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The Fats of Life

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The Fats of Life
Life is good! Warm weather has finally arrived here in Maine, and the Bosox have just swept the Yankees at Fenway Park. After the long winter, the warm weather has been a distraction. Fortunately, I have some interesting articles to share with you to keep us all focused—although, like me, you may want to sit outside in the sunshine with a beverage of your choice while enjoying the Spring issue.

Shinji Koba and colleagues from Showa University School of Medicine in Tokyo have written a brief review, and described a novel method for the measurement of small, dense LDL cholesterol. A manual precipitation step using heparin and magnesium is required to separate small LDL particles from other lipoproteins and larger LDL; the small LDL collected in the filtrate is then measured using a homogeneous LDL cholesterol assay.

Also in this issue, Jenny Hsu, Sara Agee, and John Todd from Singulex describe their highly sensitive immunoassay for troponin I. We are beginning to see the next generation of assays that can report measurable troponin concentrations in healthy subjects. Their Erenna® immunoassay system measures as little as 1.7 ng/L of troponin I with 10% CV, and the 99th percentile in a group of apparently healthy subjects was 7 ng/L.

Finally, Jim Otvos from LipoScience has contributed an interesting and provocative editorial describing another “inconvenient truth”: LDL cholesterol as a flawed measure of LDL-related risk. Some of Jim’s data may surprise you.

I look forward to seeing all of you in Chicago.

John H. Contois, Associate Editor
The Fats of Life
While we all are happily celebrating the arrival of the spring, AACC has already opened the registration for the 2009 Annual meeting in Chicago, IL. The list and description of our upcoming AACC-LVDD special events during this meeting are as follows:

LVDD Executive Committee (Board) Meeting
Sunday, July 19  8:00 am – 11:00 am  
Hyatt Regency Chicago Hotel (Columbian Room)  
Registration: not required (this meeting also serves as a division business meeting!).

Annual LVDD Dinner Meeting
Current Topics in Cardiovascular Disease  
Monday, July 20  5:30 pm – 9:30 pm  
Hyatt Regency Chicago Hotel (Columbus Hall A, B, C, D)  
Sponsored by the Lipoproteins and Vascular Diseases Division

LVDD members are invited to celebrate the division’s 20th anniversary during an evening of socializing and science. Following dinner there will be two scientific presentations. The first will address enzyme cycling and its clinical application for the homocysteine test. Homocysteine is an emerging new risk factor for cardiovascular disease and stroke that can be tested virtually on any automated clinical chemistry analyzer. The second talk, on recent advances in cardiovascular risk testing, will discuss the relative merits of alternative measures for LDL cholesterol and describe results of recent trials for the inflammatory markers CRP and Lp-PLA2.

Enzyme Cycling and Its Clinical Application for the Homocysteine Test
Chong Yuan, Ph.D., Diazyme Laboratories, San Diego, CA

Recent Advances in Cardiovascular Risk Testing: Lipids, Lipoproteins, and Inflammation
Joseph P. McConnell, Ph.D., The Mayo Clinic and Foundation, Rochester, MN

Registration:
Limited to the first 90 LVDD Members: $50
Registration includes a reception, awards presentation, and dinner followed by scientific presentations.

International Lipoprotein Standardization Forum
Tuesday, July 21  6:00 pm – 9:30 pm  
Hyatt Regency Chicago Hotel (Columbus Hall K, L)  
Sponsored by the AACC Lipoproteins and Vascular Diseases Division. Cosponsored by the Japan Healthcare Technology Foundation and Pacific Biometrics Research Foundation.

LVDD members are invited to join international leaders in a discussion of recent findings related to lipoproteins, with a focus on new technologies and standardization efforts. The first presentation will look at CETP inhibitor, which is considered a potential lipoprotein-modifying agent, and will summarize the differences of lipoprotein phenotypes by CETP inhibitor from those in genetic CETP deficiency. The second presentation will discuss the current technology of lipid/lipoprotein testing based on results of the US-Japanese cooperative study.

Prospect of CETP Inhibitor
Akihiro Inazu, M.D., Kanazawa University, Kanazawa, Japan

Performance of Current Homogeneous Methods for HDL and LDL Cholesterol: The Final Results
Greg Miller, Ph.D., Virginia Commonwealth University, Richmond, VA
Registration:
Limited to the first 60 LVDD members: $35
Registration includes a reception followed by dinner and scientific presentations.

Hope to meet as many of you as possible during the Annual meeting. And again, pictures taken during our 2008 Monday night event in Washington, DC may remind you to plan for the ever-popular LVDD events.

Sincerely,

Gyorgy (George) Csako, MD
Chair, LVDD, AACC
Small Dense LDL-Cholesterol in Determining Severe Coronary Atherosclerosis

Shinji Koba*, Yuuya Yokota*, Yasuki Ito*, Tsutomu Hirano*

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Introduction

Low-density lipoprotein (LDL) particles are heterogeneous with respect to their size, density and lipid composition, and their lipid content chiefly determines the size of LDL particles. High plasma concentration of LDL-cholesterol (LDL-C) is an established major risk factor for coronary heart disease (CHD). On the other hand, it is well accepted that not native LDL particles, but oxidatively-modified LDL particles have the potential to cause foam cell formation, which plays an important role in atherogenesis [1]. Among LDL particles, small dense LDLs have been shown to be easily oxidized in vitro and to have lower amounts of lipophilic antioxidants compared with larger LDL particles separated by density-gradient ultracentrifugation [2,3]. In addition, small dense LDL particles have a lower binding affinity for the LDL receptor and have longer clearance time compared with larger LDL. Small dense LDLS penetrate into the arterial wall much more readily and bind to the extracellular matrix more tightly than larger LDL particles [4]. This suggests that small dense LDLs are more pro-atherogenic compared with larger LDLs, and measurement of small dense LDL is useful to detect high risk patients for CHD.

Serum Lipid Biomarkers for Distinguishing CHD Patients

The Framingham Heart Study [5] has shown that the difference in distribution of LDL-C is very small between CHD patients who did not take any lipid-lowering drugs and the non-diseased population, and about 80% of CHD patients had LDL-C levels in the same range compared to healthy subjects. The distribution of high-density lipoprotein (HDL)-C concentration, on the other hand, shifts toward lower levels about 10 mg/dL compared to the controls and seemed to be a better predictor of CHD than LDL-C. More than two decades ago, Sniderman and his colleagues [6] compared the cholesterol content and apolipoprotein (apo) B content in LDL fractions separated by ultracentrifugation in 31 patients without and 59 patients with angiographically documented CHD. They showed that LDL-apo B, but not LDL-C, was the better marker to discriminate between patients with and without CHD. Thus, HDL-C or apo B appears to be a better marker to discriminate CHD than LDL-C. Figure 1 compares LDL-C and LDL particle numbers in two subjects. Subject A has a

![Figure 1](image-url)
predominance of cholesterol-rich, large LDL particles, whereas subject B has many small, cholesterol-poor LDL particles, resulting in a marked difference in apo B and small LDL-C concentration. Subject B is characteristic of patients with CHD and/or metabolic syndrome. Thus, increased numbers of small dense LDL particles seems to be a more useful biomarker than LDL-C to detect CHD risk.

**Measurement of Small Dense LDL and Small Dense LDL-Cholesterol**

LDL particle size is most often measured by gradient gel electrophoresis using non-denatured 2 to 16% polyacrylamide gel according to the procedure described by Nichols et al [7]. Two distinct LDL size phenotypes, pattern A, large buoyant LDL particles, and pattern B, small dense LDL particles, can be easily separated. Many studies have shown that the predominance of small dense LDL evaluated by this gradient gel electrophoresis is associated with CHD [8-10]. However, this method is not a quantitative assay for small dense LDL-C. We have established a simple and rapid method for measuring small dense LDL-C by heparin magnesium precipitation [11]. Briefly, the precipitation reagent containing heparin and magnesium is added to each serum sample followed by incubation, then the samples are centrifuged, the aggregates are trapped by the filter, and the pass-through fraction is collected for measurement. The clear infranatant is then analyzed by direct homogeneous LDL-C methodologies.

**Small Dense LDL-C Concentration and CHD**

We compared LDL size and small dense LDL-C concentration in 225 consecutive angiographically-documented CHD patients who were not receiving any lipid-lowering medication and 95 healthy men, aged 40 to 63 years, and 47 healthy postmenopausal women [12]. The CHD patients were classified into three groups based on the disease type, i.e., acute coronary syndrome (ACS, 73 men and 11 women), including acute myocardial infarction (MI) and unstable angina pectoris; stable CHD (103 men and 20 women), including stable effort angina pectoris and/or prior histories of MI or percutaneous coronary intervention (PCI), and coronary spastic angina (10 men and 8 women). Similar to our previous study [9], LDL particle size and HDL-C levels were significantly lower in all types of CHD compared with healthy men and women, while LDL-C levels were significantly higher only in patients with ACS (Figure 2). Small dense LDL-C levels were significantly higher in both ACS and stable CHD. On the other hand, large LDL-C, estimated by subtracting the small dense LDL-C concentration from the LDL-C concentration, was somewhat lower in coronary spastic angina and stable CHD.

![Figure 2](image-url)

**Figure 2.** Comparison of LDL-C, small dense LDL-C and large LDL-C among healthy men (N=95), healthy postmenopausal women (N=47), patients with coronary spastic angina (N=18), stable CHD patients (N=123), and ACS patients (N=84). Data are expressed as mean ± standard error. *P<0.05 vs control men, †P<0.05 vs control women, #P<0.05 vs coronary spastic angina, §P<0.05 vs stable CHD. Based on reference 12.

The small dense LDL-C concentrations were measured in 482 consecutive stable CHD patients undergoing scheduled coronary angiography [13]. Severe CHD was defined as the presence of stenosis with more than 50% narrowing of the diameter of the left main coronary artery or stenosis with more than 75% narrowing of the diameter in one or more branches of the coronary arteries. Figure 3 shows the comparison of LDL-C, small dense LDL-C, and large LDL-C in patients divided into four groups based on the severity of CHD and the use of lipid-lowering drugs. Among 263 patients on lipid-lowering drugs, 169 male and 53 female patients took statin alone, 23 male and 4 female patients took other lipid-lowering drugs such as
fibrates and eicosapentaenoic acid, and 11 male and 3 female patients were treated with the combination of statin and other lipid-lowering drugs. Patients with severe CHD exhibited significantly higher levels of LDL-C and small dense LDL-C, and similar levels of large LDL-C, irrespective of the use of lipid-lowering drugs. In addition, small dense LDL-C levels were significantly higher in the severe CHD patients treated with lipid-lowering drugs than in untreated mild CHD patients, whereas the LDL-C levels were similar and the large LDL-C levels were somewhat higher in unmedicated mild CHD patients than in medicated severe CHD patients. Figure 4 compares the LDL-C, small dense LDL-C, large LDL-C, and HDL-C levels between the mild and the severe CHD among 365 patients with histories of MI and/or PCI. Patients with severe CHD exhibited significantly higher levels of LDL-C and small dense LDL-C, significantly lower levels of HDL-C and similar levels of large LDL-C. Therefore, the increases of LDL-C levels in the severe CHD patients compared with the mild CHD patients were chiefly due to those of small dense LDL-C levels, irrespective of the use of lipid-lowering drugs and/or prior histories of MI and PCI.

Table 1 shows the results of logistic regression analysis for determining the severity of CHD among 482 CHD patients. According to our multivariate logistic regression analysis to compare small dense LDL-C with other risk factors, an elevated small dense LDL-C concentration was significantly associated with severe CHD independently of the levels of LDL-C, HDL-C, apo A-1, apo B, non-HDL-C, and HbA1c in stable CHD patients and in CHD patients not taking lipid-lowering agents. Previous case-control studies and prospective studies have shown that non-HDL-C and apo B are both stronger predictors of CHD than LDL-C [14-16]. Our study, on the other hand, identified small dense LDL-C as the most powerful determinant of severe CHD, independent of LDL-C, non-HDL-C and apo B.
Small Dense LDL-C Concentration and Severity of Coronary Atherosclerosis

The severity of coronary atherosclerosis was estimated by calculating the Gensini score by coronary arteriography, an established method for grading coronary stenosis. Small dense LDL-C gradually increased as the Gensini score increased, while large LDL-C did not differ among the different quartiles of Gensini score (Figure 5) [12]. This trend of increased small dense LDL-C along with the increased Gensini score was even more pronounced when the diabetic patients were excluded. Furthermore, the Gensini scores significantly increased along with elevated levels of small dense LDL-C among stable CHD patients untreated with lipid-lowering drugs (Figure 6). On the other hand, decreases in HDL-C levels are significantly associated with

Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Overall CHD (N=406)</th>
<th>Non-lipid-lowering group (N=198)</th>
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<tr>
<td></td>
<td>Odds</td>
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<tr>
<td>Age</td>
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<td>Hypertension</td>
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<td>Diabetes</td>
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<tr>
<td>LDL-C</td>
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<tr>
<td>Small dense LDL-C</td>
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<tr>
<td>Non-HDL-C</td>
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<td>HDL-C</td>
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Figure 6. Associations of Gensini score with small dense LDL-C levels, and small dense LDL-C and HDL-C levels. Quartiles of small dense LDL-C. (A) Quartiles of small dense LDL-C. Quartile 1 (Q1) = small dense LDL-C ≤ 20.5; quartile 2 (Q2) = 20.6 - 31.4; quartile 3 (Q3) = 31.6 - 48.0; quartile 4 (Q4) = the score ≥ 48.5. The population included 96, 90, 91, and 93 patients in Q1, Q2, Q3, and Q4, respectively. *P<0.05 vs Q1 by Tukey-Kramer post-hoc test. (B) Median levels of small dense LDL-C (sLDL-C) and HDL-C were 31 mg/dL and 43 mg/dL, respectively. Small dense LDL-C and metabolic dyslipidemia (high small dense LDL-C and low HDL-C) in subjects untreated with lipid-lowering agents. *P<0.05 vs subjects with low small dense LDL-C and high HDL-C by Tukey-Kramer post-hoc test. Based on reference 9 and 13.

Figure 7. Associations of Gensini score with HDL-C levels. (A) Quintiles of Gensini score. Quintile 1 (Q1) = Gensini score ≤ 9.0; quintile 2 (Q2) = 9.5 - 21.0; quintile 3 (Q3) = 21.5 - 39.5; quintile 4 (Q4) = 40 - 62; quintile 5 (Q5) = ≥ 63. The population included 89, 91, 96, 96, and 91 patients in Q1, Q2, Q3, and Q4, respectively. *P<0.05 vs Q1, †P<0.05 vs Q2, §P<0.05 vs Q3, by Tukey-Kramer post-hoc test. (B) Quintiles of HDL-C. Quintile 1 (Q1) = HDL-C ≤ 34; quintile 2 (Q2) = 35 - 40; quintile 3 (Q3) = 41 - 46; quintile 4 (Q4) = 47 - 54; quintile 5 (Q5) = ≥ 55. The population included 96, 97, 83, 93, and 89 patients in Q1, Q2, Q3, and Q4, respectively. *P<0.05 vs Q1, †P<0.05 vs Q2 by Tukey-Kramer post-hoc test. Based on reference 13.
increases in Gensni scores (Figure 7). The Gensini scores were significantly higher in CHD patients with higher levels of small dense LDL-C and lower levels of HDL-C compared with CHD patients with lower levels of small dense LDL-C and higher levels of HDL-C (Figure 6).

Figure 8 demonstrates the correlation between LDL-C or small dense LDL-C and HDL-C levels among 591 men and 180 women who did not take any lipid-lowering drugs. The LDL-C concentration was strongly correlated with small dense LDL-C (r = 0.604, P<0.0001). Small dense LDL-C, but not total LDL-C was significantly correlated with HDL-C levels (r=-0.384, P<0.0001). These data suggest that high levels of small dense LDL-C correlate with both atherogenic LDL and metabolic dyslipidemia such as increased triglyceride-rich lipoproteins and decreased HDL-C.

**Conclusion**
The increase in LDL-C levels in the clinically and angiographically-graded severe CHD patients compared with patients without severe coronary atherosclerosis were chiefly due to small dense LDL particles. Therefore, elevated small dense LDL-C concentration is a very promising risk marker to detect the progression of coronary atherosclerosis, to predict cardiovascular events, and to assess metabolic dyslipidemia.

**References**


Cardiovascular disease (CVD) is the leading cause of death in the United States, more prevalent than all types of cancer combined. The most recent statistics published by the American Heart Association indicate that an estimated one in three American adults suffers from one or more types of CVD (1). Traditionally, cardiovascular disease has been diagnosed during a physiological exam that includes an electrocardiogram (ECG or EKG) stress test. However, a major drawback with this procedure is that it lacks clinical sensitivity, as the false-negative rate has been reported to be as high as 28% (2). Newer, more sensitive methods utilizing protein biomarkers offer the potential for earlier detection of CVD. Changes in biomarker concentration may be indicative of disease development or risk, and may be detectable before any physical symptoms become apparent.

In this regard, cardiac troponin-I (cTnI) is widely accepted as the biomarker of choice for diagnosing acute myocardial infarction (AMI) in the context of acute coronary syndrome (ACS). Both the National Academy of Clinical Biochemistry (3) and the joint committee of the European Society of Cardiology/American College of Cardiology (4) recommend the diagnostic decision limit to be a value above the 99th percentile of a healthy reference population, as determined with an assay imprecision of ≤10% CV. Traditionally, evaluating this distribution of cTnI concentrations in a healthy reference population has been impeded by poor assay precision at low cTnI concentrations. Therefore, the diagnostic decision limit has been often defined by assay performance at 10% CV. Recently, research-grade high-sensitivity cTnI assays have been introduced (5, 6). These new assays have made available a wealth of information defining the true range of cTnI in a reference population and have provided for a true assessment of the 99th percentile. Consequently, risk stratification of individuals that present cTnI measurements at or slightly above the true 99th percentile can now be investigated (7).

The next generation of high-sensitivity immunoassays holds great promise for enabling early cardiovascular disease detection. However, these novel assays must undergo stringent analytical and biological validation prior to clinical use. In this article we will introduce a novel high-sensitivity cTnI immunoassay based upon single-molecule counting, termed molecular cTnI. A brief description of the immunoassay system and details of its analytical performance, along with clinical applications in the cardiovascular area, will be discussed in this review.

Development and Performance of a Novel Molecular cTnI Immunoassay

An advanced cTnI immunoassay developed at Singulex is capable of quantifying analyte at the sub-picogram concentration. The first generation Erenna® Immunoassay System incorporated a robust digital molecule-counting instrument with plate-based immunoassays, where fluorescence is measured in the presence of proprietary background reduction technology. The detection system enables high-resolution digital counting of cTnI molecules, with sensitivity greater than currently available commercial assays. The limit of detection (LoD) of the first generation Erenna cTnI immunoassay was determined at 1.7 ng/L with 10% CV at 1.8 ng/L (8), which was 50 times lower than the 10% CV cutoff for the first generation Bayer Centaur cTnI immunoassay. A study by Wu and colleagues showed that the 99th percentile diagnostic decision limit established from a group of 88 healthy individuals was 7 ng/L (8). This limit represented a preliminary assessment of the healthy population reference range wherein all samples tested provided quantifiable values, and that 99% of the quantified values were
below 7 ng/L. This was the first time that such an assessment of a reference range for cTnI had been made.

Using this Erenna cTnI immunoassay, two retrospective pilot studies were performed to evaluate potential utility for AMI rule-in/out in an emergency department setting. The first study investigated 15 patients who presented with suspect acute coronary syndromes, yet their first blood draw provided negative cTnI measurements, as determined by the laboratory reference method (Centaur’s 99th percentile cutoff). These patients were followed serially with 6–8 hr intervals between blood draws. All 15 patients eventually demonstrated quantifiable cTnI using the laboratory reference method, resulting in a non-ST elevation MI diagnosis. Identical samples from all patients showed quantifiable cTnI at all time points using the Erenna cTnI immunoassay. Five of the 12 Centaur-negative baseline blood samples provided cTnI measurements greater than 7 ng/L. cTnI values of three patients were in the equivocal “grey zone,” which was between the 99th percentile and the 10% CV cutoff according to the Centaur assay. All three of these were considered elevated with the Erenna cTnI immunoassay with cTnI values >7 ng/L. The second pilot study involved 50 patients who presented to the emergency department with suspect ACS, yet provided baseline cTnI concentrations <99th percentile using the Centaur method. There was no other clinical follow-up for these patients. Interestingly, with the Erenna cTnI immunoassay, all patients in this cohort provided quantifiable cTnI values and 14 patients provided cTnI concentrations >7 ng/L. Though follow-up studies of those individuals were not available, the results do suggest the possibility that these patients experienced minor cardiac injury which was not detectable either biochemically or by physiological diagnosis. Together, these preliminary studies demonstrate the potential for highly sensitive cTnI assays to provide better diagnostic information in clinical settings.

To further enhance the analytical performance of the first generation Erenna Immunoassay System, a second generation system utilizing paramagnetic microparticles (MPs) as the solid phase for immunoassay capture and detection of analyte was developed (5) (Figure 1). The improved sensitivity and precision of the second generation molecular cTnI immunoassay demonstrated an LoD of 0.2 ng/L and a 10% CV of 0.78 ng/L. Compared to the second generation Centaur assay (TnI-Ultra, 10% CV of 30 ng/L) (9), the improved molecular cTnI immunoassay was able to quantify cTnI concentration in healthy individuals (Figure 2).

**Figure 1.** The second generation Erenna Immunoassay System utilizes a modified micro-particle (MP) based sandwich immunoassay followed by single-molecule counting technology to enable high-resolution detection of important biomarkers such as cTnI.

**Analytical Validation of Assay Specificity**

The sensitivity of the molecular cTnI immunoassay raises the question whether ultrasensitive cTnI measurements are more susceptible to non-specific binding (NSB) events. In subsequent validation experiments, three possible factors of NSB were examined for the second generation molecular cTnI immunoassay (10). NSB of specimen components was first tested using four different sample types. Measurements taken from serum, EDTA, lithium heparin, and sodium-citrated plasma of 20 healthy individuals displayed no significant differences (95% CI). Secondly, NSB of capture antibodies was assessed. Uncoated MPs, or MPs coated with non-specific macrophage inflammatory protein-1 α, prostate-specific antigen, or granulocyte-colony-stimulating factor antibodies
all generated non-quantifiable results. Detected concentrations that were greater than the lower limit of quantification occurred 93% of the time when specific cTnI-coated MPs were used, and only 5% of the time when amyloid-β-42 antibodies were used. Lastly, analyte NSB was examined using non-specific skeletal troponin, which showed no reactivity in the assay. Taken together, these results have demonstrated the molecular cTnI immunoassay’s analytical specificity and clinical utility.

Application in Clinical Research: Assessing Elevated and Normal Ranges of cTnI Concentrations in Stress-Induced Ischemia Patients and Healthy Individuals

The molecular cTnI immunoassay was used by Sabatine and colleagues to assess whether changes in cTnI concentration can be quantified in patients with stress-induced transient myocardial ischemia as determined by nuclear perfusion imaging (7). Prior to stress testing, baseline cTnI concentrations in all patients were quantifiable in all patients using the molecular cTnI immunoassay. cTnI concentration was measured immediately, 2 hours and 4 hours after stress testing. Analysis of cTnI levels in patients without ischemia showed an increase of 11% (median 0.6 pg/mL). Moreover, statistically significant concentration increases of 24% (1.4 pg/mL) and 40% (2.1 pg/mL) were noted in patients with mild and with moderate-to-severe ischemia (Figure 3). However, these results were not observed with any other commercial assays tested: the ACS:180 chemiluminescence cTnI Immunoassay (Bayer Diagnostics), the cardiac troponin T assay (Roche Diagnostics), and the Tn-I Ultra assay (Siemens Healthcare Diagnostics). Thus, the molecular cTnI immunoassay provides promise for active monitoring of myocardial conditions such as transient ischemia over time.
were measured in two separate cohorts of healthy volunteers (11). The authors concluded that the natural level of biological variability for cTnI was low and that a two-fold increase in cTnI concentration, measured between two time points, would be statistically significant. These results suggest that serial assessment of cTnI concentration within an individual may be more important than applying a population-based cutoff for the purpose of identifying cardiovascular related events, stressing the importance of patient monitoring in clinical settings.

**Impact on Preclinical Studies: an Investigation of Drug Cardiotoxicity Using the Molecular cTnI Immunoassay**

Besides being the gold standard for diagnosing acute myocardial events, cTnI has recently been described as the most effective biomarker to assess myocardial injury due to drug cardiotoxicity in preclinical studies (12). cTnI measurements in animal systems have been difficult since most commercial assays lack requisite sensitivity to measure the baseline concentration and to detect cTnI increase after cardiac injury. In a cardiotoxicity study of isoproterenol hydrochloride in rats, dogs and monkeys, elevation of serum cTnI was compared to histopathologic changes after the drug has been administered for a period up to 72 hours (13). Using the molecular cTnI immunoassay, baseline concentrations were detectable in all animals prior to cardiac insults and showed direct correlation to known baseline reference ranges described in humans (Table 1). An increase in cTnI concentration after drug administration was quantifiable in all animals, at time points before histopathologic evidence of myocardial damage became apparent. This study suggests the importance of more sensitive assays for monitoring cardiotoxicity in preclinical research. Ongoing studies using the molecular cTnI immunoassay to evaluate biological variability in placebo control animals, as well as cardiotoxicity of other therapeutic compounds are in progress. These assays will be of significant value in efforts to identify drugs at market with low-level cardiotoxic effects.

**Summary**

Emerging ultrasensitive cTnI assays, like the molecular cTnI immunoassay, offer a promising new tool to enable physicians to make better clinical decisions through early detection and monitoring of cardiovascular disease. Long-term monitoring of cTnI may enable risk stratification of future onset or re-occurrence of cardiac injuries. Finally, therapeutic compounds that could potentially cause direct physical damage to the heart can be properly evaluated in preclinical studies, with results that directly translate from pre-clinical to clinical applications. As these ultrasensitive assays become more widely used, more will be learned about the clinical value of cTnI as a biomarker for cardiac disease risk, onset and diagnosis.

**References**


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**Table 1.** Analytical sensitivity of the molecular cTnI immunoassay and range of cTnI concentrations in healthy species of rat, dog and monkey.

<table>
<thead>
<tr>
<th>Species</th>
<th>LoD (ng/L)</th>
<th>Healthy Levels (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>0.2</td>
<td>1-12</td>
</tr>
<tr>
<td>Rat</td>
<td>0.2</td>
<td>9-20</td>
</tr>
<tr>
<td>Dog</td>
<td>0.1</td>
<td>1-4</td>
</tr>
<tr>
<td>Monkey</td>
<td>0.4</td>
<td>4-5</td>
</tr>
</tbody>
</table>
of Cardiology Committee for the redefinition of myocardial infarction. J Am Coll Cardiol, 36, 959-969.


Global warming took many years to be accepted as a problem deserving serious attention, and efforts are now underway to try to do something about it. What was “inconvenient” about acknowledging the truth of global warming was that it forced us out of our comfort zones. By that, I don’t mean temperate climate zones (maybe those too), but social, economic, and political comfort zones.

Of less global significance, but for those in our profession a problem just as badly in need of recognition, acknowledgement and, ultimately, a solution, is the continued reliance on LDL cholesterol (LDL-C) as the standard measure of LDL and LDL-related cardiovascular risk. The problem in a nutshell is that LDL-C is a seriously flawed measure of LDL. [By the way, the same is true for HDL cholesterol, but that’s a story for another day.] LDL-C underestimates LDL in millions of patients and overestimates LDL in millions of others. The recent LVDD position statement on apoB did an excellent job reviewing the evidence for this, but stopped short of recommending replacement of LDL-C.1 The authors’ reason was pragmatic and understandable, a reflection of the “inconvenient” part of this particular truth: “changing perceptions and practices will not be easy.” So they suggested that, for the time being, apoB or LDL particle number (LDL-P) be used along with LDL-C.

My concern is that this “along with” position perpetuates the notion that the only thing wrong with LDL-C is that it lacks something that another measure such as apoB or non-HDL-C makes up for (such as inclusion of other atherogenic lipoproteins besides LDL). As a result, apoB and LDL-P continue to be miscast as “emerging” or “novel” risk markers, which add a measure of incremental value to the “established” cholesterol markers, rather than simply being better measures of LDL. Semantics are important here. It is important to begin referring to LDL-C as “flawed” or even “defective” because it implies more clearly the need for replacement.

My objective in this article is to share new data showing that LDL-C is, beyond a doubt, a flawed measure of LDL and LDL-related risk. But mainly, I want to call attention to an important consequence of this flaw that seems to be on no one’s radar screen, namely that our understanding of the extent to which many risk markers contribute to CVD risk “beyond LDL” is distorted by the traditional use of LDL-C as the LDL representative in multivariable risk prediction models.

Before proceeding, a few words about terminology to make sure we’re all on the same page. I have already used the terms “LDL,” “LDL-C,” and “LDL-P.” LDL-C has been the standard measure of LDL and LDL-attributable CVD risk for so long that most clinicians and researchers use “LDL-C” and “LDL” interchangeably. We must break ourselves of this habit if there is ever to be understanding of this subject. Whenever I use the term “LDL,” I am referring to low-density lipoproteins. In your mind’s eye should appear a picture of a spherical object coated with a monolayer of (mainly) phospholipid and apoB protein into which is stuffed a few thousand cholesterol ester and triglycerides molecules. That’s LDL, the main culprit in atherogenesis. LDL-C and LDL-P are different laboratory measures of LDL concentration. LDL-C quantifies the cholesterol in LDL, either in mass units (mg/dL) or molar units (mmol/L). LDL-P quantifies LDL particle number (nmol/L) using NMR spectroscopy.

Not only do these two laboratory measures of LDL differ in terms of their basis of quantification (cholesterol content versus particle number), they provide results that do not agree quantitatively in many patients (that is, values are frequently discordant). The reason is well understood and documented: the cholesterol content per LDL particle varies considerably in people. Some have more
cholesterol-rich LDL particles and others have relatively cholesterol-poor LDL.

Let me now explain why I refer to LDL-C as a flawed measure of LDL. I realize this is a provocative statement given that LDL-C has been in clinical use for almost 40 years and numerous statin trials have proven unequivocally that lowering LDL-C provides clinical benefit. So how flawed could LDL-C be? First, to clarify a possible misunderstanding, the flaw I am talking about is not a flaw in the measurement of LDL-C. Certainly the Friedewald equation can be considered “flawed” in giving LDL-C values that do not always agree with those from beta-quantification. But these differences are minor and inconsequential compared to the discordance between the cholesterol in LDL (LDL-C) and the number of LDL particles.

I have already referred to LDL, not LDL-C, as the atherogenic “culprit,” which critics might say reflects an unwarranted prejudice. How can the cholesterol in LDL not be considered “atherogenic,” given that cholesterol-rich LDL particles deliver more lipid to an atheroma than cholesterol-poor particles? But, in truth, it is not clear whether this cholesterol aspect of atherogenesis outweighs others that influence the probability that a given LDL particle will ever get to the final “delivery” stage, needing to first enter the artery wall, be retained, become oxidized, and finally get taken up by a macrophage foam cell, all steps that are influenced more by the particle aspect of LDL.

Since theory does not provide the answer to which LDL measure, LDL-C or LDL-P, is the most relevant with respect to atherosclerotic risk, the question must be addressed experimentally. This is done most productively by comparing risk associations of LDL-C and LDL-P in individuals with discordant values, since risk relations should differ little or not at all among individuals with concordant values. The results of such discordance studies show unequivocally that risk tracks with LDL-P, not LDL-C, when these two measures of LDL disagree. I will cite two examples below.

The first compared the risk of future CVD events during 15-yr follow-up among participants in the Framingham Offspring Study. Figure 1 shows the survival curves of the subgroups with concordant or discordant LDL-C and LDL-P levels greater or less than the median. For the concordant individuals, as expected, risk was substantially greater in those with high versus low LDL. Among discordant individuals, the high-risk group was the one with high LDL-P, despite low LDL-C, while the low-risk group had low LDL-P and higher LDL-C. If the amount of cholesterol in LDL rather than the number of LDL particles was the major determinant of LDL-related risk, results opposite to these would have been obtained.

The second study examined cross-sectional relations of LDL-C and LDL-P with subclinical atherosclerosis (carotid IMT) in the Multi-Ethnic Study of Atherosclerosis (MESA). The (unpublished) data in Table 1 shows that overall, LDL-P

![Figure 1](image-url) Event-free survival in Framingham participants with concordant or discordant levels (above or below the median) of LDL-C and LDL-P. [adapted from Cromwell WC et al. J Clin Lipidol. 2007;1:583-92.]
was related modestly more strongly to IMT than LDL\textsubscript{C}. But among the substantial subset (22\%) of participants with discordant values of LDL\textsubscript{P} and LDL\textsubscript{C} (discordance was defined arbitrarily as values differing by ≥25 percentile), LDL\textsubscript{C} had no relation to carotid IMT whatsoever (\(P=0.68\)), whereas LDL\textsubscript{P} was as strongly associated with IMT as in the concordant subgroup. These results demonstrate that while LDL\textsubscript{C} is a perfectly adequate measure of LDL and LDL\textsubscript{related risk in the majority of people with “normal” LDL particles, it is clearly inadequate in a substantial minority with cholesterol-rich or cholesterol-poor LDL.

Having (hopefully) established that LDL-C is indeed a flawed measure of LDL, I will now move on to discuss a very interesting and important consequence of this flaw. Besides its use in risk assessment and as a treatment target, LDL-C is used universally by epidemiologists to account in multivariable statistical models for the portion of risk attributable to LDL, for the purpose of identifying “independent” CVD risk markers. There are literally hundreds of variables that are individually or in combination (e.g., metabolic syndrome) associated with CVD risk. To be taken seriously as a “novel” marker, the risk associations of these variables must first be shown not to be confounded by a known risk factor. The definition of a confounder is given below. Not only must it be associated with the candidate risk marker, it must have a causal relationship with the outcome. LDL and blood pressure (BP) have unique stature among established risk factors because there is no doubt whatsoever about their causal connection to CVD. Any novel risk marker must therefore “prove itself” in multivariable analyses by demonstrating that its association with CVD is not eliminated upon adjustment for LDL and BP.

What are the consequences of LDL or BP being poorly measured? If the measurement has poor reproducibility or is (randomly) inaccurate, risk prediction by the multivariable model will be worse than if the measurement is performed well. That’s obvious and not particularly interesting. Prediction would improve if the confounder’s measurement flaw(s) was corrected, but use of the flawed measure in a multivariable prediction model would not lead to misleading conclusions about the predictive importance of the other risk markers in the model being investigated.

### Table 1. Associations of LDL\textsubscript{C} and LDL\textsubscript{P} with Carotid IMT in Individuals with Concordant or Discordant Levels.

|                  | \(\Delta\text{IMT (SE) in }\mu\text{m per 1-SD}|| \n|-----------------|----------------------------------|-------------------|
|-----------------|----------------------|-------------------|
|                 | n (%)                | LDL\textsubscript{P} | P value | LDL\textsubscript{C} | P value |
| Overall         | 5361                 | 41.1 (4.1)          | <0.0001  | 35.7 (4.1)          | <0.0001  |
| Concordant      | 4182 (78\%)          | 41.0 (4.6)          | <0.0001  | 42.0 (4.5)          | <0.0001  |
| Discordant      | 1179 (22\%)          | 43.4 (9.0)          | <0.0001  | 3.9 (9.4)           | 0.68     |

\(\Delta\text{IMT values are change in carotid IMT in }\mu\text{m per 1-SD increment of LDL-P or LDL-C from multivariable linear regression models adjusted for age, gender, race, hypertension, and smoking. Concordant values are arbitrarily defined as within 25 percentile units and discordant values are different by ≥25 percentile. [unpublished MESA data]}

### Confounding

A confounder is **associated** with the risk marker and is **causally related** to the outcome*.

\[
\text{Risk marker (e.g., TG, HDL-C, etc.)} \rightarrow \text{CVD}
\]

\[
\text{Confounder (BP, LDL)} \rightarrow \text{Risk marker}
\]

*Katz MH, Multivariable Analysis: A Practical Guide for Clinicians

The situation is different (and much more interesting) if the measurement flaw is systematic (not random) inaccuracy and this inaccuracy is associated with another measured variable. This variable will then appear to contribute independently to risk even if it does not (this might perhaps be called “measurement confounding”). A simple, though contrived, example may help explain the concept. Imagine that, unbeknownst to anyone, standard BP measurements are accurate for people of normal height, but systematically underestimate
BP in tall people and overestimate it in short people. Adding patient height to a multivariable model with BP and other established risk markers will improve the model's prediction, leading to the conclusion that height is a "novel" independent risk marker (tall people exhibiting higher risk than short people at any given measured BP). If BP measurements had always had this flaw, the importance of height to accurate CVD risk prediction would have been discovered many years ago and height would today be considered an “established, standard risk marker” to which novel risk markers would need to demonstrate additive prediction.

Now imagine that a new method of blood pressure measurement comes along that does not have the flaw. Adding this new BP parameter to a model containing the old BP values plus height would not improve overall risk prediction at all because inclusion of height in the model had effectively “repaired” the flaw. Based solely on the criterion of improving risk assessment, the “new, improved” BP measurement would be perceived as having no clinical utility. But if the flaw in the old BP measurement was not able to be so easily “repaired” in multivariable models by a simple height measurement, but required laboratory assessment of 3 or 4 other variables, then replacement of the flawed BP measure by the new BP measure would result in equally good risk prediction with less complexity (since the 3 or 4 surrogate markers of the flaw would not be needed). But blood pressure is also a treatment target, so continued use of the flawed BP measurement would have another clinical price: tall people achieving BP goal would have “residual risk” (because their actual BP is higher than measured) and short people would be (over)treated to unnecessarily low BP levels (because their actual BP is lower than measured).

What I have described is exactly where we find ourselves today, with a flaw having been discovered in LDL-C, the “established, standard” measure of LDL. But there are important differences from the blood pressure analogy. There is not a single variable (like height) associated with the flaw, but many (triglycerides, HDL-C, obesity measures, insulin resistance, inflammation markers, etc.) And, most importantly, these are modifiable markers (unlike height) and therefore potential treatment targets. So, even if we choose to stick, for the time being, with LDL-C as a central component of multivariable primary risk prediction (Framingham score, etc.), it is essential that we reevaluate the importance of the many risk markers, “established” or “novel,” that have strong associations with the flaw (discordance between LDL-C and LDL-P). Continuing to believe many risk markers besides LDL contribute independently to a patient’s risk when some actually do not (despite appearances to the contrary), has a significant cost to both patients and the health care system.

Let me present some additional data from MESA to support these points. In Table 2 are shown correlations of LDL-C, LDL-P, and ΔLDL (the amount by which LDL-P and LDL-C differ by percentile; values range from +77 to -87 percentile units) with 7 lipid and metabolic variables that each have significant associations with CVD risk. All 7 variables are related weakly or not at all with LDL-C, but much more strongly with LDL-P and ΔLDL. For example, LDL size is associated inversely with ΔLDL (r=-0.60), meaning that individuals with small LDL size have, on average, LDL-P > LDL-C. So it may be that the higher risk associated with small LDL, “independent of LDL quantity” (because the association is independent of LDL-C) is not really independent of LDL at all (as measured by LDL-P).

**Table 2. Spearman Correlation Coefficients**

<table>
<thead>
<tr>
<th></th>
<th>LDL size</th>
<th>HDL size</th>
<th>TG</th>
<th>HDL-C</th>
<th>Waist</th>
<th>Glucose</th>
<th>HOMA-IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL-C</td>
<td>-0.00</td>
<td>-0.20</td>
<td>0.13</td>
<td>-0.05</td>
<td>0.06</td>
<td>0.06</td>
<td>0.05</td>
</tr>
<tr>
<td>LDL-P</td>
<td>-0.42</td>
<td>-0.52</td>
<td>0.42</td>
<td>-0.38</td>
<td>0.19</td>
<td>0.16</td>
<td>0.23</td>
</tr>
<tr>
<td>ΔLDL*</td>
<td>-0.60</td>
<td>-0.48</td>
<td>0.40</td>
<td>-0.48</td>
<td>0.19</td>
<td>0.14</td>
<td>0.26</td>
</tr>
</tbody>
</table>

*ΔLDL = LDL discordance in percentile units: LDL-P (percentile) minus LDL-C (percentile). [unpublished MESA data; n=5361]
Prediction of carotid IMT by two sets of predictive models is compared in Figure 2. One set (top) contains LDL-C as the LDL indicator, and the other (bottom) contains LDL-P. Six different risk markers (TG, HOMA insulin resistance index, glucose, LDL size, HDL-C, and HDL-P) added to the LDL variable were examined in separate adjusted bivariate models. As shown in the top panel, all 6 markers added significant prediction to the LDL-C model. They would therefore all be judged by traditional criteria to be independent predictors “beyond LDL.” However, a model with LDL-P alone provided comparable or better prediction than all of the LDL-C bivariate models except the one with HDL-P. Only glucose and HDL-P truly improved prediction of IMT “beyond LDL.”

Our impressions about other “novel” markers of CVD risk have almost certainly been skewed by the LDL-C measurement flaw. Which ones of these remain as important when examined in a model with LDL-P instead of LDL-C will have to be determined. Inflammation markers such as CRP and Lp-PLA₂ have received a lot of recent attention. Both are associated significantly more strongly with LDL-P than LDL-C, suggesting at least the potential of “measurement confounding,” as described above.

To conclude, I hope I have at least begun to convince you of the inconvenient truth that LDL-C has a flaw that is contributing to suboptimal management of CVD risk. Routine use of LDL-C as the proxy for LDL-related risk will continue to distort our understanding about which risk markers besides LDL need to be worried about. It is our role as clinical chemists to help educate the clinical community about analytic problems such as this, and the recent LVDD position statement made an important contribution. We simply need to take it to the next level.

References

**Title:** Low-density lipoprotein (LDL), which includes apolipoprotein A-I (apoA1-LDL) as a novel marker of coronary artery disease.

**Authors:** Ogasawara K, Mashiba S, Hashimoto H, Kojima S, Matsuno S, Takeya M, Uchida K, Yajima J.


**Comment:** Elevated serum level of oxidized LDL detected by certain antibodies has been proposed as a marker of coronary artery disease (CAD) and acute coronary syndrome (ACS). In addition, it was also shown that high-density lipoprotein (HDL), like LDL, may be oxidized in vivo. Authors of this article from the Cardiovascular Institute, Tokyo, Japan discovered that HDL oxidation in the presence of LDL results in the transfer of apolipoprotein AI (apoA-I) in HDL to LDL. They demonstrated the presence of apoA-I-containing LDL (apoAI-LDL) in the blood by purifying a 20-kDa protein from the LDL of ACS patients and identifying it as apoAI. Further, along with high-molecular-weight apoAI, fragmented apoB was also observed in the purified apoAI-containing LDL, suggesting that apoAI-LDL is a kind of oxidized LDL formed through LDL oxidation. Since immunohistochemical staining of atherosclerotic lesions in the intima of human coronary artery showed that apoAI and apoB are localized to the same site, the authors speculated that apoAI-LDL is also present in atherosclerotic lesions and is released into the blood stream, the mechanism of which needs to be further elucidated. Using a newly developed ELISA, the authors also determined the utility of the serum apoAI-LDL level as a novel coronary risk factor. Serum apoAI-LDL was measured in 473 consecutive patients who underwent diagnostic coronary angiography. Patients included 84 with unstable angina (UA), 259 with stable CAD, and 130 without CAD (controls). The serum level of apoAI-LDL was higher in CAD patients than in the control group (31.4 [22.1-41.4] μg/mL vs. 24.6 [18.4-29.2] μg/mL, respectively, P<0.001), as well as in patients with UA compared to those with stable CAD (44.5 [35.8-51.9] μg/mL vs. 27.1 [19.5-

35.6] μg/mL, respectively, P<0.0001) (data are expressed as the median [25th-75th percentiles]). By logistic regression analysis, only apoAI-LDL was an independent, significant predictor of CAD (odds ratio: 1.50, 95% CI: 1.23-1.82, P<0.001), and able to differentiate UA (odds ratio: 1.80, 95% CI: 1.48-2.17, P<0.001) after controlling for classical risk factors (C-reactive protein, serum amyloid A and LDL cholesterol). The serum level of apoAI-LDL may thus be a more sensitive marker of CAD and ACS than classical markers.

**Title:** Efficacy and safety of the cholesteryl ester transfer protein inhibitor anacetrapib as monotherapy and co-administered with atorvastatin in dyslipidemic patients.

**Authors:** Bloomfield D, Carlson GL, Sapre A, Tribble D, McKenney JM, Littlejohn TW 3rd, Sisk CM, Mitchel Y, Pasternak RC.


**Comment:** High-density lipoprotein cholesterol (HDL-C) levels are inversely associated with cardiovascular risk. Cholesteryl ester transfer protein (CETP) inhibition is one strategy for increasing HDL-C. Torcetrapib, a CETP inhibitor, has been shown to produce substantial increases in HDL-C and modest reductions in LDL-C. However, treatment with torcetrapib was associated with an increase in blood pressure, an effect that has not been reported with other CETP inhibitors in development. A clinical outcomes study of torcetrapib in high-risk patients, ILLUMINATE (Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events), was stopped early owing to an excess in cardiovascular events in patients treated with the combination of torcetrapib and atorvastatin vs. atorvastatin alone. Subsequently, 3 studies have reported that torcetrapib did not reduce (nor increase) the atherosclerotic burden assessed in the coronary arteries (by intravascular ultrasonography) and in the carotid arteries (by ultrasonography of intima-media thickness). Anacetrapib is an orally active, potent, selective CETP inhibitor. In preliminary studies in healthy subjects, anacetrapib was well-
tolerated and consistently increased serum HDL-C concentrations with no observed effect on blood pressure or heart rate. The present study, headed by an investigator from the Merck research lab, evaluated the lipid-altering efficacy and safety of anacetrapib as monotherapy or co-administered therapy with atorvastatin in patients with dyslipidemia. A total of 589 patients with primary hypercholesterolemia or mixed hyperlipidemia (53.8% of the study population had low HDL-C) were randomized equally to one of 10 groups: 5 groups received background statin therapy of atorvastatin 20 mg and 5 did not, and each of these was randomized to placebo, anacetrapib 10, 40, 150, and 300 mg once daily for 8 weeks. An equal proportion of patients had triglycerides >150 mg/dL in each group. For placebo and anacetrapib monotherapy (10, 40, 150, and 300 mg), least squares mean percent changes from baseline to week 8 for LDL-C were 2%, -16%, -27%, -40%, and -39%, respectively, and for HDL-C were 4%, 44%, 86%, 139%, and 133%, respectively (P<0.001 vs. placebo for all doses). Co-administration of anacetrapib with atorvastatin produced significant incremental LDL-C reductions and similar HDL-C increases vs. atorvastatin monotherapy. For both anacetrapib monotherapy and co-administration with atorvastatin, the LDL-C reductions were similar in patients with baseline triglyceride levels greater than and less than or equal to the median. Anacetrapib was well tolerated, and the incidence of adverse events was similar for placebo and all active treatment groups. There were no increases in systolic or diastolic blood pressure in any treatment arm. The authors concluded that anacetrapib, as monotherapy or co-administered with atorvastatin, produced significant reductions in LDL-C and increases in HDL-C. The net result of treatment with anacetrapib + atorvastatin was approximately 70% lowering of LDL-C and more than doubling of HDL-C. The potential therapeutic benefit of CETP inhibition remains a charged controversy fueled by the interplay of several factors. Definitive assessment of the potential clinical benefits associated with inhibiting CETP will require studies of a compound that does not have the off-target toxicity that was observed with torcetrapib. The present study suggests that anacetrapib may be a compound that would enable the elucidation of this important clinical and scientific question.

**Title:** Time-dependent association of total serum cholesterol and cancer incidence in a cohort of 172,210 men and women: a prospective 19-year follow-up study.

**Authors:** Strasak AM, Pfeiffer RM, Brant LJ, Rapp K, Hilbe W, Oberaigner W, Lang S, Borena W, Concin H, Diem G, Ruttmann E, Glodny B, Pfeiffer KP, Ulmer H; the VHM&PP Study Group.

**Journal:** Ann Oncol. 2009 Jan 22. [Epub ahead of print]

**Comment:** High levels of serum total cholesterol (TC) are a well-established risk factor for coronary heart disease (CHD). In contrast, the relationship between TC and cancer incidence remains controversial. In the majority of previous studies, cancer incidence and cancer mortality were lower in men with higher baseline levels of TC. Others found higher cancer risk in subjects with high TC concentrations, no relation at all, or a U-shaped association, with both low as well as high TC levels being significantly related to increased cancer risk. In addition, most previous investigations failed to detect any association of TC with cancer incidence and/or cancer mortality in women. Differences in the study populations, length of follow-up, study end-points, and statistical adjustment for confounding may all have contributed to the conflicting patterns of association seen in previous studies. Small sample sizes and infrequent events could have been additional causes of conflicting findings. Moreover, rather than reflecting a true causal relationship, the lower cancer risk seen for high TC levels may be attributable to an effect of preclinical cancer: metabolic degradation of TC due to undiagnosed malignant lesions.

The authors of this article investigated the association of TC with subsequent overall and site-specific cancer incidence in a population-based cohort of 172,210 Austrian adults, free of cancer at baseline, prospectively followed up for a median of 13.0 years. Cox regression, allowing for time-dependent effects, was used to estimate adjusted
hazard ratios (HRs) with 95% confidence intervals (95% CIs) for the association of TC with cancer. According to the results, there were pronounced short-term associations of TC with overall cancer incidence and several site-specific malignancies in both men and women. For malignancies diagnosed shortly (<5 months) after baseline TC measurement, the highest TC tertile (>235.0 mg/dL in men and >229.0 in women) compared with the lowest tertile (<194.0 mg/dL in men and <190.0 in women) was associated with a significantly lower overall cancer risk [HR = 0.58 (95% CI 0.43-0.78, P(trend) = 0.0001) in men, HR = 0.69 (95% CI 0.49-0.99, P(trend) = 0.03) in women]. However, after roughly 5 months from baseline measurement, overall cancer risk was not significantly associated with TC. The short-term inverse association of TC with cancer was mainly driven by malignancies of the digestive organs and lymphoid and hematopoietic tissues.

While further research is needed to shed light on the underlying pathophysiological mechanisms, the pattern of association seen in this study supports the hypothesis that the inverse association of high TC levels with cancer risk may largely be attributable to reverse causation due to preclinical malignancies.

**Title:** Meta-analysis of the relationship between non-high-density lipoprotein cholesterol reduction and coronary heart disease risk.

**Authors:** Robinson JG, Wang S, Smith BJ, Jacobson TA.


**Comment:** Non-HDL-C currently is recommended by the NCEP ATP-III as the second lipid target of therapy after low-density lipoprotein cholesterol (LDL-C). This study was aimed to determine the relationship between non-high-density lipoprotein cholesterol (HDL-C) lowering and coronary heart disease (CHD) risk reduction for various lipid-modifying therapies by assessing eligible randomized placebo or active-controlled trials in a meta-analysis. The effect of mean non-HDL-C reduction on the relative risk of nonfatal myocardial infarction and CHD death was estimated using Bayesian random-effects meta-analysis models adjusted for study duration. Cochrane’s Q was used to test for heterogeneity. Inclusion criteria for meta-analysis were met by 14 statin (n = 100,827), 7 fibrate (n = 21,647), and 6 niacin (n = 4,445) trials, and 1 trial each of a bile acid sequestrant (n = 3,806), diet (n = 458), and ileal bypass surgery (n = 838). For statins, each 1% decrease in non-HDL-C resulted in an estimated 4.5-year CHD relative risk of 0.99 (95% Bayesian confidence interval: 0.98 to 1.00). The fibrate model did not differ from the statin model (Bayes factor K = 0.49) with no evidence of heterogeneity. The niacin model was moderately different from the statin model (K = 7.43), with heterogeneity among the trials (Q = 11.8, 5 df; P = 0.038). The only niacin monotherapy trial (n = 3,908) had a 1:1 relationship between non-HDL-C and risk reduction. No consistent relationships were apparent for the 5 small trials of niacin in combination. The 95% confidence intervals for the single trials of diet, bile acid sequestrants, and surgery also included the 1:1 relationship. The authors concluded that most lipid-modifying drugs used as monotherapy have an approximately 1:1 relationship between percent non-HDL-C lowering and CHD reduction, making non-HDL-C an important target of therapy for CHD prevention. A recent joint consensus report by the American Diabetes Association (ADA) and the American College of Cardiology (ACC) Foundation concluded that non-HDL-C was a better measure than LDL-C for identifying patients at high risk who had multiple cardiometabolic risk factors. Further, they proposed that non-HDL-C calculation should be included on all laboratory reports, and that, in addition to LDL goals, non-HDL-C goals (<100 mg/dL for all CHD patients and diabetic patients with 1 other cardiovascular risk factor; <130 mg/dL for all cardiometabolic risk patients with 2 major cardiovascular risk factors) should be aggressively pursued. The National Lipid Association has also endorsed the importance of non-HDL-C as an additional measure of residual cardiovascular risk. The Association concluded that non-HDL-C is a robust laboratory test, incurs no additional expense in its calculation, and can be obtained in the nonfasting state. Finally, non-HDL-C not only
Title: A novel missense LIPA gene mutation, N98S, in a patient with cholesteryl ester storage disease.
Authors: Hooper AJ, Tran HA, Formby MR, Burnett JR.

AND

Title: [Cholesterol ester storage disease: a rare disease or a rare diagnosis?] [Article in German]
Authors: Weiler C, Freudenberg F, Müller-Höcker J.
Comment: These 2 recent case reports are about a relatively uncommon but apparently often under-diagnosed lysosomal enzyme deficiency that leads to cholesteryl ester storage diseases. Lysosomal acid lipase is a member of the mammalian acid lipase family, which includes human gastric lipase and rat lingual lipase. Lysosomal acid lipase plays an important role in maintaining cellular cholesterol homeostasis. When low-density lipoprotein (LDL) undergoes receptor-mediated endocytosis via the LDL receptor, lysosomal acid lipase releases cholesterol from the lysosome. As a result of the increased cellular cholesterol, expression of the LDL-receptor and cholesterol biosynthesis (HMG-CoA-reductase) is reduced and cholesterol homeostasis is maintained. If lysosomal acid lipase is deficient, cholesteryl esters accumulate and the lack of cholesterol release from lysosomes upregulates the LDL receptor and HMG-CoA-reductase, resulting in further accumulation of lysosomal lipids. Deficiency of lysosomal acid lipase is known to occur in two clinical forms. Wolman disease and cholesteryl ester storage disease (CESD) are both recessive allelic disorders caused by mutations in the lysosomal acid lipase (LIPA) gene on chromosome 10q23.2-q23.3, but are distinguished by the level of residual activity of the mutant enzyme. Since the enzyme is either not produced or has no activity due to mutations in Wolman disease, there is a complete absence of lysosomal acid lipase activity. The condition clinically manifests itself within the first few weeks of life with hepatosplenomegaly, vomiting, steatorrhea, and enlarged, calcified adrenal glands. Progressively worsening anemia follows and the infants rarely survive longer than one year. In contrast, CESD patients have residual lysosomal acid lipase activity and therefore present with a less severe phenotype. Hepatomegaly is often observed in childhood, progressively increases over time, and eventually leads to liver fibrosis. Other clinical signs are variable, with some adult patients experiencing jaundice, splenomegaly, or liver failure. Hypercholesterolemia, sometimes in combination with hypertriglyceridemia, may be present with an increased risk of atherosclerosis. The CESD case described by Hooper and co-workers from Australia involved a 26-year-old female. The patient, previously well with no significant past medical history, presented with recurrent right upper quadrant abdominal pain that was sharp, fleeting and stabbing in nature. There was a strong family history of dyslipidemia. Consistent with liver congestion, the patient had hepatomegaly and abnormal alanine aminotransferases (AST 41 U/L [N: <30], ALT 100 U/L [N: <40]). Her fasting lipid profile revealed elevated total (8.4 mmol/L [N: <5.5] or 324 mg/dL) and LDL cholesterol (6.5 mmol/L [N: <3.4] or 131 mg/dL), and elevated apolipoprotein B (1.75 g/L [N: <1.00] or 175 mg/dL). In the absence of other causes of chronic liver disease, a liver biopsy was performed and revealed features consistent with CESD. Sequencing of the LIPA gene showed that she was a compound heterozygote for the previously reported exon 8 splice junction mutation and a novel missense mutation (N98S) in exon 4. The splice junction mutation allows some (approximately 3%) normal splicing to occur, and therefore gives rise to residual lysosomal acid lipase activity. Asn98 in lysosomal acid lipase is highly conserved among species and mutation of this residue could influence catalytic activity or accessibility to the active site. The CESD case reported by Weiler and colleagues from Germany involved a 13-year-old boy with a long-standing history of nonspecific hepatomegaly, slightly elevated liver enzymes, and elevated total (295 mg/dL) and LDL cholesterol (235 mg/dL). A liver biopsy was performed for histological and electron microscopic examination. The combination of hepatomegaly...
with accumulation of macrophages and ultrastructural evidence of lysosomal lipid storage provided evidence for CESD. The probability of an underdiagnosis or false disease classification, for example for nonalcoholic steatohepatitis (NASH), is high in cases of CESD. A recent study estimated that the carrier frequency of the exon 8 splice junction mutation is about 1 in 200, giving 6 homozygote cases per million. However, only a small number of cases have been reported in the literature, suggesting that CESD indeed is largely underdiagnosed, and should be considered as a differential diagnosis in liver disease and in patients with combined hyperlipidemia.