**UTILITY OF ADVANCED LIPOPROTEIN TESTING IN CLINICAL PRACTICE**

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**ABSTRACT**

A standard lipid panel includes total cholesterol, triglycerides, and HDL-C. LDL-C can then be calculated. While the Friedewald formula is the classical method to calculate LDL-C levels recently developed formulas such as Martin Hopkins formula or Sampson-NIH formula are more accurate when triglycerides are elevated and/or LCL-C levels are low. In some instances, a direct LDL-C assay is employed, particularly when the triglyceride levels are elevated (>400mg/dL). Non-HDL-C can also be calculated (non-HDL-C = total cholesterol – HDL-C). Increasing levels of LDL-C and non-HDL-C are associated with an increased risk of atherosclerotic cardiovascular disease (ASCVD). However, numerous studies have demonstrated that the association of non-HDL-C with ASCVD is more robust. It is possible to measure apolipoprotein B and A-I levels, LDL and HDL size, LDL and HDL particle number, and Lp(a). Numerous studies have documented a link between small dense LDL particles and an increased risk of ASCVD; however, the association is markedly reduced or entirely eliminated when the analyses are adjusted for other factors that affect ASCVD risk. Similarly, there is little data demonstrating that HDL subfractions are useful in risk prediction beyond HDL and other traditional risk factors. Apolipoprotein B levels and LDL particle number are more strongly associated with ASCVD than LDL-C, particularly when the levels of LDL-C and apolipoprotein B levels or LDL particle number are discordant. Similarly, while apolipoprotein B levels or LDL particle number are significantly better than non-HDL-C in predicting ASCVD risk when the levels of non-HDL-C and apolipoprotein B levels or LDL particle number are discordant whether this will alter therapy in most patients is debated. The guidelines put forth by a variety of different expert panels and organizations do not require apolipoprotein B or LDL particle number but may use them as risk enhancing factor. It is also the author’s opinion that at this time the routine measurement of apolipoprotein B and/or LDL particle number is not required. Until data demonstrate the superiority of measuring apolipoprotein B or LDL particle number on clinical outcomes it is hard to recommend the routine use of such testing. However, in situations where there is uncertainty measurement of apolipoprotein B and/or LDL particle number can be helpful. Studies have demonstrated an association of Lp(a) with ASCVD. Many experts recommend measuring Lp(a) once in all patients while other experts recommend measuring Lp(a) more selectively in a) patients with unexplained premature CHD, b) patients with a strong family history of premature CHD, c) patients with resistance to LDL lowering with statins, d) patients with rapid unexplained progression of atherosclerosis, and e) patients with familial hypercholesterolemia. Elevations in Lp(a) will stimulate more aggressive lowering of LDL and the consideration of adding drugs that lower Lp(a) such as PCSK9 inhibitors. While routine use of advanced lipoprotein testing is not routinely recommended it should be recognized that in selected patients the additional information provided can be helpful and result in changes in treatment. As additional drugs to treat lipids are developed and our understanding of lipid and lipoprotein metabolism expands in the future the use of advanced lipoprotein analysis may assume a more important role.

**INTRODUCTION**

A variety of specialized lipid and lipoprotein tests are available and a question that is frequently asked is whether and when to utilize these tests in evaluating and treating patients with lipid disorders. The standard lipid panel includes the measurement of total cholesterol, triglycerides, and HDL cholesterol (HDL-C). The LDL cholesterol (LDL-C) can then be calculated using the Friedewald formula (LDL-C = total cholesterol – HDL-C – TG/5). In some instances, a direct LDL-C assay is employed because as the triglyceride levels increase the accuracy of the calculated LDL-C decreases and once the triglyceride levels are greater than 400mg/dl most laboratories will no longer provide a calculated LDL-C level. In patients with normal triglyceride levels and LDL levels > 100mg/dl calculated LDL-C and directly measured LDL-C are very strongly correlated and the difference between the levels is relatively small ([1-3](#_ENREF_1)). However, if the triglyceride levels are greater than 150-200mg/dl the calculated LDL-C will be lower than the directly measured LDL-C level ([1](#_ENREF_1)). Additionally, if the LDL-C level is low (<100mg/dl) the calculated LDL-C also tends to underestimate the true LDL-C level ([1-5](#_ENREF_1)). Because of the inaccuracies of LDL-C levels calculated by the Friedewald formula a new and more accurate formula (Martin Hopkins Formula) has been developed ([6](#_ENREF_6)). Several studies have demonstrated the increased accuracy of this new formula compared to the Friedewald formula with a particular advantage in settings of low LDL-C and high triglycerides ([7-12](#_ENREF_7)). Major laboratories such as Quest now calculate LDL-C levels using the Martin Hopkins formula. A disadvantage of the Martin Hopkins formula is that it is more complex than the Friedewald formula and the LDL-C cannot be simply calculated. However, there is free, online access that allows for the automated calculation of LDL cholesterol by the Martin Hopkins formula ([www.LDL-Calculator.com/](http://www.LDL-Calculator.com/)) and a smart phone application (LDL cholesterol calculator: <https://www.hopkinsmedicine.org/apps/all-apps/ldl-cholesterol-calculator>). In addition to the Martin Hopkins formula other formulas to more accurately calculate LDL-C levels have been developed. The Sampson-NIH equation in some studies was more accurate when the triglyceride levels were elevated than the Martin Hopkins formula ([13](#_ENREF_13),[14](#_ENREF_14)) but in other studies the Martin Hopkins formula was more accurate ([15](#_ENREF_15)). The key is that there are better methods to calculate LDL-C levels than the Friedewald formula when triglyceride levels are elevated or LDL-C levels are low.

Non-HDL cholesterol (non-HDL-C) levels can also be calculated from a routine lipid panel (non-HDL-C = total cholesterol – HDL-C). “Remnant cholesterol” can also be estimated from the routine lipid panel (Remnant cholesterol = total cholesterol – LDL-C (direct measurement) – HDL-C) ([16](#_ENREF_16),[17](#_ENREF_17)). High calculated remnant cholesterol levels are associated with an increased risk of ASCVD ([16](#_ENREF_16)). Whether remnant cholesterol levels provide information on ASCVD risk above that provided by non-HDL-C and triglyceride levels is not clear. It should be noted that there is no accepted standard for defining remnant lipoproteins or the methods used to accurately measure remnant particles ([18](#_ENREF_18)). Of note most guidelines and risk calculators do not require lipid and lipoprotein measurements beyond a routine lipid panel. For example, the ACC/AHA (Pooled Cohort Equations), QRISK, Reynolds, SCORE, and Framingham calculators utilize total cholesterol and HDL-C levels in order to calculate the risk of atherosclerotic cardiovascular disease (ASCVD) ([19-23](#_ENREF_19)).

In the past fasting lipid panels were exclusively recommended but recent guidelines recommend either fasting or non-fasting lipid panels (([24-26](#_ENREF_24)). Non-fasting lipid panels will increase the convenience of obtaining lipid studies. Additionally, in patients with diabetes, fasting for the lipid panel increases the risk of hypoglycemia ([27](#_ENREF_27)). Moreover, studies have shown that the ability of fasting and non-fasting lipid panels to predict ASCVD is similar ([28-32](#_ENREF_28)). Fasting and non-fasting total cholesterol, HDL-C, and non-HDL-C levels are virtually identical ([33](#_ENREF_33),[34](#_ENREF_34)). Triglyceride levels may increase in the fed state depending upon the amount of fat consumed and the time after consumption and therefore depending upon the circumstances there may be a considerable difference between fasting and non-fasting triglyceride levels in some patients ([33](#_ENREF_33),[34](#_ENREF_34)). LDL-C levels calculated by the Friedewald formula are often decreased in the fed state due to increases in triglyceride levels ([33](#_ENREF_33)). In the non-fasting state when LDL-C levels were calculated using the Friedewald formula 30% of patients had a ≥ 10 mg/dL difference compared to direct LDL-C measurements ([9](#_ENREF_9)). In contrast, when LDL-C was calculated using the Hopkins Martin or Sampson-NIH formula the results were very similar to direct measurements. Therefore, if one is using non-fasting LDL-C in decision making one should calculate the LDL-C level using the Hopkins Martin or Sampson-NIH formula to increase accuracy. It should be noted that in patients where a genetic disorder of lipid metabolism is suspected or with previously elevated triglyceride levels a fasting lipid panel is preferred. Similarly, if triglyceride levels are elevated (>175mg/dL) with a non-fasting lipid panel the lipid panel should be repeated while fasting.

**LDL CHOLESTEROL VS. NON-HDL CHOLESTEROL**

LDL-C and non-HDL-C levels are strongly correlated and increasing levels of either parameter is associated with an increased risk of ASCVD. Numerous studies have compared the ability of LDL-C and non-HDL-C to predict ASCVD events ([35](#_ENREF_35)). In general, while both LDL-C and non-HDL-C predict an increased risk, non-HDL-C levels are a better predictor ([35-42](#_ENREF_35)). For example, in the Women’s Health Study, a prospective cohort study of 15,632 initially healthy US women aged 45 years or older, the relative risk of a cardiovascular event in the top vs. bottom quintile was 1.62 for LDL-C and 2.51 for non-HDL-C ([41](#_ENREF_41)). Similarly, in the Health Professionals Follow-up Study, a study of 51,529 US male health professionals between 40 to 75 years of age, the relative risk of a cardiovascular event in the highest quintile compared with the lowest quintile was 1.81 for LDL-C and 2.76 for non-HDL-C ([42](#_ENREF_42)).

While LDL-C and non-HDL-C are strongly correlated there are some individuals where these measurements are discordant (i.e., a relatively low LDL-C and a relatively high non-HDL-C or conversely a relatively high LDL-C and a relatively low non-HDL-C). In discordant situations the non-HDL-C levels are a much better predictor of cardiovascular events than the LDL levels. For example, in a study by Mora of 27,533 healthy women, 11.6% had discordant levels with discordance defined as an LDL-C above the median and a non-HDL-C below the median or an LDL-C below the median with a non-HDL-C above the median ([43](#_ENREF_43)). Most significantly, in women with a below-median LDL-C but a non-HDL-C above the median coronary risk was underestimated by almost 3-fold for women when the LDL-C was used to predict events ([43](#_ENREF_43)). Conversely, in women with above-median LDL-C but a non-HDL-C below the median coronary risk was overestimated by almost 3-fold when their LDL-C was used to predict events ([43](#_ENREF_43)). Thus, the risk of ASCVD tracks more closely with non-HDL-C levels and these results highlight the advantage of non-HDL-C measurements compared to LDL-C measurements in determining risk of ASCVD.

In addition, this discordance between calculated LDL-C (measured by the Friedewald formula) and non-HDL-C levels can result in the misclassification of patients. For example. in patients with LDL-C levels <70 mg/dl, 15% had a non-HDL-C level ≥ 100 mg/dl and if the triglyceride levels were between 150-199mg/dl 22% had a non-HDL-C ≥ 100 mg/dl ([44](#_ENREF_44)). Thus, a significant number of patients who have reached their LDL-C goal of < 70mg/dl have not reached their non-HDL-C goal. The method used to determine LDL-C levels influences the rate of discordance between LDL-C and non-HDL-C levels. When the LDL-C levels were measured by the Friedewald formula the discordance was considerable higher than when LDL-C levels were measured using the Hopkins Martin formula (Table 1) ([45](#_ENREF_45)).

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| **Table 1. Discordance Between LDL-C and Non-HDL-C Levels** | |
|  | **Percent with Non-HDL-C > 100mg/dl** |
| LDL-C < 70mg/dL Friedewald Formula | 14-15% |
| LDL-C < 70mg/dL Hopkins Martin Formula | ~2% |
|  | **Percent with Non-HDL-C > 130mg/dl** |
| LDL-C < 100mg/dl Friedewald Formula | 8-10% |
| LDL-C < 100mg/dl Hopkins Martin Formula | ~ 1% |

Finally, studies have examined the relative utility of LDL-C and non-HDL-C levels in determining the benefits of statin therapy. A meta-analysis by Boekholdt and colleagues looked at 8 statin trials with 62,154 patients ([46](#_ENREF_46)). They found that while on treatment levels of both LDL-C and non-HDL-C were associated with the risk of future cardiovascular events the association was more robust for non-HDL-C ([46](#_ENREF_46)).

Taken together these data indicate that while both LDL-C and non-HDL-C levels are predictive of ASCVD events non-HDL-C is a better predictor. The older NCEP guidelines recommended non-HDL-C as a therapeutic target if the triglyceride levels were greater than 200mg/dl and the newer National Lipid Association and American Association of Clinical Endocrinologists recommendations consider non-HDL-C as a target along with LDL-C ([19](#_ENREF_19),[26](#_ENREF_26),[47](#_ENREF_47)). The non-HDL-C targets are 30mg/dl higher than the LDL-C targets (for example if the LDL-C target is 70mg/dl the non-HDL-C target would be 100mg/dl). It is the opinion of this author that clinicians should utilize non-HDL-C levels more frequently in the evaluation and management of patients with hyperlipidemia. Additionally, non-HDL-C levels are easily calculated when one obtains a routine lipid panel in the fed or fasted state.

**ADVANCED LIPOPROTEIN TESTS**

In addition to a routine lipid panel, it is possible for the clinician to measure a number of other parameters including apolipoprotein B and A-I levels, LDL and HDL size, LDL and HDL particle number, and lipoprotein (a) (Lp(a)) levels. A number of different tests are offered by large commercial laboratories. Currently, lipoprotein analysis by Nuclear Magnetic Resonance Spectroscopy (NMR) is offered by LabCorp and Ion-Mobility Analysis is offered by Quest Diagnostics. Density Gradient Ultracentrifugation (VAP) by Atherotec was discontinued (Feb 2016). Both, LabCorp and Quest provide routine lipid panel measurements plus LDL particle number, apolipoprotein B levels, indication of LDL and HDL size, and Lp(a) measurements.

It should be recognized that the standardization of certain of these assays is not as rigorous as the standardization of routine lipid panel assays ([3](#_ENREF_3)). The Centers for Disease Control and Prevention (CDC) maintains a Lipid Standardization Program (LSP) that provides standards for measuring total cholesterol, triglycerides, HDL-C, apolipoprotein A-I, and apolipoprotein B. Measurements of LDL and HDL size and particle number are not as standardized and studies have shown differences in results between different methods ([3](#_ENREF_3),[48](#_ENREF_48),[49](#_ENREF_49)). For example, Witte and colleagues compared LDL size using NMR and gradient gel electrophoresis and observed a correlation of only 0.39 between the two methods with an average difference in LDL size of 5.38nm with NMR values being lower ([50](#_ENREF_50)). When these investigators classified patients according to whether they had small dense LDL (Pattern B) less than 50% of patients classified as pattern B using gradient gel electrophoresis were classified as pattern B using NMR ([50](#_ENREF_50)). Similarly, Ensign et al., compared VAP, NMR, tube gel electrophoresis, and gradient gel electrophoresis to determine LDL subclasses and found a strong disagreement in patient LDL phenotyping among these four different methods ([51](#_ENREF_51)). Measurement of LDL and HDL particle number has also shown discrepant results between different methods ([52](#_ENREF_52),[53](#_ENREF_53)). These and other results highlight the lack of rigorous standardization ([54](#_ENREF_54)).

**LDL SIZE**

The size of LDL particles is heterogeneous and there are a number of different methods to determine LDL size (ultracentrifugation, gradient gel electrophoresis, ion mobility, NMR) ([55](#_ENREF_55)). As noted above, the different methods of LDL subclass analysis may produce different results and significant variations are possible even within one method ([48](#_ENREF_48)). Studies have shown that small dense LDL is more pro-atherogenic than large LDL particles. Small dense LDL are thought to be more atherogenic because they are better able to penetrate the endothelial cell barrier and enter the intima, are more susceptible to oxidation, bind to proteoglycans in the arterial wall, and have a longer half time in the circulation than large LDL particles ([56](#_ENREF_56)). It should be noted though that large LDL particles are also pro-atherogenic ([57-61](#_ENREF_57)). For example, patients with familial hypercholesterolemia tend to have large LDL particles and these patients are at high risk to develop ASCVD ([60](#_ENREF_60)). Small LDL particles are typically seen in patients with elevated triglyceride levels and decreased HDL-C levels (i.e. patients with the metabolic syndrome, obese patients, patients with diabetes) ([62](#_ENREF_62)). Numerous studies have documented a link between small dense LDL particles and an increased risk of ASCVD ([63](#_ENREF_63),[64](#_ENREF_64)). However, the association of small dense LDL with ASCVD is markedly reduced or entirely eliminated when the analyses are adjusted for other factors that affect the risk of ASCVD ([63](#_ENREF_63),[64](#_ENREF_64)). The National Lipid Association expert panel was unable to identify any patient subgroups in which measuring LDL size is necessary ([65](#_ENREF_65)). The author concurs with that viewpoint.

**HDL SIZE**

HDL particles are heterogeneous and vary in size ([66](#_ENREF_66),[67](#_ENREF_67)). The metabolism and function of the spectrum of HDL particles is poorly understood. Additionally, there are a number of different methods of measuring HDL size and the comparability of the various methods is uncertain ([54](#_ENREF_54),[66](#_ENREF_66),[67](#_ENREF_67)). Finally, and most importantly there is little data demonstrating that measurements of HDL subfractions are useful in risk prediction beyond measuring HDL and other traditional risk factors ([64](#_ENREF_64),[67](#_ENREF_67),[68](#_ENREF_68)). Because of these issues the National Lipid Association Expert Panel was unable to find situations where HDL subfraction measurements would be recommended ([65](#_ENREF_65)).

It should be recognized that the crucial issue with HDL may not be the HDL levels per se but rather the function of the HDL particles ([54](#_ENREF_54)). Assays have been developed to determine the ability of HDL to facilitate cholesterol efflux from macrophages and these studies have shown that the levels of HDL-C do not necessarily indicate the ability to mediate cholesterol efflux ([69](#_ENREF_69)). Moreover, cholesterol efflux from macrophages had a strong inverse association with both carotid intima-media thickness and the likelihood of angiographic coronary artery disease, independently of the HDL-C level ([70](#_ENREF_70)). Additionally cholesterol efflux was also inversely associated with the incidence of cardiovascular events ([71](#_ENREF_71),[72](#_ENREF_72)). These results indicate that it is the functional capability of HDL to facilitate cholesterol efflux that is important rather than simply HDL-C levels ([73](#_ENREF_73)).

Assays have also been developed to measure the ability of HDL to protect LDL from oxidation ([74](#_ENREF_74)). The ability of HDL to protect LDL from oxidation is decreased in patients with cardiovascular disease and in patients with inflammatory disorders who are at increased risk of developing cardiovascular disease ([74](#_ENREF_74),[75](#_ENREF_75)). Similar to studies of cholesterol efflux these observations suggest that HDL function is a key variable. Unfortunately assays to measure cholesterol efflux or the ability of HDL to prevent oxidation are not available outside of research laboratories.

**APOLIPOPROTEIN B**

All of the pro-atherogenic lipoproteins (chylomicron remnants, VLDL remnants, IDL, LDL, and Lp(a)) carry one apolipoprotein B on their surface such that apolipoprotein B levels reflect the total number of atherogenic particles ([76](#_ENREF_76)). Most of the circulating apolipoprotein B is associated with LDL particles ([76](#_ENREF_76)). However, the contribution of very high Lp(a) levels to total Apo B levels can be substantial (Estimated Apo B in LDL/VLDL = Apo B mg/dl – (Lp(a) mg/dl x 0.16) ([77](#_ENREF_77)). Apo B levels measured in the non-fasting state are similar to fasting values.

The levels of apolipoprotein B, LDL-C, and non-HDL-C are strongly correlated. Almost all studies have shown that apolipoprotein B levels are more closely associated with ASCVD than LDL-C levels and the general consensus is that apolipoprotein B levels are a more accurate predictor of ASCVD events than LDL-C ([41](#_ENREF_41),[42](#_ENREF_42),[65](#_ENREF_65),[78-85](#_ENREF_78)). Apolipoprotein B levels are equivalent to non-HDL-C levels in predicting ASCVD but when these measurements are discordant apolipoprotein B levels are a more accurate predictor of ASCVD.

There are two large meta-analyses that have compared the ability of non-HDL-C and apolipoprotein B to predict ASCVD. The Emerging Risks Factor Collaboration examined 22 long term perspective studies with 91,307 subjects with a large number of events (4499) ([28](#_ENREF_28)). In this study there were no differences in the ability of non-HDL-C or apolipoprotein B to predict ASCVD. The hazard ratio was increased approximately 2-fold in the upper quantile of non-HDL-C and apolipoprotein B compared to the lowest quantile. In contrast, another meta-analysis of 12 studies (not all perspective) with 233,455 subjects and 22,950 events reported slightly different results ([86](#_ENREF_86)). In this study the relative risk ratio for apolipoprotein B was 1.43 (1.35-1.51) vs. 1.34 (1.24-1.44) for non-HDL-C, indicating a slightly greater predictive ability of apolipoprotein B ([86](#_ENREF_86)).

A recent very large study has compared the predictive ability of non-HDL-C and apolipoprotein B ([87](#_ENREF_87)). In the UK Biobank study 346,686 individuals without baseline CVD and not taking statins were followed for a median of 8.9 years. Fatal or nonfatal CVD events occurred in 6216 participants (1656 fatal). The conclusion of this very large study was that measurement of non-HDL-C was sufficient to capture the lipid-associated risk in CVD prediction, with no meaningful improvement from addition of apolipoprotein B.

Studies have also examined the predictive ability of non-HDL cholesterol and apolipoprotein B during treatment of dyslipidemia. In the Heart Protection Study (placebo vs. simvastatin) with over 20,000 participants and over 5,000 events the ability of non-HDL-C and apolipoprotein B to predict cardiovascular events were virtually identical ([88](#_ENREF_88)). A meta-analysis by Boekholdt and colleagues looked at 8 statin trials with 62,154 patients and the adjusted hazard ratios for major cardiovascular events per 1-SD increase were very similar for apolipoprotein B and non-HDL-C ([46](#_ENREF_46)). A meta-analysis by Robinson et al of 25 trials (n = 131,134): 12 on statin, 4 on fibrate, 5 on niacin, 2 on simvastatin-ezetimibe, 1 on ileal bypass surgery, and 1 on aggressive versus standard low-density lipoprotein (LDL) cholesterol and blood pressure targets observed that decreases in non-HDL cholesterol levels modestly outperformed apolipoprotein B in predicting cardiovascular events ([89](#_ENREF_89)). Additionally, apolipoprotein B and non-HDL-C decreases similarly predicted cardiovascular disease risk in the statin trials.

While apolipoprotein B and non-HDL-C are strongly correlated there are some individuals where these measurements are discordant (i.e., a relatively low apolipoprotein B and a relatively high non-HDL-C or conversely a relatively high apolipoprotein B and a relatively low non-HDL-C). An analysis of the Interheart study explored the effect of discordance of apolipoprotein B and non-HDL-C ([90](#_ENREF_90)). The Interheart study is a case-control study of acute myocardial infarction with blood samples in 9345 cases and 12,120 controls from 52 countries. Concentrations of non-HDL-C and apolipoprotein B were expressed as percentiles within the population. Concordance was defined as percentile non-HDL-C = percentile apolipoprotein B. Discordance was defined as percentile non-HDL-C > percentile apolipoprotein B or percentile non-HDL-C < percentile apolipoprotein B by 5%. The results of this study demonstrated that when apolipoprotein B and non-HDL-C levels were discordant the apolipoprotein B measurement was a significantly better predictor of ASCVD ([90](#_ENREF_90)). Subjects with a low apolipoprotein B and a high non-HDL-C were at low risk (Odds Ratio 0.72 (0.67-0.77 95% CI) whereas subjects with a high apolipoprotein B and a low non-HDL-C were at a high risk (Odds Ratio 1.58 (1.38-1.58 95% CI). Similar results have recently been reported from the Women’s Health Study ([91](#_ENREF_91)). Subjects with a high apolipoprotein B level and a discordant lower non-HDL cholesterol level had an increased risk (hazard ratio 1.22 CI 1.07- 139). Of note the subjects with higher apolipoprotein B levels relative to non-HDL-C had an increased prevalence of the metabolic syndrome including higher triglyceride levels and decreased HDL-C levels. Finally, the Cardia study compared the ability of apolipoprotein B and non-HDL-C levels to predict the development of coronary artery calcium, a surrogate marker of cardiovascular events ([92](#_ENREF_92)). In this study apolipoprotein B levels were superior to non-HDL-C in predicting the development of coronary artery calcium (Table 2) ([92](#_ENREF_92)). It is worth noting that the number of subjects that are discordant is relatively small (430 discordant/ 2794 total; 15.4% discordant).

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| **Table 2. Cardia Study** | |
| **Apo B/non-HDL-C (number of subjects)** | **Odds Ratio (CI)** |
| Low/low (1184) | 1.00 |
| Low/high (213) | 1.30 (0.91-1.85) |
| High/low (217) | 1.63 (1.15-2.32) |
| High/high (1180 | 2.32 (1.91-2.83) |

A key question is whether measuring apolipoprotein B in addition to routine risk factors will significantly affect our ability to decide on whether and how to treat patients. Using data from the Framingham Heart Study it was shown that adding apolipoprotein B to non-HDL-C and standard risk factors increased the C-statistic from 0.723 to 0.730, a very small increase suggesting that routine measurements of apolipoprotein B would not be very helpful ([81](#_ENREF_81),[93](#_ENREF_93)). Similarly, the Emerging Risk Factor Collaboration group and the Women’s Health Study also examined the effect of adding apolipoprotein B results on the C-statistic and found very little change ([83](#_ENREF_83),[94](#_ENREF_94)). Additionally, the Emerging Risk Factor Collaboration modelled the effect of measuring apolipoprotein B levels on patient classification using the NCEP III guidelines. In 15,436 subjects with a cardiovascular risk of 10-20% over the next 10 years the addition of apolipoprotein B measurements would result in a change in classification in only 488 subjects (3.2%) ([94](#_ENREF_94)). Most subjects would be moved to a lower risk category (334) and a very small number would be reclassified to a higher risk category (154). These results coupled with the C-statistic results noted above suggest that the routine addition of apolipoprotein measurements in primary prevention patients would likely not have a major effect in altering patient management.

In patients treated with statins a meta-analysis has compared the association of apolipoprotein B and non-HDL-C levels on the risk of major cardiovascular events ([46](#_ENREF_46)). While both on-treatment decreases in apolipoprotein B and non-HDL-C levels were associated with a decrease in cardiovascular events the strength of the association was somewhat greater for non-HDL-C than apolipoprotein B (Table 3) ([46](#_ENREF_46)). A meta-analysis of seven randomized controlled trials comprising more than 60 000 study participants has also shown that changes in LDL-C, apoB100, and non-HDL-C all predicted similar CVD risk reduction after 1-year of statin therapy (-20, -24, and -20% risk reduction, respectively) ([95](#_ENREF_95)). Finally, in another meta-analysis of 25 trials (12 statin, 4 fibrate, 5 niacin, 2 simvastatin-ezetimibe, 1 ileal bypass, 1 intensive vs. standard statin) the authors concluded that “across all drug classes, apo B decreases did not consistently improve risk prediction over LDL cholesterol and non-HDL cholesterol decreases” ([89](#_ENREF_89)). Thus, in patients treated for hyperlipidemia the measurement of apolipoprotein B levels also does not appear to significantly contribute to the management of these patients.

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| **Table 3. Risk of Cardiovascular Disease in Statin Treated Patients (Hazard Ratios)** | | |
| **Quartiles** | **Non-HDL-C** | **Apo B** |
| 1 | 1 (reference) | 1 (reference) |
| 2 | 1.12 | 1.05 |
| 3 | 1.17 | 1.12 |
| 4 | 1.42 | 1.33 |

Another approach to addressing the question of the importance of routinely measuring apolipoprotein levels is to determine if measuring apolipoprotein B level will alter our therapeutic approach. While most guidelines have not included apolipoprotein B goals there are guidelines that do recommend apolipoprotein B levels. For example, the National Lipid Association recommends in very high risk patients a LDL-C < 70mg/dL, a non-HDL-C < 100mg/dL, and an apolipoprotein B level < 80mg/dL ([96](#_ENREF_96)). In an analysis by Sathiyakumar and colleagues if the LDL-C was < 70mg/dL and the non-HDL-C was < 100mg/dL (over 9000 subjects) fewer than 2% of the patients had an apolipoprotein B level > 80mg/dL ([45](#_ENREF_45)). These results indicate that measuring apolipoprotein B levels will not identify a large number of patients that are not meeting the proposed goals.

In summary while measurement of apolipoprotein B levels is an excellent and likely the best predictor of ASCVD events whether it provides a substantial amount of information above and beyond what is provided by LDL-C and non-HDL-C and standard risk factors to justify routine apolipoprotein B measurement remains to be definitively determined. Whether routinely measuring apolipoprotein B levels will alter management in a sufficient number of patients to justify the extra expense of measuring apolipoprotein B needs to be rigorously studied. As noted earlier many of the patients with elevated apolipoprotein B levels relative to non-HDL-C levels are obese, diabetic, and have the metabolic syndrome and it is likely that clinicians will recognize based on non-lipid risk factors that these individuals are at high risk for ASCVD. There will of course be individual patients where measuring apolipoprotein levels will be helpful in determining treatment. For example, in patients thought to have Familial Dysbetalipoproteinemia (Type 3 disease) the non-HDL-C/apolipoprotein B ratio is a simple test for selecting patients with mixed hyperlipidemia that may have Familial Dysbetalipoproteinemia for additional studies ([97](#_ENREF_97)). Similarly, in patients with high cholesterol levels and biliary obstruction a low apolipoprotein B level suggests the presence of lipoprotein X, an atypical lipoprotein particle containing unesterified cholesterol and phospholipids but not apolipoprotein B ([3](#_ENREF_3),[98](#_ENREF_98)).

**LDL PARTICLE NUMBER**

The cholesterol content of LDL is not constant and can vary greatly between individuals and can change over time in a particular individual. For example, treatments that lower serum triglyceride levels can increase the size and cholesterol content of LDL ([99](#_ENREF_99),[100](#_ENREF_100)). Measuring LDL particle number is an alternative way to quantitate LDL burden. While LDL-C and LDL particle number are strongly correlated there are some individuals who are discordant (relatively high LDL-C and relatively low LDL particle number or relatively low LDL-C and relatively high particle number). In patients with elevated triglycerides and/or low HDL levels the LDL-C levels are relatively low compared to LDL particle number ([101](#_ENREF_101),[102](#_ENREF_102)). Studies have shown that LDL particle number is more strongly associated with ASCVD than LDL-C, particularly when the levels of LDL-C and LDL particle number are discordant ([43](#_ENREF_43),[83](#_ENREF_83),[103-106](#_ENREF_103)). Whether LDL particle number is a better predictor than non-HDL-C is discussed below.

Several studies have compared the ability of LDL particle number and non-HDL-C to predict ASCVD. In the Framingham Offspring Study there were 3,066 subjects with 431 events and LDL particle number was measured by NMR ([103](#_ENREF_103)). In this study LDL particle number was more strongly associated with ASCVD than non-HDL-C (Hazard ratio 1.28 (CI 1.17-1.39) for LDL particle number vs. 1.21 (CI 1.10-1.33) for non HDL-C) ([103](#_ENREF_103)). In the Women’s Health Study there were 27,673 subjects with 1015 events and LDL particle number was also measured by NMR ([83](#_ENREF_83)). In this study the association of LDL particle number and non-HDL-C with ASCVD was very similar with the hazard ratio of 2.51 for LDL particle number and 2.52 for non-HDL-C ([83](#_ENREF_83)). Finally, in the Multi-Ethnic Study of Atherosclerosis subjects (n = 6693) no benefit of measuring LDL particle number compared to routine lipid measurements on predicting ASCVD could be demonstrated ([107](#_ENREF_107)).

While there are several studies that have examined patients discordant for apolipoprotein B levels and non-HDL-C levels (see section on apolipoprotein B) only two studies have examined discordance between LDL particle number and non-HDL-C. In the Multi-Ethnic Study of Atherosclerosis there were 6,814 men and women and LDL particle number was measured by NMR ([108](#_ENREF_108)). The endpoint in this study was carotid intima-media thickness (CIMT) and coronary artery calcium (CAC), surrogate markers for ASCVD events. When there was discordance between LDL particle number and non-HDL-C, LDL particle number was more closely associated with CIMT and CAC but the differences were very modest ([108](#_ENREF_108)). In the Women’s Health Study subjects with high LDL particle number measured by NMR that was discordant with non-HDL cholesterol levels were at increased risk of CHD (hazard ratio 1.13 CI 0.99-1.29) ([91](#_ENREF_91)).

In patients on-treatment there is only a single study comparing LDL particle number and non-HDL-C. In the Heart Protection study 20,536 subjects were treated with simvastatin or placebo and LDL particle number was measured by NMR ([88](#_ENREF_88)). The predictive strength of LDL particle number and non-HDL-C was very similar in both the placebo group and the statin group indicating no advantage of measuring LDL particle number ([88](#_ENREF_88)).

It should also be noted that while LDL particle number and Apo B levels are highly correlated there are circumstances when they are discordant ([109](#_ENREF_109)). High LDL particle number relative to Apo B levels was seen with insulin resistance, smaller LDL particle size, increased systemic inflammation, and low circulating LDL-C and HDL-C levels while high Apo B levels relative to LDL particle number was seen with larger LDL particle size and elevated levels of lipoprotein(a) ([109](#_ENREF_109)).

In summary, while measurement of LDL particle number is an excellent predictor of ASCVD events whether it provides a substantial amount of information beyond what is provided by non-HDL-C and standard risk factors to justify routine LDL particle measurement remains to be definitively determined.

**Lp(a) MEASUREMENT**

Lp(a) is an LDL particle with a single apolipoprotein B with a plasminogen like protein, apoprotein (a), attached by a disulfide bond ([110-112](#_ENREF_110)). Apoprotein (a) is genetically very heterogeneous due to variations in molecular weight (from 300-800 kDa) due to differences in the number of Kringle repeats ([110-112](#_ENREF_110)). The plasma levels of Lp(a) vary greatly with undetectable levels in some individuals (0.1mg/dl) and very high levels in others (>200mg/dl) ([113](#_ENREF_113)). Individuals with genetically determined small apoprotein (a) have high plasma levels of Lp(a) whereas individuals with genetically determined large apoprotein (a) have low levels ([110-112](#_ENREF_110)). The size of the apo(a) isoforms is inherited with an individual having two distinct apo(a) isoforms derived from apo(a) genes from their mother and father ([113](#_ENREF_113)). This results in individuals having two different size Lp(a) particles in the serum. It is estimated that up to 90% of the variation in Lp(a) levels is determined genetically with environment having minimal effects. Lp(a) levels are very stable within an individual over their lifespan. Inflammation and renal disease increase while severe liver disease decrease Lp(a) levels ([75](#_ENREF_75),[114](#_ENREF_114)).

Approximately 20% of subjects have Lp(a) levels greater than 50mg/dL and 30% have Lp(a) greater than 30mg/dL. Ethnicity greatly affects Lp(a) levels ([114](#_ENREF_114)). The levels of Lp(a) in Blacks are approximately 2-3-fold higher than in Caucasians, Caucasians and Chinese have similar levels, South Asians have levels between Blacks and Caucasians, and Mexicans have levels lower than Caucasians (Blacks> South Asians > Caucasians/Chinese > Mexicans) ([114](#_ENREF_114)). Lp(a) levels do not correlate with LDL-C, non-HDL-C, apolipoprotein B, or LDL particle number.

Several large meta-analyses have demonstrated an association of Lp(a) levels with ASCVD. For example, a meta-analysis by the Emerging Risk Factors Collaboration looked at the individual records of 126,634 participants in 36 prospective studies with 9,336 CHD outcomes, 1,903 ischemic strokes, and 8,114 nonvascular deaths ([115](#_ENREF_115)). They found a continuous association of Lp(a) with the risk of ASCVD that was not greatly affected by adjustment for other lipid levels or other established risk factors. In an analysis of 31 prospective studies with 9,870 events Bennet et al reported an odds ratio of 1.45 for individuals in the top third of Lp(a) compared with those in the bottom third ([116](#_ENREF_116)). Of note adjustment for lipid levels and other established risk factors also had little effect on this association indicating that Lp(a) is an independent risk factor ([116](#_ENREF_116)). Additionally, in patients with familial hypercholesterolemia elevated Lp(a) levels markedly increases the risk of the development of ASCVD ([117](#_ENREF_117)). Mendelian randomization studies and basic science studies including experiments in animals that overexpress apoprotein (a) have suggested that increases in Lp(a) are not just a risk factor for atherosclerosis but causative for atherosclerosis ([111](#_ENREF_111),[112](#_ENREF_112),[118-120](#_ENREF_118)). Finally, elevations in Lp(a) account for a significant proportion of the increased risk of ASCVD that is related to family history ([121](#_ENREF_121)).

While the above studies clearly indicate that Lp(a) levels are a risk factor for the development of ASCVD the significance of Lp(a) in secondary prevention is not clear ([122](#_ENREF_122)). Some studies have reported that Lp(a) is a risk factor in the setting of ASCVD ([123-127](#_ENREF_123)) while other studies have failed to demonstrate a role for Lp(a) ([128-131](#_ENREF_128)). In a meta-analysis of 11 studies with a total of 18,978 subjects the association between Lp(a) and ASCVD was significant in studies in which the average LDL cholesterol was ≥130 mg/dl (OR: 1.46, 95% CI: 1.23 to 1.73, p < 0.001), whereas this relationship was attenuated and did not achieve statistical significance for studies with an average LDL cholesterol <130 mg/dl (OR: 1.20, 95% CI: 0.90 to 1.60, p = 0.21) ([128](#_ENREF_128)). This observation suggests that in individuals with elevated LDL-C levels the impact of elevated Lp(a) levels will be magnified. However, in other studies Lp(a) was a risk factor even though LDL-C levels were relatively low ([123](#_ENREF_123),[127](#_ENREF_127)). Recently Williet and colleagues reported a meta-analysis of patient-level data from seven randomized, placebo-controlled, statin outcomes trials that included 29,069 patients with repeat Lp(a) measurements ([132](#_ENREF_132)). They found that elevated baseline and on-statin lipoprotein(a) showed an independent approximately linear relation with cardiovascular disease risk. Additionally, studies have shown that genetic variations at the LPA locus (apo(a) gene that effects Lp(a) levels) are associated with ASCVD events during statin therapy in patients ([133](#_ENREF_133)). Taken together the bulk of the data suggests that elevated Lp(a) levels increase ASCVD risk even in patients with underlying cardiovascular disease.

The Emerging Risk Factor Collaboration modelled the effect of measuring Lp(a) levels on patient classification using the NCEP III guidelines ([94](#_ENREF_94)). In 15,436 subjects with a cardiovascular risk of 10-20% over the next 10 years the addition of Lp(a) measurements would result in a change in classification in 1,517 subjects (9.8%). Most subjects would be moved to a lower risk category (962) and a number of subjects would be reclassified to a higher risk category (555) ([94](#_ENREF_94)). These results coupled with the above findings suggest that the addition of Lp(a) measurements in patients might be useful in selected patients.

The potential benefits of measuring Lp(a) levels will become clearer when drugs are developed that specifically lower Lp(a) levels and clinical trials determining the effect of these drugs on ASCVD outcomes are completed. Without definitive data from randomized outcome trials demonstrating that specifically lowering Lp(a) levels results in a reduction in ASCVD events the advantages of measuring and treating Lp(a) will remain uncertain. Therapy to specifically lower Lp(a) is under development and hopefully in the near future will provide a clear demonstration of the benefits of monitoring and treating Lp(a) levels ([134](#_ENREF_134),[135](#_ENREF_135)).

In the meantime, many experts would recommend measuring Lp(a) levels once in all patients ([136-138](#_ENREF_136)) while other experts would measure Lp(a) in selected patients (Table 4) ([65](#_ENREF_65),[139](#_ENREF_139),[140](#_ENREF_140)). Elevations in Lp(a) will stimulate more aggressive lowering of LDL levels and the consideration of adding drugs that lower Lp(a) such as PCSK9 inhibitors ([141](#_ENREF_141)).

|  |
| --- |
| **Table 4.** **WHEN TO MEASURE LP(a) LEVELS** |
| * Patients with unexplained premature CHD * Patients with a strong family history of premature CHD * Patients with a family history of elevated Lp(a) levels (Cascade screening) * Patients with resistance to LDL-C lowering with statins * Patients with rapid unexplained progression of atherosclerosis * Patients with familial hypercholesterolemia * Patients with aortic valvular stenosis of uncertain cause * Patients with intermediate risk profiles? |

Standard measurements of LDL-C (either calculated or measured) include Lp(a) cholesterol ([139](#_ENREF_139),[142](#_ENREF_142)). When Lp(a) levels are very high they can make a significant contribution to LDL-C levels. Similarly, when LDL-C levels are markedly reduced with treatment the LDL-C measured may include a significant contribution from Lp(a). The contribution of Lp(a) cholesterol to calculated LDL-C is approximately mg/dL Lp(a) x 0.3 (when both are expressed in mg/dL) ([139](#_ENREF_139),[142](#_ENREF_142)). For example, if the Lp(a) level is 100mg/dL one can estimate that approximately 30mg/dL of the calculated LDL level is due to Lp(a). Note that these estimates are not precise and the percent cholesterol per mg Lp(a) particle can vary from 5.8% to 57.3% ([143](#_ENREF_143)). Assays are underdevelopment to accurately determine the cholesterol in Lp(a) to allow for more accurate determinations of LDL-C levels ([143](#_ENREF_143)).

Accurate measurement of Lp(a) represents a formidable technical challenge, unequalled in the world of biochemical diagnostics ([139](#_ENREF_139),[144](#_ENREF_144)). This is due to the extreme length polymorphism of apo(a), whose size can vary over five-fold. Currently Lp(a) assays are not well standardized and there can be considerable variation between commercial assays. One study of 6 different assays found a variation from reference material of −8% to +22% ([145](#_ENREF_145)) and another study found considerable variation in Lp(a) levels between 5 different assays ([146](#_ENREF_146)). Hopefully more accurate assays using monoclonal antibodies will become widely available ([147](#_ENREF_147)).

Measuring Lp(a) mass (in mg/dL), as it is frequently done in commercial clinical labs, will not allow for a reliable and consistent way to convert Lp(a) concentration to nmol/l. For example, 50 mg/dL of Lp(a) with 40 kringle IV type 2 repeats is actually fewer particles than 30 mg/dL of an Lp(a) with 15 kringle IV type repeats. The solution is the adoption of an isoform-independent method that equally identifies each Lp(a) particle ([139](#_ENREF_139)). Such a method is currently approximated by the use of a spectrum of isoform-specific calibrators, and providers should, if possible, have Lp(a) measured using this method and reported as concentration in nmol/l.

**CONCLUSIONS**

While advanced lipoprotein measurements can provide additional insights and information it is not clear that for the evaluation and treatment of the vast majority of our patients that these measurements are necessary. Notably, the guidelines on the evaluation and treatment of hyperlipidemia put forth by a variety of different expert panels and organizations do not require advanced lipoprotein measurements. It is also the author’s opinion that at this time the routine use of advanced lipoprotein testing in clinical practice is not required and that LDL-C and non-HDL-C levels provide sufficient information to guide evaluation and treatment for most patients. Until clinical trial data demonstrate the superiority of utilizing advanced lipoprotein testing on clinical outcomes it is hard to recommend the routine use of such testing. However, it should be recognized that in selected patients the additional information provided can be helpful and result in changes in treatment. It is hoped that as additional drugs to treat lipids are developed and our understanding of lipid and lipoprotein metabolism expands that in the future the use of advanced lipoprotein analysis will assume a more important role in the evaluation and treatment of patients to prevent ASCVD.

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