitrary TG cutoff of > 4.5 mmol/L could be considered.

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Additional Information

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