UTILITY OF ADVANCED LIPOPROTEIN TESTING IN CLINICAL PRACTICE

Kenneth R Feingold MD, Emeritus Professor of Medicine, University of California- San Francisco, kenneth.feingold@ucsf.edu

Updated January 1, 2023

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). 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). 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). 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). 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). 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/) 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 (13,14) but in other studies the Martin Hopkins formula was more accurate (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) (16,17). High calculated remnant cholesterol levels are associated with an increased risk of ASCVD (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). 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).

In the past fasting lipid panels were exclusively recommended but recent guidelines recommend either fasting or non-fasting lipid panels ((24-26). 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). Moreover, studies have shown that the ability of fasting and non-fasting lipid panels to predict ASCVD is similar (28-32). Fasting and non-fasting total cholesterol, HDL-C, and non-HDL-C levels are virtually identical (33,34). 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 (33,34). LDL-C levels calculated by the Friedewald formula are often decreased in the fed state due to increases in triglyceride levels (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). 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). In general, while both LDL-C and non-HDL-C predict an increased risk, non-HDL-C levels are a better predictor (35-42). 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). 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).

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). 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). 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). 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). 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).

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). 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).

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 (19,26,47). 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). 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 (3,48,49). 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). 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). 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). Measurement of LDL and HDL particle number has also shown discrepant results between different methods (52,53). These and other results highlight the lack of rigorous standardization (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). As noted above, the different methods of LDL subclass analysis may produce different results and significant variations are possible even within one method (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). It should be noted though that large LDL particles are also pro-atherogenic (57-61). For example, patients with familial hypercholesterolemia tend to have large LDL particles and these patients are at high risk to develop ASCVD (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). Numerous studies have documented a link between small dense LDL particles and an increased risk of ASCVD (63,64). 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 (63,64). The National Lipid Association expert panel was unable to identify any patient subgroups in which measuring LDL size is necessary (65). The author concurs with that viewpoint.

HDL SIZE

HDL particles are heterogeneous and vary in size (66,67). 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 (54,66,67). 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 (64,67,68). Because of these issues the National Lipid Association Expert Panel was unable to find situations where HDL subfraction measurements would be recommended (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). 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). 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). Additionally cholesterol efflux was also inversely associated with the incidence of cardiovascular events (71,72). 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).

Assays have also been developed to measure the ability of HDL to protect LDL from oxidation (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 (74,75). 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). Most of the circulating apolipoprotein B is associated with LDL particles (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). 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 (41,42,65,78-85). 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). 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). 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).

A recent very large study has compared the predictive ability of non-HDL-C and apolipoprotein B (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). 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). 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). 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). 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). 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). 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). In this study apolipoprotein B levels were superior to non-HDL-C in predicting the development of coronary artery calcium (Table 2) (92). It is worth noting that the number of subjects that are discordant is relatively small (430 discordant/ 2794 total; 15.4% discordant).

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 (81,93). 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 (83,94). 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). 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). 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). 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). 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). 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.

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). 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). 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). 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 (3,98).

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 (99,100). 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 (101,102). 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 (43,83,103-106). 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). 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). In the Women's Health Study there were 27,673 subjects with 1015 events and LDL particle number was also measured by NMR (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). 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).

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). 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). 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).

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). 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).

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). 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).

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). 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). 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). 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). 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). 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 (75,114).

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). 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). 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). 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). 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). Additionally, in patients with familial hypercholesterolemia elevated Lp(a) levels markedly increases the risk of the development of ASCVD (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 (111,112,118-120). Finally, elevations in Lp(a) account for a significant proportion of the increased risk of ASCVD that is related to family history (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). Some studies have reported that Lp(a) is a risk factor in the setting of ASCVD (123-127) while other studies have failed to demonstrate a role for Lp(a) (128-131). 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). 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 (123,127). 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). 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). 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). 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). 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 (134,135).

In the meantime, many experts would recommend measuring Lp(a) levels once in all patients (136-138) while other experts would measure Lp(a) in selected patients (Table 4) (65,139,140). 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).

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 (139,142). 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) (139,142). 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). Assays are underdevelopment to accurately determine the cholesterol in Lp(a) to allow for more accurate determinations of LDL-C levels (143).

Accurate measurement of Lp(a) represents a formidable technical challenge, unequalled in the world of biochemical diagnostics (139,144). 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) and another study found considerable variation in Lp(a) levels between 5 different assays (146). Hopefully more accurate assays using monoclonal antibodies will become widely available (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). 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.

REFERENCES

1. Martin SS, Blaha MJ, Elshazly MB, Brinton EA, Toth PP, McEvoy JW, Joshi PH, Kulkarni KR, Mize PD, Kwiterovich PO, Defilippis AP, Blumenthal RS, Jones SR. Friedewald-estimated versus directly measured low-density lipoprotein cholesterol and treatment implications. J Am Coll Cardiol 2013; 62:732-739
2. Mora S, Rifai N, Buring JE, Ridker PM. Comparison of LDL cholesterol concentrations by Friedewald calculation and direct measurement in relation to cardiovascular events in 27,331 women. Clin Chem 2009; 55:888-894
3. Wilson PWF, Jacobson TA, Martin SS, Jackson EJ, Le NA, Davidson MH, Vesper HW, Frikke-Schmidt R, Ballantyne CM, Remaley AT. Lipid measurements in the management of cardiovascular diseases: Practical recommendations a scientific statement from the national lipid association writing group. J Clin Lipidol 2021; 15:629-648
4. Scharnagl H, Nauck M, Wieland H, Marz W. The Friedewald formula underestimates LDL cholesterol at low concentrations. Clinical chemistry and laboratory medicine 2001; 39:426-431
5. Meeusen JW, Snozek CL, Baumann NA, Jaffe AS, Saenger AK. Reliability of Calculated Low-Density Lipoprotein Cholesterol. Am J Cardiol 2015; 116:538-540
6. Martin SS, Blaha MJ, Elshazly MB, Toth PP, Kwiterovich PO, Blumenthal RS, Jones SR. Comparison of a novel method vs the Friedewald equation for estimating low-density lipoprotein cholesterol levels from the standard lipid profile. JAMA 2013; 310:2061-2068
7. Quispe R, Hendrani A, Elshazly MB, Michos ED, McEvoy JW, Blaha MJ, Banach M, Kulkarni KR, Toth PP, Coresh J, Blumenthal RS, Jones SR, Martin SS. Accuracy of low-density lipoprotein cholesterol estimation at very low levels. BMC Med 2017; 15:83
8. Whelton SP, Meeusen JW, Donato LJ, Jaffe AS, Saenger A, Sokoll LJ, Blumenthal RS, Jones SR, Martin SS. Evaluating the atherogenic burden of individuals with a Friedewald-estimated low-density lipoprotein cholesterol <70 mg/dL compared with a novel low-density lipoprotein estimation method. J Clin Lipidol 2017; 11:1065-1072
9. Sathiyakumar V, Park J, Golozar A, Lazo M, Quispe R, Guallar E, Blumenthal RS, Jones SR, Martin SS. Fasting Versus Nonfasting and Low-Density Lipoprotein Cholesterol Accuracy. Circulation 2018; 137:10-19
10. Martin SS, Giugliano RP, Murphy SA, Wasserman SM, Stein EA, Ceska R, Lopez-Miranda J, Georgiev B, Lorenzatti AJ, Tikkanen MJ, Sever PS, Keech AC, Pedersen TR, Sabatine MS. Comparison of Low-Density Lipoprotein Cholesterol Assessment by Martin/Hopkins Estimation, Friedewald Estimation, and Preparative Ultracentrifugation: Insights From the FOURIER Trial. JAMA Cardiol 2018; 3:749-753
11. Lee J, Jang S, Son H. Validation of the Martin Method for Estimating Low-Density Lipoprotein Cholesterol Levels in Korean Adults: Findings from the Korea National Health and Nutrition Examination Survey, 2009-2011. PLoS One 2016; 11:e0148147
12. Chaen H, Kinchiku S, Miyata M, Kajiya S, Uenomachi H, Yuasa T, Takasaki K, Ohishi M. Validity of a Novel Method for Estimation of Low-Density Lipoprotein Cholesterol Levels in Diabetic Patients. J Atheroscler Thromb 2016; 23:1355-1364
13. Sampson M, Ling C, Sun Q, Harb R, Ashmaig M, Warnick R, Sethi A, Fleming JK, Otvos JD, Meeusen JW, Delaney SR, Jaffe AS, Shamburek R, Amar M, Remaley AT. A New Equation for Calculation of Low-Density Lipoprotein Cholesterol in Patients With Normolipidemia and/or Hypertriglyceridemia. JAMA Cardiol 2020; 5:540-548
14. Ginsberg HN, Rosenson RS, Hovingh GK, Letierce A, Samuel R, Poulouin Y, Cannon CP. LDL-C calculated by Friedewald, Martin-Hopkins, or NIH equation 2 versus beta-quantification: pooled alirocumab trials. J Lipid Res 2022; 63:100148
15. Sajja A, Park J, Sathiyakumar V, Varghese B, Pallazola VA, Marvel FA, Kulkarni K, Muthukumar A, Joshi PH, Gianos E, Hirsh B, Mintz G, Goldberg A, Morris PB, Sharma G, Blumenthal RS, Michos ED, Post WS, Elshazly MB, Jones SR, Martin SS. Comparison of Methods to Estimate Low-Density Lipoprotein Cholesterol in Patients With High Triglyceride Levels. JAMA Netw Open 2021; 4:e2128817
16. Varbo A, Nordestgaard BG. Remnant lipoproteins. Curr Opin Lipidol 2017; 28:300-307
17. Nordestgaard BG, Langlois MR, Langsted A, Chapman MJ, Aakre KM, Baum H, Boren J, Bruckert E, Catapano A, Cobbaert C, Collinson P, Descamps OS, Duff CJ, von Eckardstein A, Hammerer-Lercher A, Kamstrup PR, Kolovou G, Kronenberg F, Mora S, Pulkki K, Remaley AT, Rifai N, Ros E, Stankovic S, Stavljenic-Rukavina A, Sypniewska G, Watts GF, Wiklund O, Laitinen P, European Atherosclerosis S, the European Federation of Clinical C, Laboratory Medicine Joint Consensus I. Quantifying atherogenic lipoproteins for lipid-lowering strategies:

Consensus-based recommendations from EAS and EFLM. Atherosclerosis 2020; 294:46-61

18. Krauss RM, King SM. Remnant lipoprotein particles and cardiovascular disease risk. Best Pract Res Clin Endocrinol Metab 2022:101682

19. Expert Panel on Detection E, Treatment of High Blood Cholesterol in A. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA 2001; 285:2486-2497

20. Stone NJ, Robinson JG, Lichtenstein AH, Bairey Merz CN, Blum CB, Eckel RH, Goldberg AC, Gordon D, Levy D, Lloyd-Jones DM, McBride P, Schwartz JS, Shero ST, Smith SC, Jr., Watson K, Wilson PW, Eddleman KM, Jarrett NM, LaBresh K, Nevo L, Wnek J, Anderson JL, Halperin JL, Albert NM, Bozkurt B, Brindis RG, Curtis LH, DeMets D, Hochman JS, Kovacs RJ, Ohman EM, Pressler SJ, Sellke FW, Shen WK, Smith SC, Jr., Tomaselli GF, American College of Cardiology/American Heart Association Task Force on Practice G. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation 2014; 129:S1-45

21. Hippisley-Cox J, Coupland C, Robson J, Brindle P. Derivation, validation, and evaluation of a new QRISK model to estimate lifetime risk of cardiovascular disease: cohort study using QResearch database. BMJ 2010; 341:c6624

22. Conroy RM, Pyorala K, Fitzgerald AP, Sans S, Menotti A, De Backer G, De Bacquer D, Ducimetiere P, Jousilahti P, Keil U, Njolstad I, Oganov RG, Thomsen T, Tunstall-Pedoe H, Tverdal A, Wedel H, Whincup P, Wilhelmsen L, Graham IM, Score Project Group. Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. Eur Heart J 2003; 24:987-1003

23. Ridker PM, Paynter NP, Rifai N, Gaziano JM, Cook NR. C-reactive protein and parental history improve global cardiovascular risk prediction: the Reynolds Risk Score for men. Circulation 2008; 118:2243-2251, 2244p following 2251

24. Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS, Braun LT, de Ferranti S, Faiella-Tommasino J, Forman DE, Goldberg R, Heidenreich PA, Hlatky MA, Jones DW, Lloyd-Jones D, Lopez-Pajares N, Ndumele CE, Orringer CE, Peralta CA, Saseen JJ, Smith SC, Jr., Sperling L, Virani SS, Yeboah J. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol. Circulation 2018:CIR0000000000000625

25. Nordestgaard BG, Langsted A, Mora S, Kolovou G, Baum H, Bruckert E, Watts GF, Sypniewska G, Wiklund O, Boren J, Chapman MJ, Cobbaert C, Descamps OS, von Eckardstein A, Kamstrup PR, Pulkki K, Kronenberg F, Remaley AT, Rifai N, Ros E, Langlois M, European Atherosclerosis Society, the European Federation of Clinical Chemistry, Laboratory Medicine Joint Consensus, Initiative. Fasting is not routinely required for determination of a lipid profile: clinical and laboratory implications including flagging at desirable concentration cut-points-a joint consensus statement from the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine. Eur Heart J 2016; 37:1944-1958

26. Jacobson TA, Ito MK, Maki KC, Orringer CE, Bays HE, Jones PH, McKenney JM, Grundy SM, Gill EA, Wild RA, Wilson DP, Brown WV. National Lipid Association recommendations for patient-centered management of dyslipidemia: part 1 - executive summary. J Clin Lipidol 2014; 8:473-488

27. Aldasouqi S, Sheikh A, Klosterman P, Kniestedt S, Schubert L, Danker R, Hershey DS. Hypoglycemia in patients with diabetes who are fasting for laboratory blood tests: the Cape Girardeau Hypoglycemia En Route Prevention Program. Postgrad Med 2013; 125:136-143

28. Emerging Risk Factors Collaboration, Di Angelantonio E, Sarwar N, Perry P, Kaptoge S, Ray KK, Thompson A, Wood AM, Lewington S, Sattar N, Packard CJ, Collins R, Thompson SG, Danesh J. Major lipids, apolipoproteins, and risk of vascular disease. JAMA 2009; 302:1993-2000

29. Mora S, Rifai N, Buring JE, Ridker PM. Fasting compared with nonfasting lipids and apolipoproteins for predicting incident cardiovascular events. Circulation 2008; 118:993-1001

30. Langsted A, Freiberg JJ, Nordestgaard BG. Fasting and nonfasting lipid levels: influence of normal food intake on lipids, lipoproteins, apolipoproteins, and cardiovascular risk prediction. Circulation 2008; 118:2047-2056

31. Doran B, Guo Y, Xu J, Weintraub H, Mora S, Maron DJ, Bangalore S. Prognostic value of fasting versus nonfasting low-density lipoprotein cholesterol levels on long-term mortality: insight from the National Health and Nutrition Examination Survey III (NHANES-III). Circulation 2014; 130:546-553

32. Mora S, Chang CL, Moorthy MV, Sever PS. Association of Nonfasting vs Fasting Lipid Levels With Risk of Major Coronary Events in the Anglo-Scandinavian Cardiac Outcomes Trial-Lipid Lowering Arm. JAMA Intern Med 2019; 179:898-905

33. Rahman F, Blumenthal RS, Jones SR, Martin SS, Gluckman TJ, Whelton SP. Fasting or Non-fasting Lipids for Atherosclerotic Cardiovascular Disease Risk Assessment and Treatment? Curr Atheroscler Rep 2018; 20:14

34. Cartier LJ, Collins C, Lagace M, Douville P. Comparison of fasting and non-fasting lipid profiles in a large cohort of patients presenting at a community hospital. Clin Biochem 2018; 52:61-66

35. Verbeek R, Hovingh GK, Boekholdt SM. Non-high-density lipoprotein cholesterol: current status as cardiovascular marker. Curr Opin Lipidol 2015; 26:502-510

36. Arsenault BJ, Rana JS, Stroes ES, Despres JP, Shah PK, Kastelein JJ, Wareham NJ, Boekholdt SM, Khaw KT. Beyond low-density lipoprotein cholesterol: respective contributions of non-high-density lipoprotein cholesterol levels, triglycerides, and the total cholesterol/high-density lipoprotein cholesterol ratio to coronary heart disease risk in apparently healthy men and women. J Am Coll Cardiol 2009; 55:35-41

37. Bittner V, Hardison R, Kelsey SF, Weiner BH, Jacobs AK, Sopko G, Bypass Angioplasty Revascularization I. Non-high-density lipoprotein cholesterol levels predict five-year outcome in the Bypass Angioplasty Revascularization Investigation (BARI). Circulation 2002; 106:2537-2542

38. Cui Y, Blumenthal RS, Flaws JA, Whiteman MK, Langenberg P, Bachorik PS, Bush TL. Non-high-density lipoprotein cholesterol level as a predictor of cardiovascular disease mortality. Arch Intern Med 2001; 161:1413-1419

39. Kastelein JJ, van der Steeg WA, Holme I, Gaffney M, Cater NB, Barter P, Deedwania P, Olsson AG, Boekholdt SM, Demicco DA, Szarek M, LaRosa JC, Pedersen TR, Grundy SM, TNT Study Group, Ideal Study Group. Lipids, apolipoproteins, and their ratios in relation to cardiovascular events with statin treatment. Circulation 2008; 117:3002-3009

40. Lu W, Resnick HE, Jablonski KA, Jones KL, Jain AK, Howard WJ, Robbins DC, Howard BV. Non-HDL cholesterol as a predictor of cardiovascular disease in type 2 diabetes: the strong heart study. Diabetes Care 2003; 26:16-23

41. Ridker PM, Rifai N, Cook NR, Bradwin G, Buring JE. Non-HDL cholesterol, apolipoproteins A-I and B100, standard lipid measures, lipid ratios, and CRP as risk factors for cardiovascular disease in women. JAMA 2005; 294:326-333

42. Pischon T, Girman CJ, Sacks FM, Rifai N, Stampfer MJ, Rimm EB. Non-high-density lipoprotein cholesterol and apolipoprotein B in the prediction of coronary heart disease in men. Circulation 2005; 112:3375-3383

43. Mora S, Buring JE, Ridker PM. Discordance of low-density lipoprotein (LDL) cholesterol with alternative LDL-related measures and future coronary events. Circulation 2014; 129:553-561

44. Elshazly MB, Martin SS, Blaha MJ, Joshi PH, Toth PP, McEvoy JW, Al-Hijji MA, Kulkarni KR, Kwiterovich PO, Blumenthal RS, Jones SR. Non-high-density lipoprotein cholesterol, guideline targets, and population percentiles for secondary prevention in 1.3 million adults: the VLDL-2 study (very large database of lipids). J Am Coll Cardiol 2013; 62:1960-1965

45. Sathiyakumar V, Park J, Quispe R, Elshazly MB, Michos ED, Banach M, Toth PP, Whelton SP, Blumenthal RS, Jones SR, Martin SS. Impact of Novel Low-Density Lipoprotein-Cholesterol Assessment on the Utility of Secondary Non-High-Density Lipoprotein-C and Apolipoprotein B Targets in Selected Worldwide Dyslipidemia Guidelines. Circulation 2018; 138:244-254

46. Boekholdt SM, Arsenault BJ, Mora S, Pedersen TR, LaRosa JC, Nestel PJ, Simes RJ, Durrington P, Hitman GA, Welch KM, DeMicco DA, Zwinderman AH, Clearfield MB, Downs JR, Tonkin AM, Colhoun HM, Gotto AM, Jr., Ridker PM, Kastelein JJ. Association of LDL cholesterol, non-HDL cholesterol, and apolipoprotein B levels with risk of cardiovascular events among patients treated with statins: a meta-analysis. JAMA 2012; 307:1302-1309

47. Jellinger PS, Handelsman Y, Rosenblit PD, Bloomgarden ZT, Fonseca VA, Garber AJ, Grunberger G, Guerin CK, Bell DSH, Mechanick JI, Pessah-Pollack R, Wyne K, Smith D, Brinton EA, Fazio S, Davidson M. American Association of Clinical Endocrinologists and American College of Endocrinology Guidelines for Management of Dyslipidemia and Prevention of Cardiovascular Disease. Endocr Pract 2017; 23:1-87

48. Chung M, Lichtenstein AH, Ip S, Lau J, Balk EM. Comparability of methods for LDL subfraction determination: A systematic review. Atherosclerosis 2009; 205:342-348

49. Delatour V, Clouet-Foraison N, Gaie-Levrel F, Marcovina SM, Hoofnagle AN, Kuklenyik Z, Caulfield MP, Otvos JD, Krauss RM, Kulkarni KR, Contois JH, Remaley AT, Vesper HW, Cobbaert CM, Gillery P. Comparability of Lipoprotein Particle Number Concentrations Across ES-DMA, NMR, LC-MS/MS, Immunonephelometry, and VAP: In Search of a Candidate Reference Measurement Procedure for apoB and non-HDL-P Standardization. Clin Chem 2018; 64:1485-1495

50. Witte DR, Taskinen MR, Perttunen-Nio H, Van Tol A, Livingstone S, Colhoun HM. Study of agreement between LDL size as measured by nuclear magnetic resonance and

gradient gel electrophoresis. J Lipid Res 2004; 45:1069-1076

51. Ensign W, Hill N, Heward CB. Disparate LDL phenotypic classification among 4 different methods assessing LDL particle characteristics. Clin Chem 2006; 52:1722-1727

52. Hopkins PN, Pottala JV, Nanjee MN. A comparative study of four independent methods to measure LDL particle concentration. Atherosclerosis 2015; 243:99-106

53. Matera R, Horvath KV, Nair H, Schaefer EJ, Asztalos BF. HDL Particle Measurement: Comparison of 5 Methods. Clin Chem 2018; 64:492-500

54. Ramasamy I. Update on the laboratory investigation of dyslipidemias. Clin Chim Acta 2018; 479:103-125

55. Ivanova EA, Myasoedova VA, Melnichenko AA, Grechko AV, Orekhov AN. Small Dense Low-Density Lipoprotein as Biomarker for Atherosclerotic Diseases. Oxid Med Cell Longev 2017; 2017:1273042

56. Berneis KK, Krauss RM. Metabolic origins and clinical significance of LDL heterogeneity. J Lipid Res 2002; 43:1363-1379

57. Campos H, Moye LA, Glasser SP, Stampfer MJ, Sacks FM. Low-density lipoprotein size, pravastatin treatment, and coronary events. JAMA 2001; 286:1468-1474

58. Campos H, Roederer GO, Lussier-Cacan S, Davignon J, Krauss RM. Predominance of large LDL and reduced HDL2 cholesterol in normolipidemic men with coronary artery disease. Arterioscler Thromb Vasc Biol 1995; 15:1043-1048

59. Mora S, Szklo M, Otvos JD, Greenland P, Psaty BM, Goff DC, Jr., O'Leary DH, Saad MF, Tsai MY, Sharrett AR. LDL particle subclasses, LDL particle size, and carotid atherosclerosis in the Multi-Ethnic Study of Atherosclerosis (MESA). Atherosclerosis 2007; 192:211-217

60. Patsch W, Ostlund R, Kuisk I, Levy R, Schonfeld G. Characterization of lipoprotein in a kindred with familial hypercholesterolemia. J Lipid Res 1982; 23:1196-1205

61. Rudel LL, Parks JS, Johnson FL, Babiak J. Low density lipoproteins in atherosclerosis. J Lipid Res 1986; 27:465-474

62. Krauss RM, Siri PW. Metabolic abnormalities: triglyceride and low-density lipoprotein. Endocrinol Metab Clin North Am 2004; 33:405-415

63. Ip S, Lichtenstein AH, Chung M, Lau J, Balk EM. Systematic review: association of low-density lipoprotein subfractions with cardiovascular outcomes. Ann Intern Med 2009; 150:474-484

64. Krauss RM. Lipoprotein subfractions and cardiovascular disease risk. Curr Opin Lipidol 2010; 21:305-311

65. Davidson MH, Ballantyne CM, Jacobson TA, Bittner VA, Braun LT, Brown AS, Brown WV, Cromwell WC, Goldberg RB, McKenney JM, Remaley AT, Sniderman AD, Toth PP, Tsimikas S, Ziajka PE, Maki KC, Dicklin MR. Clinical utility of inflammatory markers and advanced lipoprotein testing: advice from an expert panel of lipid specialists. J Clin Lipidol 2011; 5:338-367

66. Hafiane A, Genest J. High density lipoproteins: Measurement techniques and potential biomarkers of cardiovascular risk. BBA Clin 2015; 3:175-188

67. Savolainen MJ. Epidemiology: disease associations and modulators of HDL-related biomarkers. Handb Exp Pharmacol 2015; 224:259-283

68. Superko HR, Pendyala L, Williams PT, Momary KM, King SB, 3rd, Garrett BC. High-density lipoprotein subclasses and their relationship to cardiovascular disease. Journal of clinical lipidology 2012; 6:496-523

69. Hovingh GK, Rader DJ, Hegele RA. HDL re-examined. Curr Opin Lipidol 2015; 26:127-132

70. Khera AV, Cuchel M, de la Llera-Moya M, Rodrigues A, Burke MF, Jafri K, French BC, Phillips JA, Mucksavage ML, Wilensky RL, Mohler ER, Rothblat GH, Rader DJ. Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis. N Engl J Med 2011; 364:127-135

71. Rohatgi A, Khera A, Berry JD, Givens EG, Ayers CR, Wedin KE, Neeland IJ, Yuhanna IS, Rader DR, de Lemos JA, Shaul PW. HDL cholesterol efflux capacity and incident cardiovascular events. N Engl J Med 2014; 371:2383-2393

72. Qiu C, Zhao X, Zhou Q, Zhang Z. High-density lipoprotein cholesterol efflux capacity is inversely associated with cardiovascular risk: a systematic review and meta-analysis. Lipids Health Dis 2017; 16:212

73. Anastasius M, Kockx M, Jessup W, Sullivan D, Rye KA, Kritharides L. Cholesterol efflux capacity: An introduction for clinicians. Am Heart J 2016; 180:54-63

74. Navab M, Reddy ST, Van Lenten BJ, Anantharamaiah GM, Fogelman AM. The role of dysfunctional HDL in atherosclerosis. J Lipid Res 2009; 50 Suppl:S145-149

75. Feingold KR, Grunfeld C. The Effect of Inflammation and Infection on Lipids and Lipoproteins. In: De Groot LJ, Beck-Peccoz P, Chrousos G, Dungan K, Grossman A, Hershman JM, Koch C, McLachlan R, New M, Rebar R, Singer F, Vinik A, Weickert MO, eds. Endotext. South Dartmouth (MA) 2022.

76. Feingold KR. Introduction to Lipids and Lipoproteins. In: De Groot LJ, Beck-Peccoz P, Chrousos G, Dungan K, Grossman A, Hershman JM, Koch C, McLachlan R, New M, Rebar R, Singer F, Vinik A, Weickert MO, eds. Endotext. South Dartmouth (MA) 2021.

77. Enkhmaa B, Anuurad E, Zhang W, Berglund L. Significant associations between lipoprotein(a) and corrected apolipoprotein B-100 levels in African-Americans. Atherosclerosis 2014; 235:223-229

78. Benn M, Nordestgaard BG, Jensen GB, Tybjaerg-Hansen A. Improving prediction of ischemic cardiovascular disease in the general population using apolipoprotein B: the Copenhagen City Heart Study. Arterioscler Thromb Vasc Biol 2007; 27:661-670

79. Chien KL, Hsu HC, Su TC, Chen MF, Lee YT, Hu FB. Apolipoprotein B and non-high density lipoprotein cholesterol and the risk of coronary heart disease in Chinese. J Lipid Res 2007; 48:2499-2505

80. Holme I, Aastveit AH, Jungner I, Walldius G. Relationships between lipoprotein components and risk of myocardial infarction: age, gender and short versus longer follow-up periods in the Apolipoprotein MOrtality RISk study (AMORIS). J Intern Med 2008; 264:30-38

81. Ingelsson E, Schaefer EJ, Contois JH, McNamara JR, Sullivan L, Keyes MJ, Pencina MJ, Schoonmaker C, Wilson PW, D'Agostino RB, Vasan RS. Clinical utility of different lipid measures for prediction of coronary heart disease in men and women. JAMA 2007; 298:776-785

82. McQueen MJ, Hawken S, Wang X, Ounpuu S, Sniderman A, Probstfield J, Steyn K, Sanderson JE, Hasani M, Volkova E, Kazmi K, Yusuf S, Interheart Study Investigators. Lipids, lipoproteins, and apolipoproteins as risk markers of myocardial infarction in 52 countries (the INTERHEART study): a case-control study. Lancet 2008; 372:224-233

83. Mora S, Otvos JD, Rifai N, Rosenson RS, Buring JE, Ridker PM. Lipoprotein particle profiles by nuclear magnetic resonance compared with standard lipids and apolipoproteins in predicting incident cardiovascular disease in women. Circulation 2009; 119:931-939

84. Parish S, Peto R, Palmer A, Clarke R, Lewington S, Offer A, Whitlock G, Clark S, Youngman L, Sleight P, Collins R, International Studies of Infarct Survival Collaboration. The joint effects of apolipoprotein B, apolipoprotein A1, LDL cholesterol, and HDL cholesterol on risk: 3510 cases of acute myocardial infarction and 9805 controls. Eur Heart J 2009; 30:2137-2146

85. Shai I, Rimm EB, Hankinson SE, Curhan G, Manson JE, Rifai N, Stampfer MJ, Ma J. Multivariate assessment of lipid parameters as predictors of coronary heart disease among postmenopausal women: potential implications for clinical guidelines. Circulation 2004; 110:2824-2830

86. Sniderman AD, Williams K, Contois JH, Monroe HM, McQueen MJ, de Graaf J, Furberg CD. A meta-analysis of low-density lipoprotein cholesterol, non-high-density lipoprotein cholesterol, and apolipoprotein B as markers of cardiovascular risk. Circ Cardiovasc Qual Outcomes 2011; 4:337-345

87. Welsh C, Celis-Morales CA, Brown R, Mackay DF, Lewsey J, Mark PB, Gray SR, Ferguson LD, Anderson JJ, Lyall DM, Cleland JG, Jhund PS, Gill JMR, Pell JP, Sattar N, Welsh P. Comparison of Conventional Lipoprotein Tests and Apolipoproteins in the Prediction of Cardiovascular Disease. Circulation 2019; 140:542-552

88. Parish S, Offer A, Clarke R, Hopewell JC, Hill MR, Otvos JD, Armitage J, Collins R, Heart Protection Study Collaborative Group. Lipids and lipoproteins and risk of different vascular events in the MRC/BHF Heart Protection Study. Circulation 2012; 125:2469-2478

89. Robinson JG, Wang S, Jacobson TA. Meta-analysis of comparison of effectiveness of lowering apolipoprotein B versus low-density lipoprotein cholesterol and nonhigh-density lipoprotein cholesterol for cardiovascular risk reduction in randomized trials. Am J Cardiol 2012; 110:1468-1476

90. Sniderman AD, Islam S, Yusuf S, McQueen MJ. Discordance analysis of apolipoprotein B and non-high density lipoprotein cholesterol as markers of cardiovascular risk in the INTERHEART study. Atherosclerosis 2012; 225:444-449

91. Lawler PR, Akinkuolie AO, Ridker PM, Sniderman AD, Buring JE, Glynn RJ, Chasman DI, Mora S. Discordance between Circulating Atherogenic Cholesterol Mass and Lipoprotein Particle Concentration in Relation to Future Coronary Events in Women. Clin Chem 2017; 63:870-879

92. Wilkins JT, Li RC, Sniderman A, Chan C, Lloyd-Jones DM. Discordance Between Apolipoprotein B and LDL-Cholesterol in Young Adults Predicts Coronary Artery Calcification: The CARDIA Study. J Am Coll Cardiol 2016; 67:193-201

93. Pencina MJ, D'Agostino RB, Zdrojewski T, Williams K, Thanassoulis G, Furberg CD, Peterson ED, Vasan RS, Sniderman AD. Apolipoprotein B improves risk assessment of future coronary heart disease in the Framingham Heart Study beyond LDL-C and non-HDL-C. Eur J Prev Cardiol 2015; 22:1321-1327

94. Emerging Risk Factors Collaboration, Di Angelantonio E, Gao P, Pennells L, Kaptoge S, Caslake M, Thompson A, Butterworth AS, Sarwar N, Wormser D, Saleheen D, Ballantyne CM, Psaty BM, Sundstrom J, Ridker PM, Nagel D, Gillum RF, Ford I, Ducimetiere P, Kiechl S, Koenig W, Dullaart RP, Assmann G, D'Agostino RB, Sr., Dagenais GR, Cooper JA, Kromhout D, Onat A, Tipping RW, Gomez-de-la-Camara A, Rosengren A, Sutherland SE, Gallacher J, Fowkes FG, Casiglia E, Hofman A, Salomaa V, Barrett-Connor E, Clarke R, Brunner E, Jukema JW, Simons LA, Sandhu M, Wareham NJ, Khaw KT, Kauhanen J, Salonen JT, Howard WJ, Nordestgaard BG, Wood AM, Thompson SG, Boekholdt SM, Sattar N, Packard C, Gudnason V, Danesh J. Lipid-related markers and cardiovascular disease prediction. JAMA 2012; 307:2499-2506

95. Thanassoulis G, Williams K, Ye K, Brook R, Couture P, Lawler PR, de Graaf J, Furberg CD, Sniderman A. Relations of change in plasma levels of LDL-C, non-HDL-C and apoB with risk reduction from statin therapy: a meta-analysis of randomized trials. J Am Heart Assoc 2014; 3:e000759

96. Jacobson TA, Ito MK, Maki KC, Orringer CE, Bays HE, Jones PH, McKenney JM, Grundy SM, Gill EA, Wild RA, Wilson DP, Brown WV. National lipid association recommendations for patient-centered management of dyslipidemia: part 1--full report. J Clin Lipidol 2015; 9:129-169

97. Boot CS, Middling E, Allen J, Neely RDG. Evaluation of the Non-HDL Cholesterol to Apolipoprotein B Ratio as a Screening Test for Dysbetalipoproteinemia. Clin Chem 2019; 65:313-320

98. Arvind A, Osganian SA, Cohen DE, Corey KE. Lipid and Lipoprotein Metabolism in Liver Disease. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, de Herder WW, Dhatariya K, Dungan K, Hershman JM, Hofland J, Kalra S, Kaltsas G, Koch C, Kopp P, Korbonits M, Kovacs CS, Kuohung W, Laferrere B, Levy M, McGee EA, McLachlan R, Morley JE, New M, Purnell J, Sahay R, Singer F, Sperling MA, Stratakis CA, Trence DL, Wilson DP, eds. Endotext. South Dartmouth (MA) 2019.

99. Frost RJ, Otto C, Geiss HC, Schwandt P, Parhofer KG. Effects of atorvastatin versus fenofibrate on lipoprotein profiles, low-density lipoprotein subfraction distribution, and hemorheologic parameters in type 2 diabetes mellitus with mixed hyperlipoproteinemia. Am J Cardiol 2001; 87:44-48

100. Yuan J, Tsai MY, Hunninghake DB. Changes in composition and distribution of LDL subspecies in hypertriglyceridemic and hypercholesterolemic patients during gemfibrozil therapy. Atherosclerosis 1994; 110:1-11

101. 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

102. Otvos JD, Jeyarajah EJ, Cromwell WC. Measurement issues related to lipoprotein heterogeneity. Am J Cardiol 2002; 90:22i-29i

103. Cromwell WC, Otvos JD, Keyes MJ, Pencina MJ, Sullivan L, Vasan RS, Wilson PW, D'Agostino RB. LDL Particle Number and Risk of Future Cardiovascular Disease in the Framingham Offspring Study - Implications for LDL Management. J Clin Lipidol 2007; 1:583-592

104. Otvos JD, Mora S, Shalaurova I, Greenland P, Mackey RH, Goff DC, Jr. Clinical implications of discordance between low-density lipoprotein cholesterol and particle number. J Clin Lipidol 2011; 5:105-113

105. Blake GJ, Otvos JD, Rifai N, Ridker PM. Low-density lipoprotein particle concentration and size as determined by nuclear magnetic resonance spectroscopy as predictors of cardiovascular disease in women. Circulation 2002; 106:1930-1937

106. El Harchaoui K, van der Steeg WA, Stroes ES, Kuivenhoven JA, Otvos JD, Wareham NJ, Hutten BA, Kastelein JJ, Khaw KT, Boekholdt SM. Value of low-density lipoprotein particle number and size as predictors of coronary artery disease in apparently healthy men and women: the EPIC-Norfolk Prospective Population Study. J Am Coll Cardiol 2007; 49:547-553

107. Manickam P, Rathod A, Panaich S, Hari P, Veeranna V, Badheka A, Jacob S, Afonso L. Comparative prognostic utility of conventional and novel lipid parameters for cardiovascular disease risk prediction: do novel lipid parameters offer an advantage? J Clin Lipidol 2011; 5:82-90

108. Degoma EM, Davis MD, Dunbar RL, Mohler ER, 3rd, Greenland P, French B. Discordance between non-HDL-cholesterol and LDL-particle measurements: results from the Multi-Ethnic Study of Atherosclerosis. Atherosclerosis 2013; 229:517-523

109. Varvel SA, Dayspring TD, Edmonds Y, Thiselton DL, Ghaedi L, Voros S, McConnell JP, Sasinowski M, Dall T, Warnick GR. Discordance between apolipoprotein B and low-density lipoprotein particle number is associated with insulin resistance in clinical practice. J Clin Lipidol 2015; 9:247-255

110. Gudnason V. Lipoprotein(a): a causal independent risk factor for coronary heart disease? Curr Opin Cardiol 2009; 24:490-495

111. Koschinsky ML, Boffa MB. Lipoprotein(a): an important cardiovascular risk factor and a clinical conundrum. Endocrinol Metab Clin North Am 2014; 43:949-962

112. Lamon-Fava S, Diffenderfer MR, Marcovina SM. Lipoprotein(a) metabolism. Curr Opin Lipidol 2014; 25:189-193

113. Schmidt K, Noureen A, Kronenberg F, Utermann G. Structure, function, and genetics of lipoprotein (a). J Lipid Res 2016; 57:1339-1359

114. Enkhmaa B, Anuurad E, Berglund L. Lipoprotein (a): impact by ethnicity and environmental and medical conditions. J Lipid Res 2016; 57:1111-1125

115. Emerging Risk Factors Collaboration, Erqou S, Kaptoge S, Perry PL, Di Angelantonio E, Thompson A, White IR, Marcovina SM, Collins R, Thompson SG, Danesh J. Lipoprotein(a) concentration and the risk of coronary heart disease, stroke, and nonvascular mortality. JAMA 2009; 302:412-423

116. Bennet A, Di Angelantonio E, Erqou S, Eiriksdottir G, Sigurdsson G, Woodward M, Rumley A, Lowe GD, Danesh J, Gudnason V. Lipoprotein(a) levels and risk of future coronary heart disease: large-scale prospective data. Arch Intern Med 2008; 168:598-608

117. Alonso R, Andres E, Mata N, Fuentes-Jimenez F, Badimon L, Lopez-Miranda J, Padro T, Muniz O, Diaz-Diaz JL, Mauri M, Ordovas JM, Mata P, Safeheart Investigators. Lipoprotein(a) levels in familial hypercholesterolemia: an important predictor of cardiovascular disease independent of the type of LDL receptor mutation. J Am Coll Cardiol 2014; 63:1982-1989

118. Clarke R, Peden JF, Hopewell JC, Kyriakou T, Goel A, Heath SC, Parish S, Barlera S, Franzosi MG, Rust S, Bennett D, Silveira A, Malarstig A, Green FR, Lathrop M, Gigante B, Leander K, de Faire U, Seedorf U, Hamsten A, Collins R, Watkins H, Farrall M, Procardis Consortium. Genetic variants associated with Lp(a) lipoprotein level and coronary disease. N Engl J Med 2009; 361:2518-2528

119. Hobbs HH, Chiesa G, Gaw A, Lawn R, Maika SD, Koschinsky M, Hammer R. Apo(a) expression in transgenic mice. Ann N Y Acad Sci 1994; 714:231-236

120. Liu AC, Lawn RM. Vascular interactions of lipoprotein (a). Curr Opin Lipidol 1994; 5:269-273

121. Durrington PN, Ishola M, Hunt L, Arrol S, Bhatnagar D. Apolipoproteins (a), AI, and B and parental history in men with early onset ischaemic heart disease. Lancet 1988; 1:1070-1073

122. Boffa MB, Stranges S, Klar N, Moriarty PM, Watts GF, Koschinsky ML. Lipoprotein(a) and secondary prevention of atherothrombotic events: A critical appraisal. J Clin Lipidol 2018; 12:1358-1366

123. Albers JJ, Slee A, O'Brien KD, Robinson JG, Kashyap ML, Kwiterovich PO, Jr., Xu P, Marcovina SM. Relationship of apolipoproteins A-1 and B, and lipoprotein(a) to cardiovascular outcomes: the AIM-HIGH trial (Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglyceride and Impact on Global Health Outcomes). J Am Coll Cardiol 2013; 62:1575-1579

124. Nestel PJ, Barnes EH, Tonkin AM, Simes J, Fournier M, White HD, Colquhoun DM, Blankenberg S, Sullivan DR. Plasma lipoprotein(a) concentration predicts future coronary and cardiovascular events in patients with stable coronary heart disease. Arterioscler Thromb Vasc Biol 2013; 33:2902-2908

125. Arsenault BJ, Barter P, DeMicco DA, Bao W, Preston GM, LaRosa JC, Grundy SM, Deedwania P, Greten H, Wenger NK, Shepherd J, Waters DD, Kastelein JJ, Treating to New Targets Investigators. Prediction of cardiovascular events in statin-treated stable coronary patients of the treating to new targets randomized controlled trial by lipid and non-lipid biomarkers. PLoS One 2014; 9:e114519

126. Berg K, Dahlen G, Christophersen B, Cook T, Kjekshus J, Pedersen T. Lp(a) lipoprotein level predicts survival and major coronary events in the Scandinavian Simvastatin Survival Study. Clin Genet 1997; 52:254-261

127. Khera AV, Everett BM, Caulfield MP, Hantash FM, Wohlgemuth J, Ridker PM, Mora S. Lipoprotein(a) concentrations, rosuvastatin therapy, and residual vascular risk: an analysis from the JUPITER Trial (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin). Circulation 2014; 129:635-642

128. O'Donoghue ML, Morrow DA, Tsimikas S, Sloan S, Ren AF, Hoffman EB, Desai NR, Solomon SD, Domanski M, Arai K, Chiuve SE, Cannon CP, Sacks FM, Sabatine MS. Lipoprotein(a) for risk assessment in patients with established coronary artery disease. J Am Coll Cardiol 2014; 63:520-527

129. Zewinger S, Kleber ME, Tragante V, McCubrey RO, Schmidt AF, Direk K, Laufs U, Werner C, Koenig W, Rothenbacher D, Mons U, Breitling LP, Brenner H, Jennings RT, Petrakis I, Triem S, Klug M, Filips A, Blankenberg S, Waldeyer C, Sinning C, Schnabel RB, Lackner KJ, Vlachopoulou E, Nygard O, Svingen GFT, Pedersen ER, Tell GS, Sinisalo J, Nieminen MS, Laaksonen R, Trompet S, Smit RAJ, Sattar N, Jukema JW, Groesdonk HV, Delgado G, Stojakovic T, Pilbrow AP, Cameron VA, Richards AM, Doughty RN, Gong Y, Cooper-DeHoff R, Johnson J, Scholz M, Beutner F, Thiery J, Smith JG, Vilmundarson RO, McPherson R, Stewart AFR, Cresci S, Lenzini PA, Spertus JA, Olivieri O, Girelli D, Martinelli NI, Leiherer A, Saely CH, Drexel H, Mundlein A, Braund PS, Nelson CP, Samani NJ, Kofink D, Hoefer IE, Pasterkamp G, Quyyumi AA, Ko YA, Hartiala JA, Allayee H, Tang WHW, Hazen SL, Eriksson N, Held C, Hagstrom E, Wallentin L, Akerblom A, Siegbahn A, Karp I, Labos C, Pilote L, Engert JC, Brophy JM, Thanassoulis G, Bogaty P, Szczeklik W, Kaczor M, Sanak M, Virani SS, Ballantyne CM, Lee VV, Boerwinkle E, Holmes MV, Horne BD, Hingorani A, Asselbergs FW, Patel RS, consortium G-C, Kramer BK, Scharnagl H, Fliser D, Marz W, Speer T. Relations between lipoprotein(a) concentrations, LPA genetic variants, and the risk of mortality in patients with established coronary heart disease: a molecular and genetic association study. Lancet Diabetes Endocrinol 2017; 5:534-543

130. Schwartz GG, Ballantyne CM, Barter PJ, Kallend D, Leiter LA, Leitersdorf E, McMurray JJV, Nicholls SJ, Olsson AG, Shah PK, Tardif JC, Kittelson J. Association of Lipoprotein(a) With Risk of Recurrent Ischemic Events Following Acute Coronary Syndrome: Analysis of the dal-

Outcomes Randomized Clinical Trial. JAMA Cardiol 2018; 3:164-168

131. Puri R, Ballantyne CM, Hoogeveen RC, Shao M, Barter P, Libby P, Chapman MJ, Erbel R, Arsenault BJ, Raichlen JS, Nissen SE, Nicholls SJ. Lipoprotein(a) and coronary atheroma progression rates during long-term high-intensity statin therapy: Insights from SATURN. Atherosclerosis 2017; 263:137-144

132. Willeit P, Ridker PM, Nestel PJ, Simes J, Tonkin AM, Pedersen TR, Schwartz GG, Olsson AG, Colhoun HM, Kronenberg F, Drechsler C, Wanner C, Mora S, Lesogor A, Tsimikas S. Baseline and on-statin treatment lipoprotein(a) levels for prediction of cardiovascular events: individual patient-data meta-analysis of statin outcome trials. Lancet 2018; 392:1311-1320

133. Wei WQ, Li X, Feng Q, Kubo M, Kullo IJ, Peissig PL, Karlson EW, Jarvik GP, Lee MTM, Shang N, Larson EA, Edwards T, Shaffer CM, Mosley JD, Maeda S, Horikoshi M, Ritchie M, Williams MS, Larson EB, Crosslin DR, Bland ST, Pacheco JA, Rasmussen-Torvik LJ, Cronkite D, Hripcsak G, Cox NJ, Wilke RA, Stein CM, Rotter JI, Momozawa Y, Roden DM, Krauss RM, Denny JC. LPA Variants Are Associated With Residual Cardiovascular Risk in Patients Receiving Statins. Circulation 2018; 138:1839-1849

134. Tsimikas S, Viney NJ, Hughes SG, Singleton W, Graham MJ, Baker BF, Burkey JL, Yang Q, Marcovina SM, Geary RS, Crooke RM, Witztum JL. Antisense therapy targeting apolipoprotein(a): a randomised, double-blind, placebo-controlled phase 1 study. Lancet 2015; 386:1472-1483

135. Tsimikas S. RNA-targeted therapeutics for lipid disorders. Curr Opin Lipidol 2018; 29:459-466

136. Kronenberg F, Mora S, Stroes ESG. Consensus and guidelines on lipoprotein(a) - seeing the forest through the trees. Curr Opin Lipidol 2022; 33:342-352

137. Pearson GJ, Thanassoulis G, Anderson TJ, Barry AR, Couture P, Dayan N, Francis GA, Genest J, Gregoire J, Grover SA, Gupta M, Hegele RA, Lau D, Leiter LA, Leung AA, Lonn E, Mancini GBJ, Manjoo P, McPherson R, Ngui D, Piche ME, Poirier P, Sievenpiper J, Stone J, Ward R, Wray W. 2021 Canadian Cardiovascular Society Guidelines for the Management of Dyslipidemia for the Prevention of Cardiovascular Disease in Adults. Can J Cardiol 2021; 37:1129-1150

138. Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, Chapman MJ, De Backer GG, Delgado V, Ference BA, Graham IM, Halliday A, Landmesser U, Mihaylova B, Pedersen TR, Riccardi G, Richter DJ, Sabatine MS, Taskinen MR, Tokgozoglu L, Wiklund O, Group ESCSD. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. Eur Heart J 2020; 41:111-188

139. Tsimikas S, Fazio S, Ferdinand KC, Ginsberg HN, Koschinsky ML, Marcovina SM, Moriarty PM, Rader DJ, Remaley AT, Reyes-Soffer G, Santos RD, Thanassoulis G, Witztum JL, Danthi S, Olive M, Liu L. NHLBI Working Group Recommendations to Reduce Lipoprotein(a)-Mediated Risk of Cardiovascular Disease and Aortic Stenosis. J Am Coll Cardiol 2018; 71:177-192

140. Wilson DP, Jacobson TA, Jones PH, Koschinsky ML, McNeal CJ, Nordestgaard BG, Orringer CE. Use of lipoprotein(a) in clinical practice: A biomarker whose time has come. A scientific statement from the National Lipid Association. Don P. Wilson, MD, on behalf of the Writing group. J Clin Lipidol 2019;

141. van Capelleveen JC, van der Valk FM, Stroes ES. Current therapies for lowering lipoprotein (a). J Lipid Res 2016; 57:1612-1618

142. Yeang C, Witztum JL, Tsimikas S. 'LDL-C' = LDL-C + Lp(a)-C: implications of achieved ultra-low LDL-C levels in the proprotein convertase subtilisin/kexin type 9 era of potent LDL-C lowering. Curr Opin Lipidol 2015; 26:169-178

143. Yeang C, Witztum JL, Tsimikas S. Novel method for quantification of lipoprotein(a)-cholesterol: implications for improving accuracy of LDL-C measurements. J Lipid Res 2021; 62:100053

144. Marcovina SM, Albers JJ. Lipoprotein (a) measurements for clinical application. J Lipid Res 2016; 57:526-537

145. Scharnagl H, Stojakovic T, Dieplinger B, Dieplinger H, Erhart G, Kostner GM, Herrmann M, Marz W, Grammer TB. Comparison of lipoprotein (a) serum concentrations measured by six commercially available immunoassays. Atherosclerosis 2019; 289:206-213

146. Wyness SP, Genzen JR. Performance evaluation of five lipoprotein(a) immunoassays on the Roche cobas c501 chemistry analyzer. Pract Lab Med 2021; 25:e00218

147. Marcovina SM, Navabi N, Allen S, Gonen A, Witztum JL, Tsimikas S. Development and validation of an isoform-independent monoclonal antibody-based ELISA for measurement of lipoprotein(a). J Lipid Res 2022; 63:100239