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# The Role of Advanced Lipid Testing in the Prediction of Cardiovascular Disease

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**Abstract** Advanced lipid testing has been suggested by some experts to identify patients with substantial residual risk for more aggressive targeting of lifestyle and pharmacologic therapies. It measures the subpopulation of lipoproteins and apolipoproteins, which include lipoprotein (a), apolipoprotein A-I, and apolipoprotein B, and measures of lipoprotein particle composition such as LDL particle (LPL-P) and HDL particle (HDL-P) number and size. Obesity is associated with smaller LDL-P and HDL-P sizes. Moderate weight loss via fasting/calorie restriction is associated with LDL-P size increase, whereas moderate weight loss via endurance exercise is associated with HDL-P size increase. Diets high in carbohydrates are associated with a more atherogenic advanced lipoprotein profile characterized by smaller LDL-P and HDL-P sizes. In summary, lifestyle changes such as weight loss, exercise, and dietary modification correlate with improvement in the profile of advanced lipoproteins. Regrettably, therapies targeting HDL and HDL composition have been disappointing to date.

**Keywords** Lipoprotein · Particles · Exercise · Diet

## Introduction

On the basis of a preponderance of evidence and current National Cholesterol Education Program Adult Treatment Panel III guidelines, low-density lipoprotein (LDL) cholesterol (LDL-C) level is the primary lipid target to lower the risk of coronary heart disease (CHD), resulting in significant reductions in nonfatal and fatal CHD events [1]. Efforts are continually being made to further reduce residual CHD risk. On the basis of National Cholesterol Education Program Adult Treatment Panel III guidelines, non-high-density-lipoprotein cholesterol (non-HDL-C) is a secondary lipid target for patients with a triglyceride level above 200 mg/dL. Non-HDL-C has been shown to be superior to LDL-C in predicting secondary CHD events in patients taking a statin [2]. Unfortunately, a significant number of patients continue to have CHD events, indicating substantial residual risk. Advanced lipid testing or lipoprotein analysis has been suggested by some experts to identify these patients for more aggressive targeting of lifestyle and pharmacologic therapies.

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## What Does Advanced Lipoprotein Testing Measure?

Advanced lipoprotein testing measures the subpopulation of lipoproteins and apolipoproteins, which include lipoprotein (a) [Lp(a)], apolipoprotein A-I (apo A-I), apolipoprotein B (apo B), and measures of lipoprotein particle composition. Lipid synthesis begins in the liver and results in the formation of very low density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and LDL. VLDL, IDL, and LDL all carry apolipoprotein B on their surface in a consistent 1:1 ratio and are considered atherogenic. On the other hand, high-density lipoprotein (HDL) particles (HDL-P) carry apo A-I molecules, although not in a 1:1 ratio, and are considered antiatherogenic [3]. The composition of all lipoprotein particles (VLDL, IDL, LDL, HDL) can be characterized by the total particle number, average particle size, and proportion of small, medium, and large particles. Lp(a) is a plasma protein consisting of an LDL particle (LDL-P) and apolipoprotein (a) and is atherogenic.

LDL-P and HDL-P contain a certain amount of esterified cholesterol within their hydrophobic cores. However, there is often discordance between the total cholesterol content as measured by routine laboratory analysis and particle composition. Studies have shown that this discordance can be clinically meaningful and predictive of CHD [4••]. Therefore, advanced lipoprotein testing offers an opportunity to delineate that discordance to improve risk prediction or determine the intensity of therapy.

## How Are Advanced Lipoproteins Measured?

Apo B and apo A-I levels are most commonly measured by the vertical auto profile (VAP) test or nuclear magnetic resonance (NMR) spectroscopy. The VAP test uses a density gradient rapid ultracentrifugation technique to measure the size and charge of the apolipoproteins. The NMR method uses magnetic resonance to estimate the lipoprotein distribution using proton spectroscopy methods. Another measurement method used is an immunoassay. All three methods for the measurement of apo B and apo A-I are considered comparable by international standards [5]. However, there is significant variability among these tests. Apo B and apo A-I levels were found to be highest when measured by immunoassays, intermediate when measured by NMR spectroscopy (14 % lower than for immunoassays), and lowest when measured by the VAP test (17 % lower than for immunoassays) [6•].

In contrast, there is no international standard for lipoprotein subclass composition assessment, including HDL-P and LDL-P number and size. Currently, the following methods are available: NMR spectroscopy, VAP test, gradient gel electrophoresis, and microfluidic gel electrophoresis using a chip technology. Thus far, there is significant lack of agreement between the methods in determining particle number and size [7].

## Effects of Weight Loss and Exercise on Advanced Lipoproteins

Obesity has long been associated with unfavorable routine lipid profiles, i.e., high triglyceride and low HDL-C levels [8]. Conversely, weight loss and exercise have been associated with reduced levels of triglycerides and increased HDL-C [9]. The impact of obesity on lipoprotein particle composition is less well established. A cross-sectional study comparing obese (BMI 30–45 kg/m$^2$) and nonobese (BMI 18.5–25 kg/m$^2$) participants who were normotensive and nondiabetic found that obese participants, on average, had smaller LDL-P size ($p<0.05$) and HDL-P size ($p<0.05$), both measured by NMR spectroscopy [10].

A cohort study involving 683 adult Finnish participants with 6.5 years of follow-up examined changes in lipoprotein particle concentration and sizes (measured by NMR spectroscopy) [11]. Moderate weight loss (5 % or more) was associated with decreased particle concentrations of all apo B-containing lipoproteins, increased concentration of large HDL-P [24.1 %, 95 % confidence interval (CI) 15.8–32.5 %; $p<0.001$] and decreased concentration of small HDL-P (−9.0 %, 95 % CI −13.1 to −4.9 %; $p<0.001$). The favorable changes in lipoprotein subclass profiles highlight a potential mechanism by which weight loss can modify cardiovascular risk.

Other studies consistently show similar relationships between weight change and lipoprotein subclasses. For a period of 12 weeks, 60 overweight/obese adult participants were randomized to one of the following four groups: an alternate-day fasting (ADF) group, a calorie restriction group, an exercise group, and a control group [12]. All groups but the control group achieved moderate weight loss (mean weight loss of 5 %). HDL-P and LDL-P were measured by polyacrylamide gel electrophoresis. Remarkably, the methods to achieve weight loss affected HDL and LDL in a distinctive fashion. Relative to the baseline, ADF increased LDL-P size (265±2 Å vs 261±1 Å; $p=0.01$), decreased the proportion of small LDL-P (18±3 % vs 25±3 %; $p=0.04$), but had no impact on HDL-P size. Calorie restriction increased LDL-P size (264±2 Å vs 260±2 Å; $p=0.01$) relative to the baseline, and had no significant impact on LDL-P proportion and HDL-P size. Exercise increased the proportion of large HDL-P relative to the baseline (34±3 % vs. 28±3 %; $p=0.04$), but had no impact on LDL-P size or the proportion of small LDL-P. This result seems to indicate that LDL-P is more sensitive to dietary modification, whereas HDL-P is more sensitive to exercise.

In a follow-up study by the same investigators, 64 obese participants were randomized to one of the following four groups: a group subjected to a combination of ADF and endurance exercise (both as described above), an ADF group, an exercise group, and a control group [13]. The combination group showed an increase in LDL-P size (4±1 Å; $p<0.001$) and a decrease in the proportion of small HDL-P (11±1 % vs 15±2 %; $p=0.007$). The ADF-only group showed a similar significant increase in LDL-P size (5±1 Å; $p<0.001$), but no impact on HDL-P size. This study supports the synergistic effects of both calorie restriction and exercise on lipoprotein subclasses.

On the other hand, the Studies of a Targeted Risk Reduction Intervention Through Defined Exercise (STTRIDE) trial showed a negligible effect of diet in the setting of active exercise on lipoproteins [14]. In this study, obese participants were randomized to an aerobic exercise program or inactivity for 6 months. There were a total of 204 participants in which all nutrition and lipid parameters were available. Exercise,

independent of dietary changes, was found by NMR spectroscopy to decrease the concentration of LDL-P ($p$=0.03), increase LDL-P size ($p$=0.009), and increase HDL-P size ($p$=0.05). Close adherence to American Heart Association diet was not associated with any significant changes in the advanced lipoprotein profile.

In summary, obesity is associated with smaller LDL-P and HDL-P sizes. Moderate weight loss has been shown to reverse these effects by increasing LDL-P and HDL-P sizes. Moderate weight loss via fasting/calorie restriction is associated with LDL-P size increase, whereas moderate weight loss via endurance exercise is associated with HDL-P size increase. The combined effects of dietary changes and active exercise on lipoprotein subclasses seem to be heterogeneous thus far (Table 1).

## Effects of Diet on Advanced Lipoproteins

As discussed already, weight loss and exercise appear to improve the advanced lipoprotein profile in obese individuals, but the role of diet independent of weight loss and exercise remains unclear.

A randomized, double-blind, crossover study subjected 12 nonobese adult participants with normal lipid profiles to high-fat and low-fat diets for 3 days [15]. Both diets were isocaloric. High-fat diet was defined as 37 % energy from fat and 50 % from carbohydrates, whereas low-fat diet was defined as 25 % energy from fat and 62 % from carbohydrates. After 3 days, fasting serum lipid levels and LDL-P size (assessed by polyacrylamide gradient gel electrophoresis) were obtained. In only 3 days of feeding, participants subjected to the high-fat diet showed a significant increase in the size of LDL-P (255.0 Å vs 255.9 Å; $p$=0.01) and a decrease in the proportion of small LDL-P (smaller than 255.0 Å; 50.7 % vs 44.6 %; $p$=0.01).

Another randomized, crossover study subjected 63 healthy, nonobese adult participants to 4 weeks of a high-fat, low-carbohydrate (HFLC) diet and 4 weeks of a low-fat, high-carbohydrate (LFHC) diet (in random order) [16]. The HFLC diet consisted of 40 % fat, 45 % carbohydrate, and 15 % protein, whereas the LFHC diet consisted of 20 % fat, 65 % carbohydrate, and 15 % protein. Compared with the HFLC diet, participants receiving the LFHC diet had higher Lp(a) levels (19.9±13.7 mg/dL vs 17.8±12.8 mg/dL; $p$<0.01) and a smaller LDL-P peak size (256.5±8.3 Å vs. 261.6±9.5 Å; $p$<0.0001) as measured by polyacrylamide gel electrophoresis. This is consistent with the previously mentioned study in that high-fat diet is associated with increased LDL-P size. Another study randomized 35 overweight/obese adults to a reduced-fat or reduced-carbohydrate diet for 9 months [17]. Reduced fat was defined as fat approximating 30 % of daily caloric intake; whereas, reduced carbohydrate was defined as carbohydrate approximating 20 % of daily caloric intake. Again, consistent with previously mentioned studies, the reduced-carbohydrate diet showed significant increases in mean LDL size and the levels of large LDL-P and large and small HDL-P (measured by NMR spectroscopy).

Along the same lines, a single-blinded, parallel design study randomized 37 adult participants with metabolic syndrome to a whole-egg diet or a yolk-free egg substitute diet for 12 weeks [18]. Both groups were also a part of a moderately carbohydrate restricted diet. Both groups showed a reduction in LDL-P size and an increase in HDL-P size (measured by NMR spectroscopy). However, the increase in HDL-P size was greater in the whole-egg diet group (+0.22±0.30 for whole-egg diet vs +0.05±0.22 nm for yolk-free egg substitute, $p$<0.05).

Of note, a monozygotic twin cohort study from Finland studied the impact of omega-3 polyunsaturated fatty acids on the advanced lipoprotein profile [19]. The participants in this study included 24 healthy monozygotic twin pairs aged 23–33 years. The results showed significantly higher proportions of large HDL-P and lower proportions of smaller HDL-P (measured by polyacrylamide gel electrophoresis) in co-twins who had higher intake of omega-3 polyunsaturated fatty acids.

In conclusion, diets high in carbohydrates are associated with a more atherogenic advanced lipoprotein profile characterized by smaller LDL-P and HDL-P sizes (Table 1).

## Effects of Drugs on Advanced Lipoproteins

A recent systematic review examined the effects of commonly used lipid-lowering agents, i.e., statins and fibrates [20]. Statins (pravastatin, simvastatin, atorvastatin, and pitavastatin) were found to be associated with a mean 30 % decrease of LDL-P concentration (1,346±226 nmol/L vs 1,942±380 nmol/L) and a mean 27 % decrease of apo B concentration (103±21 mg/dL vs 144±31 mg/dL). Despite that, the levels of both LDL-P and apo B were still in the 42th and 54th percentile, respectively, in the population, perhaps indicating a residual CHD risk with statin therapy. Fibrates (fenofibrate, bezafibrate, and gemfibrozil) were found to decrease the levels of LDL-P (mean 10 %) and apo B (mean 6 %). Lipoproteins were measured by NMR spectroscopy.

Niacin is the most effective clinically available agent in raising HDL-C levels. It also helps lower triglyceride and LDL-C levels. However, the Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides (AIM-HIGH) trial, which compared the combination of niacin and simvastatin with simvastatin alone, was stopped for futility owing to a lack of clinical benefit [21, 22]. Furthermore, the HPS2-THRIVE study recently released its results, which showed the combination of niacin and lapiropiprant,

**Table 1** The effects of weight loss, exercise, and dietary interventions on lipoprotein composition

| Authors | Participants | Intervention | Study type | Subfraction method | Results |
|---|---|---|---|---|---|
| Weight loss and exercise | | | | | |
| Mantyselka et al. [11] | 683 | Moderate weight loss | Cohort | NMR spectroscopy | Moderate weight loss: increase in concentration of large HDL-P, decrease in concentration of small LDL-P |
| Varady et al. [12] | 60 | ADF; CR; EE | Randomized controlled | Polyacrylamide gel electrophoresis | ADF: increase in LDL-P size, decrease in concentration of LDL-P.<br>CR: increase in LDL-P size.<br>EE: increase in concentration of large HDL-P |
| Bhutani et al. [13] | 64 | ADF+EE; ADF; EE | Randomized controlled | Polyacrylamide gel electrophoresis | ADF+EE: increase in LDL-P size, decrease in concentration of small LDL-P.<br>ADF: increase in LDL-P size.<br>EE: insignificant |
| Huffman et al. [14] | 204 | Aerobic exercise | Randomized controlled | NMR spectroscopy | Decrease in concentration of LDL-P, increase in LDL-P size, increase in HDL-P size |
| Diet | | | | | |
| Guay et al. [15] | 12 | High-fat diet; low-fat diet | Randomized, double-blind, crossover | Polyacrylamide gel electrophoresis | High-fat diet: increase in LDL-P size, increase in concentration of large LDL-P, decrease in concentration of small LDL-P |
| Faghihnia et al. [16] | 63 | High-fat diet; low-fat diet | Randomized crossover | Polyacrylamide gel electrophoresis | High-fat diet: increase in LDL-P size, increase in concentration of medium-sized LDL-P, decrease in concentration of very small LDL-P, decrease in concentration of Lp(a) |
| LeCheminant et al. [17] | 35 | Low-fat diet; low-carbohydrate diet | Quasi-experimental[a] | NMR spectroscopy | Low-carbohydrate diet: increase in LDL size, increase in concentration of large LDL-P, increase in concentration of large HDL-P, increase in concentration of small HDL-P |
| Blesso et al. [18] | 37 | EGG; SUB | Single-blind, parallel design | NMR spectroscopy | EGG: decrease in LDL-P size, large increase in HDL-P size.<br>SUB: decrease in LDL-P size, increase in HDL-P size |
| Bogl et al. [19] | 48 | Omega-3 polyunsaturated fatty acid intake | Cohort twins | Polyacrylamide gel electrophoresis | Higher omega-3 polyunsaturated fatty acid intake: increase in concentration of large HDL-P, decrease in concentration of small HDL-P |

*ADF* alternate-day fasting, *CR* calorie restriction, *EE* endurance exercise, *EGG* whole eggs plus low-carbohydrate diet, *HDL-P* high-density lipoprotein particle, *LDL-P* low-density lipoprotein particle, *Lp(a)* lipoprotein (a), *SUB* yolk-free egg substitute pus low-carbohydrate diet

[a] Each of the six clinic sites was assigned as either low carbohydrate or low fat.

in addition to simvastatin, did not reduce the incidence of CHD events and was associated with increased incidence of adverse events, including incident diabetes and diabetes complications [23]. Niacin has been shown to increase HDL2 (large HDL, measured by ultracentrifugation or electrophoresis) and increase LDL-P size (measured by gel electrophoresis) [24] as well as to lower Lp(a) levels [25•].

Cholesteryl ester transfer protein (CETP) inhibitors are novel lipid-modifying agents that have been the focus of many recent trials and studies. CETP is an enzyme that is involved in HDL maturation. It removes cholesterol esters from HDL and redistributes them into VLDL and LDL in exchange for triglycerides. CETP inhibition markedly increases HDL-C levels by 50-100 %. Unfortunately, clinical trials involving

CETP inhibitors thus far have not been shown to be beneficial. The Investigation of Lipid Level Management to Understand Its Impact in Atherosclerotic Events (ILLUMINATE), which studied torcetrapib, had to be terminated early owing to an increase in the incidence of CHD events and mortality, thought to be due in part to an off-target effect on aldosterone levels and development of hypertension [26]. Of note, torcetrapib was found to increase HDL-C levels by over 60 % and increased the levels of both large HDL2 and small HDL3 particles [27]. The dal-OUTCOMES trial studied dalcetrapib, which also increases the levels of both HDL-2 and HDL-3 subfractions (measured by ultracentrifugation) [28]. This trial was terminated early for futility without any safety concerns [29]. There are two other CETP inhibitors, anacetrapib and evacetrapib, that are still in phase III trials. They likely represent the last hope for whether CETP inhibitors can be a part of the armamentarium in combating CHD.

Metformin is not typically thought of as a lipid-lowering medication. However, it is one of the few diabetic medications that actually improve CHD outcomes. Type 2 diabetes mellitus has been associated with elevated levels of small, dense LDL-P in some studies [30]. The Diabetes Prevention Program randomized 3,234 participants with impaired glucose tolerance to one of the following: metformin, 850 mg, twice daily, or intensive lifestyle changes with the goal of 7 % weight loss through a low-fat diet and exercise, or placebo twice daily. Compared with placebo, metformin was found to decrease small LDL-P concentrations (711±354 nmol/L vs 793±394 nmol/L; $p<0.01$) and increase large HDL-P concentrations (4.6±2.7 nmol/L vs 4.1±2.5 nmol/L; $p<0.01$). LDL-P and HDL-P were measured by NMR spectroscopy [31••].

## Advanced Lipoproteins and Prediction of CHD

The Stop Atherosclerosis in Native Diabetics Study (SANDS) evaluated changes in carotid intima–media thickness (CIMT) and lipid composition, including advanced lipoprotein analysis. SANDS randomized 418 diabetic adult participants with no prior CHD to aggressive (LDL-C concentration 70 mg/dL or lower, non-HDL-C concentration 100 mg/dL or lower, and systolic blood pressure 115 mmHg or lower) versus standard treatment (LDL-C concentration 100 mg/dL or lower, non-HDL-C concentration 130 mg/dL or lower, and systolic blood pressure 130 mmHg or lower). The aggressive-treatment group showed significant regression in CIMT, and it was significantly associated with decreases in LDL-C ($p<0.005$) and non-HDL-C ($p<0.001$) concentrations. LDL-P and apo B concentrations (measured by NMR spectroscopy) were not significantly decreased, but showed a trend towards significance with $p$ values of 0.07 and 0.09, respectively [32]. The role of advanced lipoprotein testing in patients with diabetes has been reviewed elsewhere [33].

The Multi-Ethnic Study of Atherosclerosis (MESA) set out to evaluate the associations between HDL-C and HDL-P concentrations with CIMT and CHD events [4••]. The study followed 5,598 adult participants without baseline CHD for a mean of 6 years of follow-up. HDL-P concentration was measured by NMR spectroscopy. HDL-C and HDL-P concentrations correlated with each other ($r=0.69$) and LDL-P concentration ($r=-0.38$, $-0.25$, respectively), $p<0.05$ for all. For one standard deviation higher HDL-C (15 mg/dL) or HDL-P (6.64 μmol/L) concentration, the CIMT differences were −26.1 μm (95 % CI −34.7 to −17.4 μm) and −30.1 μm (95 % CI −38.8 to −21.4 μm), and the CHD hazard ratios were 0.74 (95 % CI 0.63–0.88) and 0.70 (95 % CI 0.59–0.82), respectively. Adjusted for each other and LDL-P concentration, HDL-C concentration was no longer associated with CIMT [2.3 μm (95 % CI −9.5 to 14.2 μm)] or CHD [hazard ratio 0.97 (95 % CI 0.77–1.22)], but HDL-P concentration remained independently associated with CIMT [−22.2 μm (95 % CI −33.8 to−10.6 μm)] and CHD [hazard ratio 0.75 (95 % CI 0.61–0.93)]. Interactions by sex, ethnicity, diabetes and high-sensitivity C-reactive protein were not significant.

The (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) trial [34•] evaluated alternative HDL measures such as HDL-P size and HDL-P concentration (measured by NMR spectroscopy) as markers of residual CHD risk. The study randomized 10,866 adult participants with CHD to rosuvastatin at 20 mg/day or placebo. After the first CHD event, HDL-P size and HDL-P, HDL-C, and apo A-I concentrations were measured ($n=234$). The rosuvastatin group was associated with increases in apo A-I concentration (2.1 %; $p<0.0001$), HDL-P concentration (3.8 %; $p<0.0001$), and HDL size (1.2 %; $p<0.0001$). Among the placebo group, apo A-I, and HDL-P concentrations showed similar inverse associations with CHD. The risk-factor-adjusted hazard ratio, 95 % CI per standard deviation, and $p$ value are as follows: 0.79, 0.63-0.98, and 0.03 for HDL-C, 0.75, 0.62-0.92, and 0.004 for apo A-I, and 0.81, 0.67-0.97, and 0.02 for HDL-P. However among the treatment group, only HDL-P concentration remained significantly inversely associated with CHD. The risk-factor-adjusted hazard ratio, 95 % CI per standard deviation, and $p$ value are as follows: 0.73, 0.57-0.93, and 0.01 for HDL-P, 0.82, 0.63-1.08, and 0.16 for HDL-C, and 0.86, 0.67-1.10, and 0.22 for apo A-I .

## Conclusions

Advanced lipoproteins, in particular apo B, total LDL-P, and total HDL-P, have been shown to predict CHD at baseline and on treatment, independently of traditional lipid measurements. Lifestyle changes such as weight loss, exercise, and dietary modification correlate with improvement in advanced

lipoprotein profiles. Therapies targeting HDL and HDL composition have been disappointing to date. Although there is not sufficient evidence to support advanced lipoprotein testing broadly, future studies may elucidate specific clinical scenarios well suited for measurement of apo B, apo A-I, Lp(a), and HDL/LDL particle composition.

**Compliance with Ethics Guidelines**

**Conflict of Interest** Alvin Chandra declares he has no conflict of interest.

Anand Rohatgi has received a research grant from Merck (significant conflict of interest), and is on the Advisory Board of Aegerion (modest conflict of interest).

**Human and Animal Rights and Informed Consent** This article does not contain any studies with human or animal subjects performed by any of the authors.

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