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Atherosclerotic cardiovascular disease is rapidly overtaking infectious diseases worldwide as the major contributor to mortality. That is already the case in developed countries. This epidemic drives a need for effective cardiovascular risk reduction therapies and, hence, practice guidelines for routine clinical implementation. In the United States, throughout most of our careers, the characterization and treatment of hyperlipidemic patients has been dominated by consensus guidelines developed by the National Cholesterol Education Program. Based on striking morbidity and mortality benefits in multiple large-scale clinical trials, the original and successive NCEP Adult Treatment Panels focused on LDL-C as the proven atherogenic lipoprotein to target for lipid-lowering therapy. As a consequence, statins, the class most effective in reducing LDL-C, have become among the most widely prescribed drugs available. The NCEP focus drove routine laboratory measurements to the lipid panel as a convenient means of estimating LDL-C. Today, characterization and treatment based on estimated LDL-C is mainstream, a story familiar to all. Many interests have converged to maintain this status quo for over two decades.

In retrospect, some aspects of the guideline-driven prevailing practices are open to question. For example, most of the LDL-lowering data from intervention studies were derived using research-grade analyses of LDL-C by beta-quantification, which is reliable in experienced hands. In routine laboratory practice today, LDL-C, as the primary focus of CVD risk reduction therapy, is either estimated by the Friedewald equation, notoriously unreliable, or by direct homogeneous assays, even less reliable. This measurement drives the statin-centric approach to lipid therapies so common in today’s clinical practice patterns. Regarding the statin drugs, careful review of the intervention trials reveals that statins reduce CHD events by only about 25-35% at best, i.e., most of the treated patients, even many at “goal” by updated NCEP Guideline recommendations, will continue to experience adverse events. As examples, in PROVE-IT, while statistically significant, there was actually only a 16% event reduction benefit at two years in the aggressive therapy group. In TNT, there were 584 CVD events in the low-dose group, but there were still 434 events in the high-dose group. History repeats itself. As far back as 1984, in the original Coronary Prevention Trial, it was concluded, and well remembered, that for every 1% reduction in LDL there was a 1-2% reduction in CHD events. What was roundly forgotten for years, from a routine therapeutic perspective, is that in the 1986 substudy it was reported that for every 1 mg/dL increase in HDL, there was a much larger 5.5% risk reduction. Consistently, studies suggest that low or abnormal HDL contributes more to CHD than high LDL, yet HDL-directed therapies have mostly been the ugly stepsister for guideline and therapeutic option targets. Where lies the best therapeutic value? And, how to best measure it? It was just reported that torcetrapib, the first of a new class of cholesteryl ester transferase inhibitors that dramatically increases HDL-C, statistically increased rather than decreased CVD events (mortality). This suggests that the traditional measurement of total HDL-C will not be adequate to clinically characterize the known cardioprotective effect of HDL for upcoming
therapies. Look to the science, not always the guidelines, to determine the appropriate metric.

The mainstream emphasis on LDL-C has roots going back over 50 years. In the early 1950s, John Gofman and colleagues at the Donner Laboratory, UC Berkeley, used the laborious and technically demanding analytical ultracentrifugation lipoprotein separation technology to demonstrate the differential association of the various lipoproteins with atherosclerosis. At the time, other expert laboratories attempted with mixed success to duplicate the Donner findings. In 1956, the recently organized predecessor to the National Heart, Lung and Blood Institute convened a Consensus Conference of lipid experts. They chose to reject available scientific data and the clinical relevance of lipoprotein measurements and concluded that the measurement of total cholesterol was wholly adequate to characterize and monitor CVD risk. The consensus, in essence, also ignored the highly important inverse association of HDL with CVD risk which, as discussed above, was largely forgotten until rediscovered more than two decades later. With significant historical impact, guidelines trumped science. In retrospect, it is clear that Gofman and co-workers were right and the “experts” were mistaken, a decision that has misdirected major studies, CVD guidelines and treatment right up to the present era. Even today, fifty years later, HDL-C takes a back seat to LDL-C, which eventually supplanted total cholesterol as the primary risk reduction guideline target. Until the mid-80s, HDL-C, if measured, was primarily determined in order to estimate LDL-C, the focus of all the major intervention studies and therapeutic guidelines.

The consequence of the historical conservatism of guideline recommendations is that, today, many patients under mainstream monotherapy treatment with statins continue to be at risk for and experience CHD events. In truth, dramatic therapeutic benefit, well supported by published studies, can be affected by applying currently available knowledge about CVD-associated risk metrics beyond the guidelines to direct more effective treatments. For example, niacin, quite effective in increasing HDL was largely ignored for many years until the pharmaceutical industry gained a proprietary position. Now, combination therapies to increase HDL and decrease LDL are available and effective, although not entirely proven in large-scale studies. Therapies are also available to alleviate the lethal, often unrecognized and increasingly common pathology of insulin resistance as a precursor of CVD, yet are not fully accepted by experts in the diabetes arena. As a consequence of not being incorporated into consensus guidelines, many scientific advances, including leading-edge therapies, while clearly effective, are poorly and lately adopted into mainstream clinical practice.

The recent Beckman Conference presented proposed draft guidelines for emerging markers of cardiovascular disease and stroke developed by an ad hoc panel convened by the National Academy of Clinical Biochemistry. Many new biomarkers have become available in recent years and expert consensus opinion as to their utility is useful. The process for developing consensus guidelines is arduous; getting wide input is challenging. For that reason the fall issue of FATS included a link to the draft guidelines. For those desiring an overview of the meeting, the invited speakers at the Beckman Conference have also made their presentations available online.

After the conference, the draft guidelines remain open for comment for several weeks while the panel continues the development process, which includes consideration of comments. Based on feedback from participants at the Beckman Conference, the draft guidelines for some emerging markers seemed to be thorough and balanced with little comment during the meeting. However, other drafts for some markers seemed cursory and/or controversial with considerable and sometimes intense feedback.

Draft guidelines that seemed to generate the most controversy were those regarding the utility of apo B versus LDL-C, the lipoprotein subclasses and inflammation markers. In order to give some visibility to the more controversial guidelines, this
issue of FATS includes an overview of some of these comments. Drs. Walldius and Jungner weigh in on the utility of apo B. They have been leaders in conducting studies, compiling reviews and advocating the advantages of apo B as an indicator of atherogenic particles. In this regard, Dr. Seccombe and colleagues have kindly provided a summary from their survey programs using commutable materials of the relative analytical performance for apo B, calculated LDL-C and direct LDL-C methods. Considering the clinical utility of lipoprotein subclasses by certain measurements, Drs. Otvos and Contois and Dr. French provide respective overviews of their comments to the draft guidelines. Also, Drs. Wolfert and Lanman include comments on LpPLA₂, a more specific marker of arterial inflammation.

This FATS also includes an overview of a new assay for oxidized LDL, another emerging marker and perhaps risk factor for CVD. Besides the usual informative literature reviews, there is also an explanation and link to an FDA position statement regarding clearance of lipoprotein subclass methods.

Russ Warnick, Editor
The Fats of Life

2 http://www.aacc.org/AACC/events/meeting_proceeding/
Welcome readers to the winter 2007 issue of the *Fats of Life*. I hope your Holidays were filled with the Joy of the season. It's hard to believe 2007 is here and January is already passed. 2006 was an exciting and successful year with lots of activities and events for the Division. The planning for 2007 is now underway. Fortunately, we have some additional help this year, as a new individual has taken my place as Finance Chair for the division. Dr. Sridevi Devaraj has agreed to take over this important leadership position and we are excited to have her as a member of our leadership group. She has already participated and contributed to the Division management efforts, participating in conference calls and planning for upcoming meetings. Welcome Sridevi and thanks for your efforts!

Regarding upcoming meetings, the LVDD is collaborating with the Northern California Section of AACC to sponsor two workshops during the Hawaii ASCLS/CLMA Annual Meeting. The meeting will be held in Honolulu at the Marriott Waikiki Beach Resort, Tuesday May 15th through Thursday May 17th, with the AACC-sponsored workshops taking place on the 17th. The workshops are entitled “Current Issues in Cardiovascular Risk Assessment” and “Special Topics in Cardiovascular Disease.” AACC speakers include Drs. Sridevi Devaraj, Dan Hoefner, John Contois, Joe McConnell, Amar Sethi, George Csako, Alan Wu, and Russ Warnick. The Hawaii ASCLS/CLMA group puts on an excellent educational program, so plan to attend.

The LVDD-sponsored programs at the AACC National Meeting in San Diego this year are also being planned—the events include a collaborative Saturday evening symposium on Personalized Medicine sponsored by the Toxicology, Nutrition, Molecular, and LVD Divisions. On Monday, the LVDD Dinner and Mixer will be held, and on Tuesday evening, the LVDD and the Japanese Lipoprotein Standardization group will jointly sponsor an International Lipoprotein Standardization Forum. These meetings and others sponsored by the LVDD will be listed and described in the AACC program brochure and I encourage your attendance. 2007 promises to be a great year and I’m looking forward to the many interactions I’ll have with all my colleagues.

Best regards,

Joseph P. McConnell
Chair, LVDD
Oxidized LDL—The Atherogenic Form of LDL

Patrik Lindstedt, Jeanette Nikus, and Emma Ryan
Mercodia AB, Uppsala, Sweden

Low-density lipoprotein (LDL) cholesterol has long been recognized to play an important role in the development of atherosclerosis, and still remains the primary target of therapy for the prevention of coronary heart disease (CHD).

Nevertheless, it has been suggested for more than 20 years that oxidation of lipoproteins is central in the initiation and progression of atherosclerosis, from the early stage conversion of monocytes/macrophages into lipid-laden foam cells and fatty streaks to the late-stage development of coronary artery stenosis, plaque instability, plaque rupture, coronary thrombosis, and myocardial infarction.

The oxidative modification hypothesis is based on the concept that LDL in its native form is not atherogenic, and that oxidation of LDL lipids and ApoB-100 is central in the pathogenesis of vascular disease.

Background

Low-density lipoprotein (LDL) refers to a class of lipoproteins which main function is to transport cholesterol and triglycerides in the blood for use by various cells. Due to the high blood pressure, plasma constituents continuously seep into the intima of arteries and, at reasonable blood levels, LDL particles can pass in and out of the vessel wall. In the blood, LDL particles may be protected from oxidation by blood antioxidants. In excess, LDL tends to get trapped in the matrix, by proteoglycans and other extracellular matrix constituents, where it is subjected to modifications (1). Native (unmodified) LDL lacks inflammatory properties, whereas the modified LDL particles are sensed by the cells as foreign and the immune system is activated and inflammation initiated (2).

During inflammation a variety of cells (vascular endothelial cells, smooth muscle cells, fibroblasts, neutrophils, monocytes, macrophages) produce inflammatory mediators like oxidants as a defense against disease-causing substances (viruses, bacteria, parasites, tumors, harmful agents) (3). Enzymes like lipoxygenase, cyclooxygenase, phospholipase A₂, and myeloperoxidase are believed to be involved in lipid oxidation and results in the generation of aldehydes that substitute lysine residues in the ApoB-100 moiety of LDL (4), and thereby generating oxidized LDL (oxLDL). However, lipid peroxidation is not required. Indeed, aldehydes that are released by endothelial cells under oxidative stress or by activated platelets may also induce the oxidative modification of ApoB-100 in the absence of lipid peroxidation of LDL (7).

Macrophage scavenger receptors are involved in the removal of oxLDL deposited in the blood vessel wall. Uptake of LDL-cholesterol via the native LDL receptor is subjected to negative feedback regulation. In contrast, the uptake of oxLDL via scavenger receptors is not down-regulated with increasing intracellular cholesterol content and results in a massive cholesterol uptake by macrophages, which become foam cells. Foam cells that constitute the fatty streaks in early steps of atherosclerosis, induce activation of the immune system by the release of inflammatory cytokines (5).

In the progression of atherosclerosis, an increasing thickening of the intima (plaque formation) is, among other things, due to the intra- and extracellular lipid accumulation and the recruitment of monocytes and T-lymphocytes to the artery wall. Smooth muscle cell proliferation and migration to the top of the inflamed intima is stimulated by factors secreted from macrophages, endothelial cells and smooth muscle cells. There they synthesize matrix molecules like collagen, which together with the smooth muscle cells, form a plaque-stabilizing fibrous cap (2). The fibrous cap may subsequently be degraded by oxidized LDL-induced secretion of matrix metalloproteinases. If the weakened plaque ruptures, tissue factor, induced during inflammation, will interact with clot-promoting elements in the blood, causing a thrombus to form (6).
Measurement of oxidized LDL

Given the suggested pathophysiological significance of oxLDL, there has been a significant increase in the research of the pathogenic role of circulating oxLDL, giving new insights and questions in regards to the role of oxLDL in cardiovascular disease. Previously indirect measures were used to assess the LDL oxidation, mainly by measurement of susceptibility of LDL to oxidation and measurement of autoantibodies to oxLDL. The introduction of specific monoclonal antibodies raised against oxLDL, recognizing different distinct oxidation epitopes has enabled the development of specific and sensitive immunoassays to measure the levels of circulating oxLDL.

The body of evidence on circulating oxLDL and its clinical significance to the diagnosis and prognosis of cardiovascular disease has grown substantially over the past 10 years. Three different monoclonal antibodies that bind various epitopes of oxLDL have been described in detail. All three are murine monoclonal antibodies (DLH3, E06 and 4E6) and assays based on all three antibodies have been used in the investigational arena. The monoclonal antibody 4E6 (3,8,9) is directed against a conformational epitope in the ApoB-100 moiety of LDL that is generated as a consequence of substitution of at least 60 lysine residues of ApoB-100 with aldehydes. This number of substituted lysines also corresponds to the minimal number required for scavenger-mediated uptake of oxLDL. The oxLDL specificity of antibody 4E6 is different from the antibody DLH3 (10) or the antibody E06 (11, 12). DLH3 and E06 are reported to be directed against epitopes of oxidized phosphatidylcholine and to the phosphocholine head group of oxidized but not native phospholipids, respectively.

In 1998, Holvoet et al. (3) demonstrated for the first time that elevated circulating levels of oxLDL were found in most untreated patients with both stable CAD and acute coronary syndromes (unstable angina; acute myocardial infarction). In this study, oxLDL levels were measured in plasma with an ELISA procedure using the oxidized LDL-specific, murine monoclonal antibody 4E6, as developed by professor Holvoet and colleagues at the University of Leuven, Belgium. Since Holvoet’s landmark discovery and the commercial introduction of the Mercodia oxLDL ELISA, based on the 4E6 antibody, the literature has been extensive in providing further support for Holvoet’s 1998 findings and expanding on the importance of oxLDL as a marker of cardiovascular disease.

Subclinical atherosclerosis: oxLDL as a marker of asymptomatic cardiovascular disease

In 2002, Hulthe and Fagerberg (13) tested 391 clinically healthy 58-year old Swedish men from the AIR study and found that oxidized LDL was associated with subclinical atherosclerosis (clinically silent ultrasound assessed atherosclerotic changes in the carotid and femoral arteries). Otherwise healthy patients with at least one plaque in the carotid or femoral arteries had higher oxidized LDL levels compared to patients with no plaques. The results support the concept that oxidatively modified LDL may play a major role in the development of atherosclerosis.

Furthermore, baseline oxidized LDL levels have also been found to predict the progression of subclinical atherosclerotic disease. In a study done by Wallenfeldt and colleagues in 2004 (14), the oxLDL levels at entry of the study correlated significantly with the number and size of plaque at 3-year follow-up, demonstrating that oxLDL in plasma is a prognostic biomarker of the subclinical atherosclerosis development. Oxidized LDL at entry, but not LDL cholesterol, was associated with the number and size of plaques present, and proved to be a strong predictor of the progression of atherosclerosis in the carotid arteries.

Liu and colleagues (15) analyzed the potential determinants of circulating oxLDL in FCHL family members without clinical CAD. FCHL is associated with early atherosclerosis and is responsible for >10% of premature CAD and is characterized by enhanced generation of free radicals. Oxidized LDL was found to be independently
associated with carotid IMT in asymptomatic FCHL family members as well as a potential marker for early atherosclerosis in FCHL.

**Oxidized LDL improved identification of coronary artery disease patients**

In 2006, Johnston et al. (16) demonstrated that the oxLDL/HDL ratio discriminated the best between apparently healthy men and women (control subjects; n = 431), without clinical evidence of coronary artery disease, and coronary artery disease patients (n = 490), who participated in the Second Fragmin and Fast Revascularization During Instability in Coronary Artery Disease (FRISC-II) trial. This study showed that the oxLDL/HDL ratio could identify more patients with coronary artery disease than any other currently available blood lipid biomarker test, including, total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol, total cholesterol/HDL-cholesterol ratio test, and Lp-PLA₂.

**OxLDL predicts future cardiovascular events**

In 2005, Meisinger and colleagues (17) studied 346 apparently healthy men from the MONICA/KORA study. Compared to controls, baseline mean plasma levels of oxLDL were significantly higher in subjects who subsequently experienced a CHD event. Plasma levels of oxLDL were the strongest predictor of future CHD events, and the association was independent of the conventional lipoprotein profile and other traditional risk factors for CHD such as CRP. Thus the additional measurement of oxLDL may improve prediction of atherosclerotic CHD complications.

In 2006, Johnston et al. (18) examined the relationship between levels of circulating oxLDL and outcomes in patients with unstable CAD at long term follow-up, and compared the prognostic value of oxidized LDL at 2 years follow-up with that of other well established cardiovascular risk markers in patients with unstable CAD included in the FRISC-II trial. Oxidized LDL proved to be an important independent predictor of myocardial infarction, but not mortality, and the findings also suggested that oxLDL might identify unstable CAD patients at risk for future myocardial infarction, particularly in the absence of myocardial necrosis.

**The Diabetes Connection**

The association between diabetes and cardiovascular disease has emerged. The prevalence of, incidence of, and mortality from all forms of cardiovascular disease are two- to eight-fold higher in persons with diabetes than in those without diabetes (19,20). The pathogenesis of atherosclerosis in diabetes is complex and multifactorial, but one thing is very clear, there is an increase in lipid and lipoprotein peroxidation within the arterial wall of diabetic patients (19), which could result in the enhanced biosynthesis of oxLDL within the atherosclerotic lesion. In 543 middle-aged subjects from the RIAD cohort, Kopprasch (21) and investigators used the oral glucose tolerance test to distinguish between normal glucose tolerance and impaired glucose tolerance. They found that the metabolic situation of impaired glucose tolerance and newly diagnosed diabetes is associated with diabetic dyslipidemia that particularly affects the level of circulating oxLDL.

The presence of an atherogenic lipid profile is common in diabetic patients. Scheffer and colleagues (22) found that patients with higher HbA₁c and higher fasting glucose levels also had higher levels of small, dense LDL. The researchers also found that the prevalence of small, dense LDL particles correlated with high circulating levels of oxLDL in Type 2 diabetic patients. Together, these data suggest that measuring oxLDL may be useful in identifying type 2 diabetic patients with accelerated atherosclerosis.

**The Metabolic Syndrome**

The metabolic syndrome is associated with high risk for CHD, and persons with the metabolic syndrome are at increased risk for developing CHD as well as increased mortality from CHD and all causes. The relationship between the metabolic syndrome components and circulating oxLDL was
examined in the Health ABC cohort by Holvoet et al. (23). They showed for the first time that in a population cohort the metabolic syndrome is associated with higher levels of circulating oxLDL and that this association was consistent across gender and race. As in previous studies the metabolic syndrome showed predictive value for CHD and increased the risk for incident myocardial infarction. However, a new and important finding was that oxLDL increased the risk of incident myocardial infarction, suggesting an effect of oxidized LDL on myocardial infarction independent of the metabolic syndrome, adding prognostic information concerning future risk for myocardial infarction.

Sigurdardottir and colleagues (24) reported on subjects from the AIR study looking at the link between the presence of oxLDL in these healthy patients and factors related to the metabolic syndrome. They found that baseline levels of oxLDL add clinically important prognostic information to the metabolic syndrome. The metabolic syndrome was found to be associated with high levels of circulating oxLDL with the underlying mechanism seeming to be linked to the occurrence of small, dense LDL particles. The proposed proatherogenic properties of small LDL particles relate to their ability to penetrate the arterial wall, to bind more easily to arterial proteoglycans, and thus be more susceptible to oxidation.

Conclusion

Circulating oxLDL has been found to be additive to the global risk score based on age, sex, total and HDL cholesterol, diabetes, hypertension and smoking, suggesting it might be a useful marker for identifying persons at risk for coronary artery disease.

The association of oxLDL with subclinical CVD, even after adjustment for CVD risk factors, indicates that elevated levels of circulating oxLDL might be related to the risk of developing CVD rather than a consequence of having CVD. This is further supported by studies showing that measurement of circulating levels of oxidized LDL can predict future cardiovascular events and as shown in the Health ABC, the MONICA/KORA Augsburg and the FRISC-II cohort.

Several recent reviews of the available world literature confirm that circulating oxLDL levels are independently associated with different forms of coronary artery disease and peripheral arterial disease, and that oxLDL might be considered a suitable marker for identifying patients at risk for cardiovascular events in addition to markers of inflammation such as CRP and fibrinogen.

References


The following comments are in regard to the recommendations on apoB, apoA-I, and the apoB/apoA-I ratio as presented in the AACC draft on “Emerging Cardiac Markers,” which was presented and discussed in Baltimore, US, on October 20-21, 2006. As presented here, we include and number 52 new references in a separate reference list. After that list we also include the references we have cited from the reference list as it was presented in the NACB draft.

Our critique is focused on the following points:

First: the methods used for measuring LDL and HDL. The errors of these methods, and the variability between results obtained by different methods for measuring LDL and HDL, can be very large. Furthermore, LDL and HDL methods are not internationally standardized like methods for measuring apoB and apoA-I.

Second: we also believe that too little attention has been paid to what has been published on recent risk studies as well as on results from lipid-lowering treatment, especially during the last few years. In the drafted NACB document, 22 out of 63 references are from 2001 or later. In our present discussion of pros and cons, we add 52 references from 2001-2006, all of which contain substantial information relevant to the importance and advantages of apoB and apoA-I as new, strong risk markers/factors for cardiovascular disease.

Third: it also occurs to us that in the drafted NACB document there are some inconsistencies in how the apolipoproteins have been evaluated in the different sections. If apoB and the apoB/apoA-I ratio are accepted under certain circumstances, how can apoB be so negatively judged in the first section when apoB is compared with LDL-cholesterol (LDL-C) and non-HDL-C?

Fourth: there was no evaluation on apoA-I in the summary report or recommendations.

Fifth: there are few comments on the favorable prediction of risk reduction obtained during lipid-lowering therapy using the apoB/apoA-I ratio compared to LDL-C or other lipids.

In recent years, five major review papers have analyzed pros and cons for apolipoproteins versus lipids in prediction of cardiovascular (CV) risk. Two are mentioned in the NACB Draft document (Sniderman et al., NACB ref. 13, Barter et al., NACB ref. 37). The others are from Walldius and Jungner 2004 (1) and 2006 (2) and Sniderman and Marcovina (3).

Methods used for measuring LDL and HDL

From a number of papers during the last few years (4-8), it is evident that there is a major concern about the methodological errors obtained based on calculating LDL-C by the Friedewald formula, which is the most commonly used method in clinical practice. The authors conclude that estimation of LDL-C by the Friedewald equation results in a substantial level of misclassification with serious impact on clinical decisions (4). The errors are large at all levels of LDL, especially when low values of LDL have to be determined as in lipid-lowering therapy—target values in the range of 70-100 mg/dL. Hypertriglyceridemia, non-fasting, etc., are other major pitfalls giving inaccurate LDL values. No values for LDL can be given if triglycerides (TG) levels are above 4.5 mmol/L (400 mg/dL).

A large number of other so-called “direct” LDL methods have been developed. The major problem is that these methods do not measure the same parts or qualities (size, density, epitopes, etc.) of LDL (4,5,9-11). Although these methods correlate well, and they also correlate with calculated LDL-C values according to Friedewald, with r-values of about 0.9, they do not always show the same levels
or values. As pointed out by Evan Stein in his lecture at the Baltimore meeting, values for LDL-C directly determined could vary between 127-174 mg/dL! The lack of standardization and normalization between the methods is unacceptable. Moreover, it should be noted that the inaccuracies of the direct HDL-C and direct LDL-C methods are not primarily the result of inaccuracy of the assay calibration, but result of inherent problems in each of the assays (4). Furthermore, there are no common reference materials for HDL and LDL as there is for the WHO-IFCC program on apoA-I and apoB (Marcovina et al., NACB ref. 40,41).

These methodological issues for both LDL and HDL, which are serious, not often talked about, not even realized by several “experts,” and not generally known by authorities, have to be dealt with in much more detail in the drafted document.

There are also some biological advantages of using apoB over LDL-C. Thus, apoB is the major protein in LDL particles, as well as in other potentially atherogenic particles (VLDL and IDL). In conditions where small, dense LDL (sdLDL) particles are prevalent, apoB is recognized as a valid index of number of sdLDL particles, identified as the most atherogenic particles within the LDL species. This is commonly the case in subjects with the metabolic syndrome and in diabetic patients who often have normal to low LDL-C with hypertriglyceridemia and hyperapoB (12). Although NMR techniques can distinguish between different sizes of both LDL and HDL species, the size of a given particle is a risk in itself, it is not a major determinant of CV risk. It is rather the number of sdLDL particles that can be measured more easily by apoB and cheaper than by NMR techniques.

There are also methodological advantages speaking in favor of using apoA-I rather than HDL, since the variability in the values obtained using different methods for determining HDL-C are also unacceptably high (4), especially between homogeneous HDL methods. Even in this respect, Evan Stein, at the Baltimore meeting, showed less CV% errors using apoA-I than HDL methods.

Recent data also support the use of apoA-I-based methods over HDL-C-based methods (Gotto et al., NACB ref. 34; Simes et al., NACB ref. 61; 12-15). In studies by Luc et al. (13) it was found that apoA-I was the single best of four HDL parameters, i.e., HDL-C, apoA-I, LpA-I, LpA-I:A-II, in predicting outcome. In a follow-up to be published soon from the first INTERHEART study (NACB ref. 36), that study also finds apoA-I to be more closely related to risk of myocardial infarction than HDL-C (Yusuf, Sniderman, McQueen et al., personal communication).

At the Baltimore meeting, Evan Stein presented an overview of the variability of all different methods used to measure/calculcate LDL and HDL and directly determine apoB and apoA-I. Clearly, the methods used in about 120 laboratories around US to determine apoB and apoA-I had lower CV% errors. Therefore, these methods ought to be preferred, and, in fact, also be recommended more frequently than is the case today. Furthermore, Stein also pointed out in his lecture that small bias in LDL and HDL will result in both over or under treatment. He concluded that CAP survey data (NACB ref. 42) demonstrate that apoB measurements are well standardized and reproducible between laboratories and that apoB measurements are widely available. ApoA-I measurements are also widely available and commercial methods and calibration materials are easily adapted to current automated analyzers in hospitals and routine laboratories.

In the general discussion at the meeting, several scientists recommended that these methodological facts and issues should be dealt with much more in the written document/recommendations. In fact, many of the debaters viewed these LDL and HDL methodological issues as very critical. They therefore supported the use of apoB and apoA-I methods. Thus, the presently written text stating that apoB is only “marginally better than LDL” is
not an adequate reflection of these methodological issues. Furthermore, no comments were made on methodological issues of HDL and apoA-I despite the fact that the apoB/apoA-I ratio was advocated over the TC/HDL ratio in the NACB draft document. Therefore, comments on HDL and apoA-I ought to be made.

Scott Grundy, in his editorial in Circulation 2002 (NACB ref. 53), stated—as he also did in Baltimore—that apoB is an established risk factor, i.e., apoB is not only an emerging risk factor. He also recommended target values for therapy based on three levels of global risk for a given patient. In fact, in the drafted NACB-document there are already tables referring to such target values. Also, for these two reasons, apoB should be accepted as a relevant risk factor also in the conclusions and recommendations from NACB/AACC.

Our recommendations:

Considering all these methodological issues, we would like to propose that not only apoB but also apoA-I are accepted as valid measures that give valuable and accurate clinical risk information beyond that of LDL-C and HDL-C. From the practical point of view, sampling of blood in the non-fasting state is a clinical advantage over most LDL- and HDL-methods (see also the advantages for the apoB/apoA-I ratio, below).

We also would like to give another general comment on methodology. We realize that apoB and apoA-I methods are not yet available for GPs in US healthcare, although scientists like Evan Stein in his lecture stated that apoB and apoA-I methods are “widely available.” In addition, the price for measuring these apolipoproteins is not yet negotiated, and price may be a critical obstacle for widespread clinical use, at least in the US. However, these shortcomings should not be used as an argument for turning down the scientific evidence and the quality of apo-analyses. Hopefully, the document will highlight and stimulate scientists and clinicians to set up and use these methods in order to obtain a better quality of diagnostic precision beyond those methods used to calculate/determine LDL and HDL. Evolution and development should be stimulated rather than prohibited.

Epidemiological evidence in favor of apoB, apoA-I, and the apoB/apoA-I ratio

NACB reported in the draft and reference list four prospective studies in which apoB was a stronger predictor than LDL-C (Lamarche/Québec, NACB ref. 10; Talmud/NPSH, NACB ref. 11; Walldius/AMORIS, NACB ref. 15; Moss/THROMBO, NACB ref. 33); five other studies reported apoB to be a better marker than traditional lipid parameters (Rahmani, NACB ref. 14; Meisinger/MONICA-KORA, NACB ref. 27; Gotto/AFCAPS, NACB ref. 34; van Lennep/LEIDEN, NACB ref. 35; Simes/LIPID, NACB ref. 61); the recent literature-based meta-analysis of associations between apoB, apoA-I, ratio B/A and CHD (Thompson and Danesh, NACB ref. 28) and the Yusuf/INTERHEART study, NACB ref. 36, which strongly supported apoB/apoA-I as the best marker in cohorts from 52 countries. We extend the NACB reference list with 52 more references, all from the last 5 years (2001-2006) and 22 from 2006. Several of these new studies are commented on below.

ApoB versus LDL cholesterol

During the last few years, several risk studies, including also relations to various manifestations of atherosclerosis, have been published showing that apoB is superior to LDL-C (17-26). To our knowledge there is virtually no documentation on the opposite, i.e., LDL-C better than apoB. In some reports apoB and LDL-C are equivalent in predicting risk. Even if so, that should not distract from the general conclusion of more favorable risk prediction using apoB as reviewed in detail (Barter et al., NACB ref. 37; 2). To us, these conclusions indicate that the phrase “marginally better,” as written in the drafted document presented at the meeting, is not justified for the comparison between apoB and LDL-C.
ApoB and the apoB/apoA-I ratio in metabolic syndrome and diabetes

In patients with the metabolic syndrome and diabetes, LDL-C is not often high, but rather normal. Many of these patients have moderately high TG values and a low HDL-C. Increased levels, i.e., number of sdLDL particles, which is identified by high apoB values, is a common manifestation in these patients with the metabolic syndrome. Although apoB has not been directly compared with LDL-C in these studies, the strength of apoB as a risk maker in these patients is compelling (Rahmani et al., NACB ref. 14; 18,27-31). We interpret the aggregated epidemiologic evidence so that apoB is clearly superior to LDL-C in predicting risk.

ApoB or apoB/apoA-I ratio versus non-HDL cholesterol

In his editorial, Scott Grundy (NACB ref. 53) also commented and recommended the use of non-HDL cholesterol. In fact, non-HDL-C has less methodological variability than LDL-C since both total and HDL-C can be directly determined with low CV% for both these determinations. Since the methods used for determining HDL-C can vary much between laboratories (per Stein’s lecture at NACB), the values for calculated non-HDL-C (total cholesterol minus HDL-C) can also vary significantly. Although there is a strong correlation between non-HDL-C and apoB with r-values about 0.85-0.90, there are many publications indicating that apoB is superior to non-HDL-C in predicting risk (Sniderman et al., NACB ref. 13); 17,20,22-24,32,33), although a few studies have previously indicated similar prediction of outcome (Shai et al., NACB ref. 23; Meisinger et al., NACB 27; Ridker et al., NACB ref. 24). Besides these facts, it is most likely easy to explain the rational of measuring apoB—that is the number of atherogenic particles, which all contain “bad” cholesterol. Furthermore, the apoB value is determined directly with little error, and fasting is not needed. It is probably more difficult to explain to patients that non-HDL-C is “all bad cholesterol” that is not within the “good HDL cholesterol.” A variable

defined as a “non-variable,” i.e., a negative definition, is hard to understand for laymen and patients, at least in our opinion.

The apoB/apoA-I ratio versus the lipid ratios; LDL-C/HDL-C, TC/HDL-C and non-HDL-C/HDL-C

In the NACB document, as presently drafted, the apoB/apoA-I ratio is highlighted to be better than the TC/HDL-C ratio. This is in agreement with almost all published comparisons from many studies (Sniderman et al., NACB ref. 13; Rahmani et al., NACB ref. 14; Walldus et al., NACB ref. 26; Gotto et al., NACB ref. 34; 17,20,34-37). There are clear advantages to using the apoB/apoA-I ratio over LDL-C, non-HDL-C or lipid ratios, not only for identifying risk of non-fatal and fatal myocardial infarction, but also for stroke, heart failure, aortic aneurysms, type 2 diabetes, and in patients with the metabolic syndrome and several other clinical conditions characterized by ischemia based on atherosclerosis (2). Thus, the apoB/apoA-I ratio is also stronger related to high IMT values in the carotid artery and impaired endothelial function than conventional lipids (38,39). A high apoB/apoA-I ratio also predicts progress in IMT development over a three-year period better than conventional lipids (38). A high apoB/apoA-I ratio predicts presence of femoral plaques and increased risk of AMI (40). In the elderly patients the apoB/apoA-I ratio—not LDL-C—was inversely related to endothelium-dependant vasodilation (41) and apoB/apoA-I was also a useful determinant of carotid artery atherosclerosis (42). The apoB/apoA-I ratio was associated with both myocardial infarction and stroke in a nationally representative sample of US adults from NHANES III (43).

The results from INTERHEART (Yusuf et al., NACB ref. 36), a case-control study, clearly indicate the strong impact of the apoB/apoA-I ratio as the major risk factor. This ratio was the strongest of all 9 conventional risk factors (all risk factors were independent) in 15,000 patients with AMI (52 countries, males and females of all ethnicities—similar results in all studied cohorts). The apoB/apoA-I ratio explained 50% of the worldwide
variability of AMI based on determination of Population Attributable Risk—the highest of all 9 risk factors.

Our recommendation:

Since this apo-ratio is judged and accepted in the drafted document, it would be logical that also both apoB and apoA-I would have been accepted as valid single determinations of risk beyond both LDL-C, HDL-C, and lipid ratios (see above).

ApoB, apoA-I and the apoB/apoA-I ratio as targets during lipid-lowering therapy

Since apoB has been accepted in the drafted document as a marker of therapeutic effects during lipid-lowering treatment, it seems logical also to accept apoB as being better than LDL-C in predicting risk. These facts are further strengthened in new publications. Thus, even if treatment successfully reduced LDL-C levels to “normal / target levels,” the treatment effects only reduced apoB levels to about the levels of the 50th percentile of a population (44). This means that the patients are not optimally treated and that there most likely remains an increased number of untreated sdLDL particles in the circulation (45).

Baseline as well as on-treatment values for the apoB/apoA-I ratio have also been found to be better indicators of outcome/treatment effects than LDL-C in lipid-lowering trials such as the AFCAPS/TexCAPS (Gotto et al., NACB ref. 34), CARDS (18), and IDEAL (34). At the AHA 2006, Kastelein et al. (16) also presented evidence in favor of the apoB/apoA-I as the best lipid-related marker of treatment effects in the pooled analyses of the data from the IDEAL and TNT trials. It is likely that both methodological as well as biological factors contribute to the better and more precise results obtained by apolipoproteins than by conventional lipids (see 2).

Comments on publications on debates

In the drafted NACB document, some rather old publications on debates about apolipoproteins versus lipids are referenced. In recent years, additional scientific information on the importance of apolipoproteins has been debated which also ought be considered (46-50).

Concluding remarks—our recommendations and actions:

In the detailed analysis of facts presented in a full book forming the basis for the NCEP-ATP III document, there were some short comments on apoB and apoA-I as possible risk factors that might be used in the future (some advantages for apolipoproteins were commented and appreciated). This document was mainly put together by the expert committees during 2000-2001. At that time, the apolipoprotein methods were not readily available in clinical practice as they are today. Therefore, it was advised that LDL-C, non-HDL-C, and HDL-C should be used. In 2002, Scott Grundy, in his editorial, included apoB as a tentative risk variable and he presented reference-target values in relation to the calculated global risk for a given patient. Reference values for both apoB and apoA-I have also previously been recommended (see 1,2); six are already mentioned in the drafted NACB ref. list (NACB ref. 47-52). New data about reference distribution values in 82 different populations are also found (51,52). In these documents, apoB, apoA-I as well as the apoB/apoA-I ratio are favorably commented and recommended.

During the last 6-7 years since NCEP-ATP III was published, many new results from prospective risk studies and other types of trials including lipid-lowering trials have been published. Thus, the current knowledge is now much greater and it is widespread. Altogether, the integrated results from these studies, in our opinion, speak in favor of using apoB and apoA-I, as they are more accurate and stronger risk factors than conventional lipids.

In order to be accepted as new risk factors according to NCEP-ATP III criteria (taken from the full NCEP book), the following criteria for any such factor should be fulfilled:

1) have significant predictive power that is independent of the other major risk factors.
2) they should have a relatively high prevalence in the population (justifying routine measurement in risk assessment).

3) laboratory or clinical measurement must be widely available, well standardized, inexpensive.

4) have accepted population-reference values.

5) be relatively stable biologically.

6) preferably, but not necessarily, modification of the risk factor in clinical trials will have shown reduction in risk.

It is our opinion that all these criteria are fulfilled for apoB, apoA-I, and the apoB/apoA-I ratio. It is our hope and recommendation that the new and updated document will rephrase the judgment on apoB and apoA-I so that the scientific evidence is properly taken into account. Although there is still a long way to go until this can be implemented in some countries, in our view, it would be wrong to neglect this new knowledge and not make proper reference to what has been achieved in science. What is written in official US documents is, in fact, read and cited all over the world. The practical aspects about lack of availability and validation of laboratory methods should not be referred to as an obstacle for using such methods. We believe, and propose, that these new tests at least can be used as optional tests under given circumstances. Regulating authorities like the FDA and the European EMEA would then acknowledge that clinical trials are using apoB and apoA-I as better markers of CV risk and as targets during therapy.

Additional comments and information

In Sweden, we are now introducing the apolipoprotein concept for clinicians. We are using the apoB/apoA-I ratio as the major determinant of risk, and we also give the values for apoB and apoA-I on the laboratory list. One risk line is drawn showing the apoB/apoA-I ratio in deciles on the x-axis versus CV risk on the y-axis (see figure), based on the AMORIS (2) and INTERHEART data (Yusuf et al., NACB ref. 36). In these figures the apoB/apoA-I ratio varies from 0.4 to 1.6 (no sort is needed, an advantage) versus CV risk (myocardial infarction on the y-axis in a scale from 1 to about 6).

It is easy to find out where on the risk graph a given patient value is located, and to explain for the patient to which lower level treatment is targeted. Both physicians and patients find this very simple and easy to monitor over time. The methods for analyzing apoB and apoA-I are now being implemented and validated by an external group of experts. The costs for the analyses are in most laboratories lower than for conventional lipids. This program is now in operation in at least six major regions in Sweden in academic hospitals as well as in general health care served by GPs. The experience is very positive from both patients and physicians. They understand well the new concept; the new value shows the balance between the “bad” and the “good” cholesterols, fasting is not needed. In fact, one reason why we find the apoB/apoA-I ratio better than the other lipids and lipid ratios is probably due to the information embedded in the apoA-I value. Although it contains information on anti-inflammatory and anti-oxidant as well as stimulating effects on NO and prostacyclin, it also reflects the reverse cholesterol transport. All that information is obviously not included in our simple explanation to the patients: “the cholesterol balance, i.e., the balance between the “bad” and the “good” cholesterols. However, for sake of simplicity we stick to that simple explanation (compared to more difficult way of explaining non-HDL-C).
References:


34. Olsson A, Holme I, Pedersen TR - for The IDEAL Steering Committee and Investigators. Apolipoprotein B/A ratio is a better discriminator of risk of coronary heart disease than is LDL/HDL cholesterol ratio in the IDEAL study. Abstract. XIV


51. Ritchie RF, Palomaki GE, Neveux LM, Leduc TB, Marcovina S, Navolotskala O. Reference Distributions for Apolipoproteins AI and B and


References cited from NACB Draft Reference list:


The Canadian External Quality Assessment Laboratory (www.ceqal.com) operates a reference method laboratory that is part of the Cholesterol Reference Method Laboratory Network (CRMLN) (www.cdc.gov/labstandards/crmln.htm International Members). In collaboration with HealthMetrx Canada (www.digitalPT.com) CEQAL provides an external proficiency-testing program to clinical laboratories for the measurement of lipids and apoproteins. This program uses commutable test samples consisting of fresh human serum that has been collected from normal and dyslipidemic donors according to the NCCLS C37-A guideline. The samples that are used in this proficiency testing survey are processed without freezing and sent by courier on ice at 4°C to the participating laboratories. Target values for lipids and apoproteins are assigned by reference methods as operated within the CRMLN at CEQAL and at the University of Washington, Department of Medicine, Northwest Lipid Metabolism and Diabetes Research Laboratories (NWLRC) in Seattle.

In this report, we examine the performance of 18 laboratories that have subscribed to this proficiency testing program and have submitted test results for the measurement of total cholesterol, HDL cholesterol, net triglycerides, apoprotein A-1, apoprotein B and LDL cholesterol using a direct LDL measurement method.

The data were obtained from 6 samples that were recently tested over a 6-month period. The triglycerides values in these samples were all less than 234 mg/dL (2.64 mmol/L). Tukey’s outlier rule was applied and any result that was deemed to be a statistical outlier was removed from the data set prior to analysis.

Results

When the performance of these laboratories was assessed on the basis of the NCEP total error performance goals for the measurement of TC, HDL, TG and LDL, they had a 100% passing rate for the measurement of total cholesterol, 99% for the measurement of triglycerides and HDL and 98.2% for the estimation of LDL by the Friedewald formula. Table 1 summarizes the average performance data from these laboratories across these six samples.

Table 1

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Bias (%)</th>
<th>CV (%)</th>
<th>TE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol</td>
<td>1.2</td>
<td>1.6</td>
<td>4.3</td>
</tr>
<tr>
<td>HDL Cholesterol</td>
<td>-3.0</td>
<td>5.6</td>
<td>14.0</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>-1.2</td>
<td>5.7</td>
<td>12.5</td>
</tr>
<tr>
<td>LDL (calculated)</td>
<td>4.9</td>
<td>3.9</td>
<td>12.5</td>
</tr>
<tr>
<td>LDL (direct)</td>
<td>5.3</td>
<td>6.7</td>
<td>18.4</td>
</tr>
<tr>
<td>Apoprotein B</td>
<td>-1.3</td>
<td>6.0</td>
<td>13.5</td>
</tr>
<tr>
<td>Apoprotein A-1</td>
<td>-1.4</td>
<td>7.1</td>
<td>15.9</td>
</tr>
</tbody>
</table>

The lowest total error for the estimation of LDL was obtained using the laboratories reported results for TC, TG and HDL and the Friedewald formula. In Figure 1, the lipid results from each laboratory were used to calculate LDL according to Friedewald and the calculated LDL was then compared to the BQ-LDL reference value. It should be pointed out that the triglycerides levels in these samples were not elevated and as such the...
superior performance of the calculated LDL may not extrapolate to samples with higher levels of triglycerides.

Apolipoprotein B had a lower measurement error associated with it than did the direct measurement of LDL. The measurement of LDL (direct method) had an average positive bias of 5% whereas the average bias for the measurement of apolipoprotein B was -1.3%. This may reflect a difference between these two methods with respect to the transfer of “trueness” from the “in-house” calibration processes to field methods. These two methods had virtually the same precision and \( r^2 \) with respect to their reference values (see Figures below).

The direct LDL method had a better correlation \( (r^2 = 0.8581) \) with BQ-LDL than did apoprotein B \( (r^2 = 0.7355) \).

The measurement of apoprotein A-1 had a higher total error (15.9%) and imprecision (CV = 7.1%) associated with it than did the routine measurement of HDL. HDL cholesterol was also better correlated with the reference value for HDL cholesterol.
Discussion

There are approximately 300 clinical laboratories that subscribe to this proficiency-testing program. It was interesting to note that, of these, there were only 18 laboratories that were measuring apoprotein A-1, B and direct LDL (using a direct method). The quality of routine lipid testing (TC, HDL, TG) in these laboratories was better than average and it is likely that their performance for the measurement of apoprotein A-1, B and direct LDL is a true reflection of the field performance of these methods in routine clinical laboratories. In samples without elevated triglycerides a calculated LDL would appear to provide the best estimation of LDL cholesterol.

It is often assumed that once a method has been standardized that the benefits will automatically transfer to and be reflected in field methods. Proficiency testing programs that use commutable test samples with reference values assigned by credentialed reference methods are essential for confirming that the standardization process has successfully transferred to the field and that the test results as reported by the clinical laboratory are in fact accurate (“true”) and traceable to the defined accuracy base.
The impetus to reach consensus about the clinical utility of various “emerging” cardiovascular disease (CVD) risk factors comes from 1) the inability of conventional risk factors to fully account for the observed incidence of cardiovascular disease and 2) the long list of biomarkers exhibiting an association with CVD and thus having the potential to improve primary risk prediction. The conventional risk factors include two lipoproteins, LDL and HDL, which have a central position in current guidelines because their causative contributions to atherosclerotic disease are well understood and accepted. LDL and HDL are quantified in routine practice by measuring the amount of cholesterol they contain: LDL-C and HDL-C. The implicit assumption is that LDL-C and HDL-C provide an accurate assessment of these atherogenic and antiatherogenic lipoprotein particles and account fully for the CVD risk these lipoproteins confer. With this as a starting point, it is logical and reasonable that the primary criterion used by the NACB Panel for assessing the clinical relevance of the emerging risk factors is whether they contribute to risk “beyond LDL and HDL,” as shown by having independent relations with CVD in multivariable analyses adjusted for LDL-C and HDL-C.

What are the implications of LDL-C and HDL-C not providing an accurate measure of LDL and HDL in all patients?

Measuring the cholesterol contained within LDL and HDL is but one way to quantify these lipoproteins and their associated CVD risk. Measurement of the protein moiety(ies) of the particles provides an alternative basis of quantification: apoB for LDL and apoA-1 for HDL. NMR spectroscopy provides a third means of quantification, in effect “counting” numbers of LDL and HDL particles. The existence of alternative measures of LDL and HDL, which are not necessarily equivalent analytically or clinically, invites two important questions that deserve consideration by the NACB:

1) Are LDL-C and HDL-C the best laboratory measures of LDL and HDL and the CVD risk these lipoproteins confer?

2) Does achieving LDL-C and HDL-C treatment goals ensure that all patients have achieved adequate LDL lowering and HDL raising?

These questions may be beyond the scope of the 2006 Beckman Conference, since they do not relate to “emerging” risk factors, but to alternative ways to quantify established risk factors. Different criteria need to be used to evaluate their clinical utility, since they play important roles in patient management as targets of treatment, not just as primary risk predictors. Viewed in this light, we propose that apoB and apoA-1 be removed from the list of emerging risk factors and that consideration of the above questions be deferred to a later date. We also suggest that the NACB Panel consult with the AACC LVDD about this proposal. If it is decided to address at the Conference questions related to potential alternatives to LDL-C and HDL-C for use in risk assessment and (especially) patient management, consideration must include not only apoB and apoA-1 but also NMR measures of LDL and HDL particle number.

Additional Comments

Remaining comments are restricted to LDL particle number (assessed by NMR or apoB) and are made to give visibility to possible confounding of data from outcome studies relating emerging biomarkers to CVD. It is well documented that the cholesterol content of LDL particles varies between individuals because of differences in particle size as well as relative content of cholesterol ester and triglycerides. When triglycerides are elevated, LDL-C generally underestimates the number of LDL particles and, arguably, LDL-related CVD risk. As a consequence, it is at least plausible that the observed elevated CVD risk of individuals with elevated triglycerides derives less (or not at all) from the triglycerides, and more from high LDL
particle numbers that are not reflected by the measured LDL-C (since the LDL particles are smaller and relatively cholesterol-poor). The relation of triglycerides with CVD is thus potentially confounded by the association of triglycerides with LDL particle number. Employing multivariable analyses adjusted for LDL-C, but not LDL particle number, may misleadingly give the impression that triglycerides are a more important risk factor than they actually are.

This issue is being raised because many biomarkers are associated with the phenotype of LDL-C providing an underestimate of LDL particles and the CVD risk these particles confer. A partial list of these biomarkers is given below:

- Triglycerides (high)
- HDL-C (low)
- LDL size (small)
- VLDL cholesterol (high)
- Non-HDL-C (high)
- Obesity (high BMI; high waist)
- Diabetes/glucose
- Insulin (high)
- CRP (high)
- Lp-PLA₂ (high)

All of these biomarkers have significant associations with CVD risk and are considered to contribute to risk “beyond LDL” because they are independent of LDL-C. But in most cases, we do not yet know the extent to which they truly confer risk beyond LDL because the appropriate analyses adjusting for LDL particle number have not been performed (HDL-C and diabetes are confirmed to be related to CVD independent of LDL particle number). It is possible that with the use of alternative measures of LDL that more completely account for all LDL-related risk, fewer additional risk factors may be needed to optimally assess and manage CVD risk, thereby simplifying clinical practice and improving adherence to guidelines.
Comments on the NACB Draft Guidelines on Emerging Biomarkers of Cardiovascular Disease, Part 2

James Otvos and John Contois
LipoScience, Inc.

Organization of Lipoprotein Topics

The Lipoprotein section of the draft guideline document gives separate consideration to 3 topics: lipoprotein subclasses and particle size, apolipoproteins A-1 and B, and lipoprotein(a). We believe that the evidence for the potential clinical value of these markers would be more easily evaluated and understood by explicitly distinguishing between two categories of markers:

1. emerging markers of cardiovascular disease, which may enhance primary risk prediction when added to traditional risk factors, and

2. emerging markers of the quantity of LDL and HDL, which have potential utility as alternatives or adjuncts to the traditional markers (LDL-C and HDL-C) for assessing risk and as treatment targets of LDL-lowering and HDL-raising therapies.

Lipoprotein subclasses/particle size and lipoprotein(a) fit logically into the first category of emerging risk markers. ApoB and apoA-1 belong in the second category as alternative quantitative measures of LDL and HDL, respectively.

We think it is conceptually more clear to consider the evidence for apoB and apoA-1 independently as markers of atherogenic and antiatherogenic lipoproteins, rather than tying them together in a discussion of “apolipoproteins” versus “lipids.” Laboratories can measure both, so the subject should not be framed as having to choose one and give up the other, but around their specific analytic and clinical advantages for the uses to which they are put in clinical practice. We recommend consideration of the evidence for apoB and apoA-1 in separate chapters devoted to “Alternative Quantitative Measures of LDL” and “Alternative Quantitative Measures of HDL.”

Evaluation of combinations of lipoprotein markers (i.e., ratios) should also be considered separately and not from the perspective of “lipids” versus “apolipoproteins.” It should be kept in mind that ATP III did not advocate use of LDL/HDL ratios for risk assessment, presumably because these variables are already combined in the Framingham Risk Score. Nor were ratios recommended as treatment targets. One of the challenges in evaluating data for the apoB/apoA-1 ratio is lack of comparative data for “mixed ratios” such as apoB/HDL-C and any clear indication about whether enhanced prediction given by the various ratios is due to the numerator, the denominator, or an interaction between the two.

2. LDL Particle Number is Separate and Distinct From LDL Subclasses/Size

Some confusion appears to exist about where LDL particle number fits into the biomarker discussion. The introduction to the draft guidelines states that the NACB panel selected “lipoprotein subclasses and particle concentration” among the biomarkers to be considered. But, Chapter 3 in the full draft document is entitled “Lipoprotein Subclasses and Particle Size and CVD Risk” and makes no mention of particle number in the recommendations. Peter Wilson’s presentation at the Beckman Conference was titled “Lipoprotein Particle Size and CVD Risk”, but included many slides showing relations of LDL particle number with CVD.

The likely reason for the confusion is that one analysis method, NMR spectroscopy, produces both subclass/size information as well as LDL (and other lipoprotein) particle numbers, and many published studies with CVD endpoints have include both types of data. To alleviate the confusion, we urge that a clear distinction be made between markers of lipoprotein quantity (LDL and HDL particle number) and quality (lipoprotein subclass distribution or particle size).

LDL and HDL particle numbers (LDL-P and HDL-P) are simply alternative quantitative measures of LDL and HDL, just as are apoB and
apoA-1. A consideration of the available evidence addressing comparative relations of CVD with LDL-C, LDL-P, and apoB should be given in one section, with HDL-C, HDL-P, and apoA-1 being compared in a separate section.

Evaluation of the potential clinical utility of lipoprotein subclass/size biomarkers should be undertaken in a separate section with the issue framed as to whether markers of lipoprotein “quality” enhance risk prediction compared to that given by traditional or emerging markers of lipoprotein “quantity.” Concerning specifically the association of small LDL size with CVD risk, it is important to appreciate that at a given level of LDL cholesterol, persons with small LDL have higher numbers of LDL particles than those with large LDL. So, it is unclear whether the higher risk of persons with small LDL is due to particle size or to particle number (or both). Evidence for the independent contribution of LDL size to CVD risk must come from multivariate analyses that have been adjusted for LDL quantity as assessed by LDL-P or apoB.

3. Linkage of the Evidence with the Clinical Application

In Tom Pearson’s introductory overview at the Beckman Conference, he included the following objective: “Consider specific applications of the markers in clinical practice and the evidence supporting those applications.” In the context of LDL and HDL markers of CVD risk, we believe it is important to make a distinction between evidence supporting use of the marker for risk assessment (identifying persons at high risk for CVD) as opposed to risk management (identifying persons with acceptably low “residual” risk after treatment has been initiated).

In the case of LDL, evidence supporting the risk assessment application would logically include data showing that the alternative marker of LDL quantity (apoB or LDL-P) adds prediction to the Framingham Risk Score, or that risk is better predicted when apoB or LDL-P is substituted for LDL-C in a multivariate risk assessment model. Our assessment of the available literature agrees with that of the NACB Panel in indicating there is substantial evidence that LDL particle number (assessed by NMR or apoB) is at least equal to LDL cholesterol as a marker of high CVD risk. We agree that the evidence at this time does not support replacement of LDL cholesterol by LDL particle number for this risk assessment application, but there is certainly no justification for a category III recommendation against its measurement. In selected patients at moderate risk for whom LDL cholesterol is likely to underestimate LDL particle number and its associated CVD risk (such as those with hypertriglyceridemia and/or metabolic syndrome), measurement of apoB or LDL-P would help assign the level of risk more precisely and provide a better baseline indication of the required magnitude of LDL lowering.

Evidence relating to the use of alternative measures of LDL for risk management (as treatment targets) should focus not on how well high levels of LDL predict the presence of CVD risk, but on how well low levels of the various LDL markers predict the absence of CVD risk. A review of the literature indicates that when levels of LDL cholesterol are not elevated (<130 mg/dL), CVD risk is related substantially more strongly to measures of LDL particle number (assessed by apoB or NMR) than to LDL cholesterol. It follows that LDL particle number would provide a more discriminating index than LDL cholesterol of the adequacy of LDL lowering therapy. Clinical understanding and acceptance of LDL particle treatment targets should benefit from the known mechanism of statin action, which is to lower LDL particle concentrations by increasing the rate of particle removal from the circulation, which results in lower levels of LDL cholesterol.

The draft guidelines regarding LDL management (Recommendation 3) are, in our opinion, unnecessarily restrictive. A category IIb; level C recommendation is given for the measurement of apoB as an alternative to non-HDL cholesterol to monitor efficacy of lipid lowering therapy in patients with elevated triglycerides. We suggest a revision of the recommendation to at least category IIa; level A with wording to the effect that measure-
4. Additional Publications for Consideration by the Panel

**LDL subclasses/size**

**LDL Particle Number**
Otvos JD, Jeyarajah EJ, Cromwell WC. Measurement issues related to lipoprotein heterogeneity. Amer J Cardiol 2002;90(suppl):22i-29i.


Cromwell WC, Otvos JD. Heterogeneity of Low Density Lipoprotein Particle Number in Patients with Type 2 Diabetes Mellitus and Low Density Lipoprotein Cholesterol <100 mg/dL. Am J Cardiol 2006;98:1599-1602.
Special Considerations in Four Method Comparison

Kenneth French

There were several points made and questions asked that were, unfortunately, left unresolved at the NACB conference this past October.

A question posed by the NACB group was, “Are there any head to head studies that show the four techniques in agreement?” The NACB group looked only at one limited study before drawing a conclusion that there is no good agreement on particle size/density classification between ultracentrifugation, gradient gel electrophoresis (GGE) and nuclear magnetic resonance (NMR).1 Earlier head-to-head comparisons such as Dorman’s comparison of single spin density gradient ultracentrifugation to (GGE) were omitted, as were other studies.2 Even more relevant earlier work correlating the three different methodologies’ results to clinical findings was omitted from the NACB report. A prospective trial by Greg Brown3 showing high agreement between the three major technologies was accepted by the AHA in 2003. Tube Gel Electrophoresis (TGE) was not considered in this particular study. When treatment with simvastatin and niacin moved coronary heart disease patients into the fourth quartile (Q4) for large/buoyant LDL by any of the three major methodologies’ testing, regression (a negative % change in stenosis (S)) was achieved and these findings were highly significant.3

The Ensign et al. study1 referred to earlier, comparing the results of four methods and their pattern differentiation, will be the focus of this paper. There are several fundamental concerns with the study design of which one should be aware.

1. All of the technologies outlined in the Ensign et al. paper measure LDL pattern and the outcomes of the different sizes are based on defined cut points that are slightly different between the four groups. The difference in nomenclature is not related to the technologies’ inability to measure LDL-size.

2. This study only compared 34 of the 40 samples that were analyzed with the different technologies.

3. NMR does not report A/B pattern, and TGE does not report pattern but % per fraction; the authors therefore created their own designation scheme to decipher the TGE results. Quantimetrix Corporation (Redondo Beach, CA) has defended their position before the NACB panel on this concern, as they believed they were misrepresented in this study.

4. The Ensign et al. paper stated “apparently healthy” patients were chosen, but close inspection of the data contradicts this premise, as what can be seen are several cholesterol results that are abnormal according to the cutoffs established by the National Cholesterol Education Program (NCEP) Adult Treatment Program III (ATP III) guidelines. A problem with the types of patients selected for this study is that these patients represent a population of individuals with the greatest LDL diversity and change. For example, patients who do not have “clinical signs” of overt metabolic syndrome or diabetes can still be developing insulin resistance; this can be seen in patients with Triglycerides >80 mg/dL and/or HDL <40 mg/dL. This means most of these patients should be A/B or B pattern LDL, the phenotype seen in most of the Ensign patients. A review of the work done by Austin et al.4 quickly shows why this population selected by Ensign should be mostly A/B or B. As seen below, triglycerides >150 mg/dL are an easy way to determine Pattern B, but >50% of patients with triglycerides between ∼75 mg/dL and 160 mg/dL have Pattern B LDL, or at least Pattern A/B, representing a state of change from Pattern A towards Pattern B. Krauss reported a significant percentage of pattern B patients with normal triglycerides in the SCRIP study in Circulation in 1996.5 Indeed, a study at Hopkins of an African American population found that 20% of pattern B patients had normal triglycerides, as did a study by Benton and Hanak in a more general population.6,7
Acknowledging Austin’s data (figure), patients with triglycerides >75 mg/dL should indicate a pattern shift towards Pattern B or Pattern A/B. In the Ensign data set, 29 of the 34 patients had a triglycerides value >75 mg/dL. Using the same scheme of elevated triglycerides >75 mg/dL, of the 29 patients with elevated triglycerides, 17 out of the 29 indicated an abnormal pattern size of either Pattern A/B or B in all technology groups excluding TGE. This results in a homology of 59% of samples comparable across the board and is dramatically different than the homology of only 8% as re-reported in the Ensign article. On a side note, it is interesting to see how well the three technologies matched in six patients by defining the LDL subclass as abnormal, either A/B or B, using the ATP III guidelines definition for atherogenic dyslipidemia.

Another consideration should be given the data by Ensign et al., that is, if one is allowed to lump Pattern A/B with Pattern A or Pattern B, one will see a very different outcome. It should be understood that just because the pattern was reported A/B, that does not mean it was not very close to the cut point for pattern A or B. Using these criteria and excluding NMR results (as the technique does not report an intermediate small/dense or A/B pattern), the VAP and GGE results match on 28 of the 34 samples. This is >80% homology as opposed to 8%. Again, as stated earlier, this is a difference in definitions, not measurements.

These findings do leave some differences between the technologies, and this could be a resolution issue with the GGE and NMR techniques. NMR does not appear to be able to resolve Lp(a) or IDL well; while GGE can separate out Lp(a) from the LDL, it appears that IDL interference with overall LDL distribution is problematic. Because ultracentrifugation utilizes two physical properties to separate LDL particles by both density and size, it is theoretically likely to be a more accurate means of particle size/density separation than other methods relying upon a single physical attribute or chemical property for separation. It should be made known that these differences are small, and the “global” analysis of CAD risk would more than likely lead a physician to the same end point in risk assessment, independent of the test used.

5. Ensign et al. made a comparison of the three methods based on differing definitions of Pattern A or B, and did not compare the quantitative individual subclass measurements, which would have been ideal. For example, what the VAP method called Pattern A/B based on the peak particle size could have been classified as Pattern B using other definitions, such as high LDL3 and LDL4 with GGE. All three tests are correct, but the research design did not allow for this heterogeneity, rather the conclusion appeared to be that patients are either all large, medium, or small size LDL with no “in-between,” which is not what is seen in a normal population distribution.

6. The authors also compared the LDL-C measurements made by these tests; the serious concern with this approach is that NMR and GGE do not measure cholesterol. Whereas VAP direct LDL cholesterol measurement has repeatedly been validated by beta quantification as the gold standard for LDL cholesterol measurement, the other methods produce an LDL value that is calculated using various algorithms, not a measured LDL cholesterol. Therefore, this comparison is inappropriate as it is not a true one-to-one comparison.

7. It is well understood that one should never view pattern B by itself clinically; rather, it should be viewed as a part of the whole in cardiovascular heart disease (CHD) risk assessment. Rather than focusing on pattern A/B outcome, selection should have been based on a more heterogeneous population, i.e., Pattern A, A/B, and B. The patients selected for this study did not represent
the wide distribution seen with different LDL phenotypes. In order to evaluate the performance of different types of tests a wider range of samples should be used. To make an assumption of “apparently healthy” with no entry criteria negatively impacts the ability to draw a proper conclusion. The patients selected for this study appear to be those who would have the greatest LDL diversity.

In spite of the high percentage of pattern A/B, the casual comparison provided in this rebuttal letter suggests excellent agreement between VAP (density gradient ultracentrifugation) and GGE. Had a more comprehensive assessment of the tests been used, the overall conclusion for each patient would most likely be the same for all technologies.

One can understand Dr. Stein’s concerns, but full disclosure of the Ensign data might have provided a different story. However, there is agreement that the use of LDL subclasses should not be used for the purposes of screening, but reserved for “at-risk” patients; said screening should always begin first with the ATP III lipoprotein cholesterol targets, e.g., LDL and non-HDL cholesterol. One can appreciate the work done by Dr. Ensign et al., and hopes are high that full resolution will occur between all technologies so that this information can be used to address the heart disease epidemic in the U.S. and worldwide.

References:


To the NACB LMPG Committee Members:

We appreciate and congratulate you on the hard work and effort the committee has gone to in making its recommendations. We further understand how difficult it is to forge a guideline when the landscape of clinical information and scientific publications is evolving so rapidly. At the same time, we feel the need to encourage you to re-examine your position on Lp-PLA$_2$, which you have identified as one of the promising biomarkers for cardiovascular disease, but “had insufficient data to assess” clinical utility. We feel that the guideline report has prematurely dismissed Lp-PLA$_2$ from consideration for lack of study evidence when, in fact, coincident to the writing group’s tenure, a large number of important Lp-PLA$_2$ studies have emerged. In fact, the results for Lp-PLA$_2$ mass and/or activity as a CV risk predictor are positive for 21 of 22 extant epidemiological studies on Lp-PLA$_2$. The only negative study was a 123 patient/123 control case-control subset of the Women’s Health Study, conducted at the Brigham and Women’s Hospital (Blake et al., 2001). In addition to the consistency of positive findings regarding Lp-PLA$_2$, there is the important consideration that Lp-PLA$_2$ is the only serological biomarker with FDA clearance to be used as an aid in ascertaining risk of ischemic stroke. For these reasons and others listed below, we would like to review the recent literature and make some specific recommendations to the writing group.

Our first area of concern is that the report and Reference List do not reflect many of the recently published important studies demonstrating the clinical utility of Lp-PLA$_2$. In the report, only about half of the positive studies (12 of 21) of Lp-PLA$_2$’s association with cardiovascular risk are cited. To say that “most, but not all” Lp-PLA$_2$ studies are positive may give the wrong impression that there are many conflicting results when, in fact, only one study out of 22 was negative. Ten of the 22 Lp-PLA$_2$ epidemiology studies are in primary prevention populations. We are not aware of any cardiovascular inflammatory risk marker which has demonstrated such consistency of positive findings in the CV epidemiological literature. These studies have all been conducted over wide patient populations at a wide variety of academic institutions. Moreover, we have remained blinded to all of these studies and have consistently waived editorial rights. We submit that, even in advance of the additional studies and the ongoing meta-analysis identified in the draft guideline document, a compelling consistency of positive results has already been established.

Secondly, Lp-PLA$_2$ satisfies many clinical test criteria that no other inflammatory biomarker does. No other inflammatory biomarker has demonstrated statistical independence from all other traditional risk factors, including not only the Framingham risk factors (smoking, hypertension, low HDL, family history of premature CVD and age) but also insulin resistance. In addition, no other biomarker has shown independence from virtually all other inflammatory markers. No other biomarker has demonstrated such low bio-variability, comparable to the biovariability of commonly measured lipids, enabling one to follow Lp-PLA$_2$ over time. Lp-PLA$_2$ was the only inflammatory marker to demonstrate a statistically significant raising of the area under the curve (AUC), additive to traditional risk factors in receiver operating characteristic analysis in a recently published study on biomarkers from the large Atherosclerosis Risk in Communities (ARIC) primary prevention study, as well as in the secondary prevention from the KAROLA cohort and a recent study on Olmsted County residents, conducted by investigators at the Mayo Clinic. No other biomarker, including hsCRP, has shown consistency in its ability to predict cardiovascular disease in the elderly as has Lp-PLA$_2$, which was positive in both PROSPER and CHS. No other inflammatory biomarker has FDA labeling for use as an aid in the determination of risk of coronary events and ischemic stroke (because no other blood test has a clinical
It is important to consider that no inflammatory biomarker has such strong evidence for causality of cardiovascular events. The evidence for this includes:

1. Lp-PLA₂’s position as the enzyme solely responsible for hydrolysis of oxidized LDL and producing well-established inflammatory mediators lysophosphatidylcholine and oxidized free fatty acid.²⁶

2. Histopathological staining using antibodies for Lp-PLA₂ demonstrate staining intensity in advanced, rupture-prone plaques but not in early, stable plaques.²⁷

3. In vitro studies of human white blood cells spiked with oxidized LDL resulted in increased production of cytokines by the leukocytes. However, when a specific inhibitor of the Lp-PLA₂ enzyme was added, cytokine production by the leukocytes was abrogated.²⁹

Evidence has also been accumulating that Lp-PLA₂ has important predictive value for recurrent CV events, compared to other biomarkers, in high-risk populations. For example, in the PROVE IT-TIMI 22 study, an achieved LDL cholesterol of 62 mg/dL was still associated with a 22.4% risk of recurrent CV events. In contrast, an Lp-PLA₂ mass concentration of <200 ng/mL in the Mayo Heart Study,⁹ and a level below 223 ng/mL in KAROLA¹⁴ were associated with only a 5% risk of recurrent fatal and nonfatal MI and/or stroke. Although the endpoints are not identical, even considering only the combined endpoints of MI and cardiac death (death from CHD or MI) in PROVE IT, there are almost three times the 5% recurrence risk in the two cited Lp-PLA₂ studies. Thus, the negative predictive value of the Lp-PLA₂ biomarker and its low biovariability may make the marker useful to physicians trying to determine which high-risk persons, already treated to the ATP III optimal LDL cholesterol of 100 mg/dL, actually require even more aggressive treatment, because the plaque is still unstable.

Finally, stroke is the third biggest killer of Americans. One-third of strokes occur in persons aged 45-64 years old. More than 85% of strokes are ischemic. Stroke is preventable with lifestyle changes and statin treatment. Yet, LDL cholesterol has not been shown to be a robust predictor of stroke risk. Strong additivity of hypertension and even prehypertension, and Lp-PLA₂ were demonstrated in the ARIC study (PLAC test package insert, data on file with FDA). The ATP III Framingham risk calculator was developed for estimation of coronary event risk. It was not developed for and never intended to be used to estimate risk of stroke. Although an earlier Framingham stroke risk calculator has been promulgated, we know of no clinicians that utilize it. Therefore, Lp-PLA₂ fills a very important void in the early identification of stroke risk. In the ARIC study, persons in the top tertiles of systolic blood pressure (>130 mm Hg) and Lp-PLA₂ had a 6.8-fold increased risk of ischemic stroke compared to the lowest tertiles for blood pressure and Lp-PLA₂.

There are now four positive epidemiological studies for Lp-PLA₂ as a stroke risk predictor (ARIC, Rotterdam, VA-HIT and NOMAS).¹⁰,¹¹,²⁰,³⁰ To omit Lp-PLA₂ from the NACB emerging risk factor guideline may severely compromise adoption of the only serologic marker that is cleared by the FDA as an aid in ischemic stroke risk assessment.

With these points in mind, we make the following suggestions:

1. Amend the current one paragraph discussion of Lp-PLA₂, which implies that the test is novel and experimental with insufficient documented study evidence to support it, and fully reflect the predominantly positive data.

2. Revise the current reference list on Lp-PLA₂ by including all 22 epidemiological studies (references 12-24 in the original draft replaced with references 1 to 22, including titles, in the list below).

3. Incorporate into the final document an acknowledgement that, during the course of writing the current guideline, there has not been sufficient time to review the rapidly emerging study evidence around the Lp-PLA₂ biomarker; a critical mass of study evidence may have already been reached;
several major studies are anticipated shortly; and, therefore, a documented plan (also written into the guideline document) to reconsider Lp-PLA₂ for evaluation and recommendation will commence in 2007, upon having these new data available for review.

Again, we thank you for your invitation to respond and comment on these draft guidelines and sincerely appreciate your serious consideration of our position and recommendations.

Respectfully yours,

Robert L. Wolfert, Ph.D.
Richard B. Lanman, M.D.
diaDexus, Inc.
The following statement regarding lipoprotein subfractionation was issued from the U.S. Food and Drug Administration on December 6, 2006. The website for this summary can be accessed at the URL, http://www.fda.gov/cdrh/meetings/120606-summary.html.

Clinical Chemistry and Clinical Toxicology Devices Panel - December 6, 2006 (Summary)

On December 6, 2006, the Clinical Chemistry and Clinical Toxicology Devices Panel discussed general issues concerning lipoprotein (HDL and LDL) subfraction assays. The Panel addressed potential uses of the assays, potential impact on treatment, how to establish accuracy and determine reference ranges, and lack of standardization and any risk this may cause to patients. The Panel also considered the value of particle size versus particle number in assessing lipid subfractions.

In general, the Panel felt lipid subfractions have some utility in assessing a patient's risk of developing cardiovascular disease and in aiding in the diagnosis of dyslipidemia, with certain populations. However, they felt that the tests should be used in conjunction with other traditional risk assessment tools and clinical judgment.

Contact: Veronica Calvin, Executive Secretary, at 240-276-0491 ext 161 or veronica.calvin@fda.hhs.gov

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Freedom of Information Staff (HFI-35)
5600 Fishers Lane, Rockville, MD 20857
301-443-1726
Title: Are lipid-lowering guidelines evidence-based?
Authors: Abramson J, Wright JM.
Comment: This opinion article deals with an intriguing and critical question regarding the evidence behind current lipid lowering guidelines. The last major revision of the US guidelines (NCEP ATP III) in 2001 has increased the number of Americans for whom statins are recommended from 13 million to 36 million. Most of these subjects do not yet have but are estimated to be at moderately elevated risk of developing coronary heart disease. In support of statin therapy for the primary prevention of this disease in women and people aged over 65 years, the guidelines cite seven and nine randomized trials, respectively. Review of these studies led the authors to conclude that not one of them provides such evidence. Based on the lack of evidence, they suggest that lipid-lowering statins should not be prescribed for true primary prevention in women of any age and for men older than 69 years. Further, they propose that high-risk men aged 30-69 years should be advised that about 50 patients need to be treated for 5 years to prevent one event. Regarding the question “why the disagreement?”, they suggest that current guidelines are based on the assumption that cardiovascular risk is a continuum and that evidence of benefit in people with occlusive vascular disease (secondary prevention) can be extrapolated to primary prevention populations. This assumption, plus the assumption that cardiovascular risk can be accurately predicted, leads to the recommendation that a substantial proportion of the healthy population should be placed on statin therapy. They also suggest that similar assumptions underlie the conclusions of the Cholesterol Treatment Trialists’ (CTT) collaboration, a group that undertakes periodic meta-analyses of individual participants’ data on morbidity and mortality from all relevant large-scale randomized trials of lipid-modifying treatment. Based on the weakness of currently available evidence, the authors believe that the assumption that the benefits for secondary prevention populations can be extrapolated to primary prevention populations may be false and the cholesterol treatment guidelines based on this assumption may require revision.

Title: Inhibition of microsomal triglyceride transfer protein in familial hypercholesterolemia.
Comment: Patients with homozygous familial hypercholesterolemia have markedly elevated cholesterol levels, which respond poorly to drug therapy, and carry a very high risk of premature cardiovascular disease (CVD). This preliminary study explored a new therapy based on the use of an inhibitor of microsomal triglyceride transfer protein (BMS-201038). Six patients with homozygous familial hypercholesterolemia were enrolled into a dose-escalation study. All lipid-lowering therapies were suspended 4 weeks before treatment. The patients received BMS-201038 at four different doses (0.03, 0.1, 0.3, and 1.0 mg per kilogram of body weight per day), each for 4 weeks, and returned for a final visit after a 4-week drug-washout period. Analysis of lipid levels, safety laboratory analyses, and magnetic resonance imaging of the liver for fat content were performed throughout the study. All patients tolerated titration to the highest dose, 1.0 mg per kilogram per day. Treatment at this dose decreased LDL-C by 50.9% and apolipoprotein B levels by 55.6% from baseline (P<0.001 for both comparisons). Kinetic studies showed a marked reduction in the production of apolipoprotein B. The most serious adverse events included elevation of liver amino-transferase levels and accumulation of hepatic fat, which at the highest dose ranged from less than 10% to more than 40%. In summary, inhibition of the microsomal triglyceride transfer protein by BMS-201038 resulted in the reduction of LDL-C
in patients with homozygous familial hypercholesterolemia, owing to reduced production of apolipoprotein B. Practical application of this therapy may be limited, however, by substantial accumulation of hepatic fat and elevation of liver aminotransferase levels.

Title: Telomere length, risk of coronary heart disease, and statin treatment in the West of Scotland Primary Prevention Study: a nested case-control study.


Comment: Telomeres are TTAGGG DNA repeats at the ends of chromosomes. Because they shorten with every cell division, they are thought to be the internal biological clock of a living organism. Telomeres in human nucleated blood cells contain about 10,000-20,000 nucleotides at birth. Because DNA polymerase cannot fully complete the replication of the 3' end of linear DNA, telomeres are estimated to decrease by about 50 bp a year and are reduced to a few thousand bp in elderly individuals. Since shortened telomeres may be functionally deficient, the aim of this study was to determine whether inter-individual differences in telomere length and biological aging could affect susceptibility to coronary heart disease (CHD). The mean leukocyte telomere lengths were compared at recruitment in 484 individuals in the West of Scotland Primary Prevention Study (WOSCOPS) who went on to develop CHD events with those from 1058 matched controls who remained event-free. The authors also investigated whether there was any association between telomere length and observed clinical benefit of statin treatment in WOSCOPS. Mean telomere length decreased with age by 9% per decade (95% CI 3.6-14.1; P=0.001) in controls; much the same trend was seen in cases (-5.9% per decade, 95% CI -3.1 to 14.1; P=0.1902). Individuals in the middle and the lowest tertiles of telomere length were more at risk of developing a CHD event than were individuals in the highest tertile (odds ratio [OR] for CHD: 1.51, 95% CI 1.15-1.98; P=0.0029 in the middle tertile; OR 1.44, 95% CI 1.10-1.90, P=0.0090 in the lowest). In placebo-treated patients, the risk of CHD was almost double in those in the lower two tertiles of telomere length compared with those in the highest tertile (OR 1.93, 95% CI 1.33-2.80, P=0.0005 in the middle tertile; OR 1.94, 95% CI 1.33-2.84, P=0.0006 in the lowest). In contrast, pravastatin treatment substantially attenuated the increased risk with shorter telomeres (OR 1.12, 95% CI 0.75-1.69, P=0.5755 in the middle tertile; OR 1.02, 95% CI 0.68-1.52, P=0.9380 in the lowest). Based on these findings, the authors suggested that the mean leukocyte telomere length is a predictor of future CHD events in middle-aged, high-risk men and could identify individuals who would benefit most from statin treatment. Further, they believe that these findings lend support to the hypothesis that differences in biological aging might contribute to the risk and variability in age of onset of CHD.

Title: Can telomere length predict cardiovascular risk?

Authors: Spyridopoulosa I, Dimmelera S.


Comment: This comment about the study of Brouilette et al. (Lancet 2007;369:107-14) pointed out that critically short telomeres are assumed to have functional implications, such as the induction of cellular senescence that is characterized by the expression of specific markers of aging and the inability of the cell to divide. Although age is an important independent predictor for the development of cardiovascular disease, shortening of age-corrected telomere length in leukocytes exposes individuals to an additional substantial risk of mortality from cardiovascular and infectious complications. In the West of Scotland Primary Prevention Study (WOSCOPS), the use of pravastatin was tested in men with raised concentrations of LDL-C to prevent cardiovascular events. The recent substudy by Brouilette et al. has found that individuals with shortened telomere length in leukocytes exposes individuals to an additional substantial risk of mortality from cardiovascular and infectious complications. In the West of Scotland Primary Prevention Study (WOSCOPS), the use of pravastatin was tested in men with raised concentrations of LDL-C to prevent cardiovascular events. The recent substudy by Brouilette et al. has found that individuals with shortened telomere length have about a two-fold increased risk of developing coronary heart disease (CHD) in the 5 years from the start of treatment and pravastatin completely attenuates this telomere-attributed risk. Although the randomized case-control design of the study was
better than all previous studies on the association of telomere length and cardiovascular risk, several comments appeared to be warranted. The first is the potential mechanism behind the correlation between mean telomere length and CHD. Functional changes within cell populations coupled with higher inflammatory cytokine production, lessened repair of vessel wall, dysfunctional circulating endothelial progenitor cells, and increased oxidative stress are among candidate mechanisms. A second is the effect of statins on telomere-attributed risk. In addition to reducing cardiovascular risk as a consequence of lipid lowering, statins are known to exert multiple actions on vascular and inflammatory cells. Reduction of oxidative stress and protection of telomeres from shortening may be possible mechanisms but the present study did not give insight into this intriguing question. The third issue is the value of telomere length as an individual prognostic marker. The study by Brouilette et al. may have given the impression that telomere length can be used to identify individual patients at risk for coronary events, which is not the case. The high genetic variability of telomere length between individuals at birth prevents judgment on individual telomere length. Although the study convincingly showed that biological aging—as indicated by telomere shortening—could contribute to the risk of CHD, we do not know what the individual telomere length means. This is in contrast with current biomarkers (e.g., “high-sensitivity” C-reactive protein) that have distinct cut-off values. Nevertheless, the findings by Brouilette et al. should motivate the search for marker(s) for individual telomere shortening, those for which absolute numbers predict absolute risk.

**Title:** ANP T2238C, C-664G gene polymorphism and coronary heart disease in Chinese population.  
**Authors:** Zhang L, Cheng L, He M, Hu B, Wu T.  
**Comment:** There is growing evidence that atrial natriuretic peptide (ANP) plays an important part in coronary blood flow regulation and in atherosclerosis. Transition T2238 → C in the atrial natriuretic peptide (ANP) precursor gene, which leads potentially to the translation of ANP with 2 additional arginines, has been suggested to be associated with salt-sensitive hypertension. Gruchala et al. (Am Heart J 2003;145:125-31) were the first to describe an association of the Scal ANP gene polymorphism (T2238C) with the history of non-fatal myocardial infarction and the extent of coronary heart disease (CHD) in a Caucasian population. Authors of the present article further studied this relationship in a Chinese population. They detected genotypes of ANP T2238C and ANP C664G by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) methods in 158 consecutive CHD patients and 165 controls. They found that the distribution of A2A2 T2238C genotype in CHD group was significantly higher than that in control group (P<0.05). According to stepwise logistic regression analysis, male gender, smoking, history of hypertension, history of diabetes, family history of hypertension, high level of serum cholesterol, and ANP T2238C polymorphism were the possible risk factors in patients with CHD (P<0.05). However, they found no significant difference between the patients with CHD and the control group in the distribution of ANP C664G polymorphism (P>0.05). The results suggest that, similar to Caucasians, the A2A2 T2238C genotype could be one of the risk factors for CHD (P<0.05, odds ratio 1.80, 95% CI: 1.03-3.15) in Chinese subjects. The precise mechanism of this association, however, remains to be determined.  

**Title:** High plasma level of remnant-like particles cholesterol in familial combined hyperlipidemia.  
**Authors:** de Graaf J, van der Vleuten GM, Ter Avest E, Dallinga-Thie GM, Stalenhoef AF  
**Journal:** J Clin Endocrinol Metab 2007; Jan 16 [Epub ahead of print]  
**Comment:** The traditional lipid and lipoprotein levels in patients with familial combined hyperlipidemia (FCH) are relatively mildly elevated and do not fully explain the increased risk of CVD. In other populations, high remnant-like particle cholesterol (RLP-C) levels are an independent risk factor for CVD. This paper investigates whether plasma RLP-C concentrations are elevated in patients with FCH and contribute to the increased
prevalence of CVD. This is a cross-sectional study examining RLP-C levels in 37 FCH families comprising 582 subjects, of whom 134 subjects were diagnosed FCH based on total cholesterol, triglycerides and apoB levels. Plasma RLP-C concentrations were determined using an immunoseparation technique. They report that for both men and women, the mean plasma RLP-C concentration (mmol/L) was two-fold elevated in FCH patients (0.59 (0.54-0.66) and 0.40 (0.37-0.43), respectively) compared to both normolipidemic relatives (0.27 (0.26-0.29) in male and 0.22 (0.21-0.23) in female, all \( P<0.05 \)) and spouses (0.27 (0.23-0.31) in male and 0.24 (0.21-0.27) in female, all \( P<0.05 \)). Plasma RLP-C levels above the 90th percentile predicted prevalent CVD, independently of non-lipid cardiovascular risk factors (OR 2.18 [1.02-4.66]) and independently of triglyceride levels (OR 2.35 [1.15-4.83]). However, in both FCH patients and controls, RLP-C did not provide additional information about prevalent CVD over and above non-HDL cholesterol levels. The authors conclude that patients with FCH have two-fold elevated plasma RLP-C levels, which add to the atherogenic lipid profile and contribute to the increased risk for CVD. However, for clinical practice, non-HDL cholesterol is the best predictor of prevalent CVD.

Title: Anti-bis(monoacylglycero)phosphate antibody induces the accumulation of acetylated-low density lipoprotein-derived cholesterol in cultured macrophages.

Authors: Delton-Vandenbroucke I, Bouvier J, Makino A, Besson N, Pageaux JF, Lagarde M, Kobayashi T.


Comment: Bis(monoacylglycero)phosphate (BMP), also called lysobisphosphatidic acid (LBPA), is a phospholipid highly enriched in the internal membranes of multivesicular late endosomes, in which it forms specialized lipid domains. It has been suggested that BMP-rich membranes regulate cholesterol transport. In this paper, the authors examine the effect of an anti-BMP antibody on cholesterol metabolism and transport in two macrophage cell lines, RAW 264.7 and THP-1, during loading with acetylated LDL (acLDL). Anti-BMP antibody was internalized and accumulated in both macrophage cell types. Cholesterol staining with filipin and mass measurements indicated that acLDL-stimulated accumulation of free cholesterol was enhanced in macrophages that had accumulated the antibody. Unlike the hydrophobic amine, U18666A, esterification of acLDL-derived cholesterol by ACAT was not modified after anti-BMP treatment. AcLDL loading led to an increase of free cholesterol in the plasma membrane. This increase was further enhanced in anti-BMP-treated macrophages. However, cholesterol efflux to high density lipoproteins (HDL) was reduced in antibody-treated cells. The present results suggest that the accumulation of anti-BMP antibody alters cholesterol homeostasis in acLDL-loaded macrophages.

Title: Group V secretory phospholipase A2 promotes atherosclerosis. Evidence from genetically altered mice.

Authors: Bostrom MA, Boyanovsky BB, Jordan CT, Wadsworth, MP, Taatjes DJ, de Beer FC, Webb NR.


Comment: Group V secretory phospholipase A2 (GV sPLA2) has been detected in both human and mouse atherosclerotic lesions. This enzyme has potent hydrolytic activity toward phosphatidylcholine-containing substrates, including lipoprotein particles. Numerous studies in vitro indicate that hydrolysis of high density lipoproteins (HDL) and low density lipoproteins (LDL) by GV sPLA2 leads to the formation of atherogenic particles and potentially proinflammatory lipid mediators. However, there is no direct evidence that this enzyme promotes atherogenic processes in vivo. The authors performed gain-of-function and loss-of-function studies to investigate the role of GV sPLA2 in atherogenesis in LDL receptor-deficient mice. Compared with control mice, animals overexpressing GV sPLA2 by retrovirus-mediated gene transfer had a 2.7-fold increase in lesion area in the ascending region of the aortic root. Increased atherosclerosis was associated with an increase in lesional collagen deposition in the same region. Mice deficient in bone marrow-derived GV sPLA2
had a 36% reduction in atherosclerosis in the aortic arch/thoracic aorta. The data in these mouse models provide the first in vivo evidence that GV sPLA2 contributes to atherosclerotic processes, and draw attention to this enzyme as an attractive target for the treatment of atherosclerotic disease.

**Title:** A novel compound, R-138329, increases plasma HDL cholesterol via inhibition of scavenger receptor BI-mediated selective lipid uptake.

**Authors:** Nishizawaa T, Kitayamaa K, Wakabayashib K, Yamadac K, Uchiyamac M, Abec K, Ubukataa N, Inabad T, Odac T, Ameniya Y.

**Journal:** Atherosclerosis 2006; Dec 12 [Epub ahead of print].

**Comment:** In this paper, the authors describe a new compound that elevates the plasma HDL cholesterol (HDL-C) levels and which could be a promising anti-atherosclerotic agent. They examined a novel compound, R-138329, that increased HDL-C by 41% in normolipidemic hamsters at a dose of 100 mg/kg. To investigate the mechanism of action of R-138329, they examined the effect of R-138329 on the clearance of [3H]cholesterol ether ([3H]COE)-labeled and [125I]-labeled HDL in mice. R-138329 delayed the clearance of [3H]COE-labeled HDL and reduced accumulation of tracer HDL in the liver, whereas the clearance of [125I]-labeled HDL particles was unaffected by the compound. In vitro analysis showed that R-154716, a metabolite of R-138329, dramatically inhibited the uptake of [3H]COE-labeled HDL in McA-RH 7777 rat hepatoma cells. Furthermore, 100 nM of R-154716 completely inhibited [3H]COE-labeled HDL uptake induced by overexpression of scavenger receptor BI (SR-BI) in HEK293 cells. These findings suggest that the mechanism by which R-138329 elevates HDL-C in vivo is principally involved in the inhibition of SR-BI-mediated selective lipid uptake in the liver.

**Title:** Fcγ receptor deficiency confers protection against atherosclerosis in apolipoprotein E knock-out mice.


**Comment:** IgG Fc receptors (FcγRs) play a role in activating the immune system and in maintaining peripheral tolerance, but their role in atherosclerosis is unknown. The authors in this paper generated double-knockout (DKO) mice by crossing apolipoprotein E-deficient mice (apoE–/–) with Fcγ chain-deficient mice (γ–/–). The size of atherosclerotic lesions along the aorta was approximately 50% lower in DKO compared with apoE–/– control mice, without differences in serum lipid levels. The macrophage and T-cell content of lesions in the DKO were reduced by 49±6% and 56±8%, respectively, compared with the content in apoE–/– lesions. Furthermore, the expression of monocyte chemoattractant protein-1 (MCP-1), RANTES (Regulated on Activated Normal T-cell Expressed and Secreted), and intercellular adhesion molecule-1 (ICAM-1) and the activation of nuclear factor-κB (NF-κB) were significantly reduced in aortic lesions from DKO mice. In vitro, vascular smooth muscle cells (VSMCs) from both γ–/– and DKO mice failed to respond to immune complexes, as shown by impaired chemokine expression and NF-κB activation. ApoE–/– mice have higher levels of activating FcγRI and FcγRIIIA, and inhibitory FcγRIIB, compared with wild-type mice. The DKO mice express only the inhibitory FcγRIIB receptor. In conclusion, the authors predict that the FcγR deficiency limits development and progression of atherosclerosis. Furthermore, the expression of monocyte chemoattractant protein-1 (MCP-1), RANTES (Regulated on Activated Normal T-cell Expressed and Secreted), and intercellular adhesion molecule-1 (ICAM-1) and the activation of nuclear factor-κB (NF-κB) were significantly reduced in aortic lesions from DKO mice. In vitro, vascular smooth muscle cells (VSMCs) from both γ–/– and DKO mice failed to respond to immune complexes, as shown by impaired chemokine expression and NF-κB activation. ApoE–/– mice have higher levels of activating FcγRI and FcγRIIIA, and inhibitory FcγRIIB, compared with wild-type mice. The DKO mice express only the inhibitory FcγRIIB receptor. In conclusion, the authors predict that the FcγR deficiency limits development and progression of atherosclerosis. In addition to leukocytes, FcγR activation in VSMCs contributes to the inflammatory process, in part, by regulating chemokine expression and leukocyte invasion of the vessel wall. These results underscore the critical role of FcγRs in atherogenesis and support the probable use of immunotherapy in the treatment of this disease.

**Title:** Angiotensin II type 1-receptor antagonism prevents type IIA secretory phospholipase A2-dependent lipid peroxidation.
Authors: Luhtefeld M, Bandlow N, Tietge UJ, Grote K, Pfeilschifter J, Kaszkin M, Beck S, Drexler H, Schieffer B.


Comment: Accumulation and modification of low density lipoproteins (LDL) within the vessel wall represent key events in atherogenesis. Secretory phospholipase A\(_2\) type IIA (sPLA\(_2\)-IIA) modulates the enzymatic process of LDL-modification and was recently identified as an independent predictor of coronary events in patients with coronary artery disease (CAD). Angiotensin II (ANG II) type 1 (AT\(_1\))-receptor blockade reduces LDL-modification and atherosclerotic plaque formation in rodent and primate models of atherosclerosis. Therefore, this paper assessed whether ANG II via its AT\(_1\)-receptor enhances sPLA\(_2\)-IIA-dependent lipid peroxidation in vitro and in patients with CAD. Stimulation of rat aortic smooth muscle cells with ANG II (10\(^{-7}\) mol/L) enhanced sPLA\(_2\)-IIA protein expression, activity as well as LDL-peroxidation, determined by western blot, activity assay and malondialdehyde (MDA)-assay and diene formation, respectively, and were blunted by AT\(_1\)-receptor blockade (Losartan, 10\(^{-5}\) mol/L). In addition, ANG II-induced sPLA\(_2\) activity and LDL-peroxidation were abolished by the sPLA\(_2\)-IIA activity inhibitor LY311727 (10\(^{-5}\) mol/L). To evaluate a potential clinical implication, patients (n=18) with angiographically documented CAD were treated with the AT\(_1\)-receptor blocker Irbesartan (IRB; 300 mg/d) for 12 weeks. Blood samples were obtained from patients pre- and post-treatment and from healthy volunteers. sPLA\(_2\)-IIA serum level and activity, circulating antibodies against oxidized LDL (oxLDL), oxLDL and MDA were determined in patients and found to be significantly increased compared to healthy volunteers. IRB therapy reduced these markers of inflammation, whereas total cholesterol, HDL- and LDL-fractions remained unchanged. ANG II may elicit pro-atherosclerotic effects via type IIA sPLA\(_2\)-dependent LDL-modifications. Chronical AT\(_1\)-receptor blockade reduces sPLA\(_2\)-IIA level and activity and subsequently lipid peroxidation. These findings represent a novel anti-atherosclerotic mechanism and imply that AT\(_1\)-receptor blockade elicits anti-atherosclerotic potencies even in the absence of plasma cholesterol reduction.