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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 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 such as LDL and HDL particle number and particle 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 advanced lipoproteins. Regrettably, therapies targeting HDL and HDL composition have been disappointing to date.

Keywords

lipoprotein; particles; exercise; diet

Introduction

Based on a preponderance of evidence and current NCEP-ATP III guidelines, low density lipoprotein cholesterol (LDL-C) level is the primary lipid target to lower risk of coronary heart disease (CHD), resulting in significant reductions in non-fatal and fatal CHD events.^[1] Since then, efforts are continually being made to further reduce residual CHD risk. Based on NCEP-ATP III guidelines, non-high density lipoprotein cholesterol (non-HDL-C) is a secondary lipid target for patients with triglyceride level above 200 mg/dL. Non-HDL-C has

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been shown to be superior to LDL-C in predicting secondary CHD events while 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.

What does Advanced Lipoprotein Testing Measure?

Advanced lipoprotein testing measures 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, intermediate density, and low density lipoproteins (VLDL, IDL, 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, HDL particles carry apo A-I molecules, though not in a 1:1 ratio, and are considered anti-atherogenic.^[3] The composition of all lipoprotein particles (VLDL, IDL, LDL, HDL) can be characterized by 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 and an apolipoprotein(a) [apo(a)] and is atherogenic.

LDL and HDL particles contain a certain amount of esterified cholesterol within their hydrophobic cores. However, there often is discordance between 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 determination of intensity of therapy.

How are Advanced Lipoproteins Measured?

Apo B and apo A-I levels are most commonly measured by vertical auto profile (VAP) or nuclear magnetic resonance (NMR). VAP uses a density gradient rapid ultracentrifugation technique to measure size and charge of the apolipoproteins. NMR uses magnetic resonance to estimate the lipoprotein distribution using proton spectroscopy methods. Another measurement method used is an immunoassay. All 3 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 the highest when measured by immunoassays, intermediate by NMR (14% lower than immunoassays), and lowest by VAP (17% lower than immunoassays).^[6]

In contrast, there is no international standard for lipoprotein subclass composition assessment, including HDL-P number and size, LDL-P number and size. Currently, the following methods are available: NMR, VAP, 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.[⁸] Conversely, weight loss and exercise have been associated with reduced triglycerides and increased HDL-C.[⁹] The impact of obesity on lipoprotein particle composition is less established. A crosssectional study comparing obese (BMI 30-45) and non-obese (BMI 18.5 – 25) participants who were normotensive and non-diabetic 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 follow up examined changes in lipoprotein particle concentration and sizes (measured by NMR).[¹¹] Moderate weight loss (5%) was associated with decreased particle concentrations of all apo B-containing lipoproteins, increased concentration of large HDL-P (24.1%, 95% CI 15.8% – 32.5%; $p<0.001$), and decreased concentration of small HDL-P (–9.0%, 95% CI –13.1% – –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 1 of the 4 following groups: alternate day fasting (ADF), calorie restriction (CR), exercise, and control.[¹²] All 3 groups achieved moderate weight loss (mean weight loss = 5%). HDL-P and LDL-P were measured by polyacrylamide gel electrophoresis. Remarkably, the methods to achieve weight loss affected these lipoproteins in a distinctive fashion. Relative to baseline, ADF increased LDL-P size ($265 \pm 2 \text{ \AA}$ vs. $261 \pm 1 \text{ \AA}$; $p=0.01$), decreased proportion of small LDL-P ($18 \pm 3 \%$ vs. $25 \pm 3 \%$; $p=0.04$), but had no impact on HDL-P size. CR increased LDL-P size ($264 \pm 2 \text{ \AA}$ vs. $260 \pm 2 \text{ \AA}$; $p=0.01$) relative to baseline, and had no significant impact on LDL-P proportion and HDL-P. Exercise increased proportion of large HDL-P relative to baseline ($34 \pm 3 \%$ vs. $28 \pm 3 \%$; $p=0.04$), but had no impact on LDL-P size or proportion of small LDL-P. This result seems to indicate that LDL particles are more sensitive to dietary modification while HDL particles are more sensitive to exercise.

In a follow-up study by the same investigators, 64 obese participants were randomized to 1 of the 4 following groups: a combination of ADF and endurance exercise (both as described above), ADF, exercise, and control.[¹³] The combination group showed increase in LDL-P size ($4 \pm 1 \text{ \AA}$; $p<0.001$) and decrease in proportion of small HDL-P ($11 \pm 1 \%$ vs. $15 \pm 2 \%$; $p=0.007$). The ADF-only group showed similar significant increase in LDL-P size ($5 \pm 1 \text{ \AA}$; $p<0.001$), but had 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 STTRIDE (Studies of a Targeted Risk Reduction Intervention through Defined Exercise) study 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 to decrease concentration of LDL-P ($p=0.03$), increase LDL-P size ($p=0.009$), and increase HDL-P size ($p=0.05$) by NMR spectroscopy. Interestingly, close adherence to American Heart Association (AHA) diet was not associated in 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.

Effects of Diet on Advanced Lipoproteins

As discussed above, weight loss and exercise appear to improve 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 non-obese adult participants with normal lipid profiles to high 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 particle size (assessed by polyacrylamide gradient gel electrophoresis) were obtained. In only 3 days of feeding, participants from high fat diet showed significant increase in size of LDL-P (255.0 vs. 255.9 Å; $p=0.01$) and decrease in proportion of small LDL-P (<255.0 Å; 50.7% vs. 44.6%; $p=0.01$).

Another randomized, crossover study subjected 63 healthy, non-obese 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] HFLC diet consisted of 40% fat, 45% carbohydrate, and 15% protein; whereas LFHC diet consisted of 20% fat, 65% carbohydrate, and 15% protein. Compared to HFLC diet, participants on LFHC diet had higher Lp(a) (19.9 ± 13.7 vs. 17.8 ± 12.8 mg/dL; $p<0.01$) and smaller LDL-P peak size (256.5 ± 8.3 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 reduced fat (RF) or reduced carbohydrate (RC) diet for 9 months.^[17] RF was defined as fat approximating 30% of daily caloric intake; whereas, CF was defined as carbohydrate approximating 20% of daily caloric intake. Again, consistent with previously mentioned studies, reduced carbohydrate diet showed significant increases in mean LDL size, large LDL, and large and small HDL (measured by NMR spectroscopy).

Along the same lines, a single-blinded, parallel design study randomized 37 adult participants with metabolic syndrome to whole eggs diet (EGG) or yolk-free egg substitute (SUB) diet for 12 weeks.^[18] Both groups were also a part of moderately carbohydrate-restricted diet. Both groups showed reduction LDL-P size and increase in HDL-P size

(measured by NMR spectroscopy). However, the increase in HDL-P size was greater in the EGG group (EGG: $+0.22 \pm 0.30$ vs. SUB: $+0.05 \pm 0.22$ nm, $p < 0.05$).

Of note, a monozygotic twin cohort study from Finland studied the impact of omega-3 polyunsaturated fatty acids (n-3 PUFAs) on advanced lipoprotein profile.^[19] The participants in this study included 24 healthy monozygotic twin pairs aged 23-33 years. Their 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 n-3 PUFA.

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

Effects of Drugs on Advanced Lipoproteins

A recent systematic review examined other commonly used lipid lowering agents, i.e. statins and fibrates.^[20] Statins (pravastatin, simvastatin, atorvastatin, and pitavastatin) were found to be associated with mean 30% decrease of LDL-P (1346 ± 226 vs. 1942 ± 380 nmol/L) and mean 27% decrease of apo B (103 ± 21 vs. 144 ± 31 mg/dL). Despite that, both LDL-P and apo B were still 42th and 54th percentile respectively in the population, perhaps indicating a residual CHD risk on statin therapy. Fibrates (fenofibrate, bezafibrate, and gemfibrozil) were found to decrease 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. It also helps lower TG and LDL-C. However, the AIM-HIGH (Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides) trial, which compared the combination of niacin and simvastatin with simvastatin alone, was stopped for futility due to lack of clinical benefit.^[21, 22] Furthermore, the HPS2-THRIVE study recently released its results which showed the combination of niacin and lapiropirant, in addition to simvastatin, did not reduce CHD events and was associated with increased adverse events including incident diabetes and diabetes complications.^[23] Niacin has been shown to increase HDL2 (measured by ultracentrifugation or electrophoresis) and increase LDL-P size (by gel electrophoresis)^[24] as well as lower lp(a) levels.^[25]

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 by 50-100%. Unfortunately, clinical trials involving CETP inhibitors thus far have not shown to be beneficial. The ILLUMINATE (Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events) trial which studied torcetrapib had to be terminated early due to an increase in 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 by over 60% and increased both large HDL2 and small HDL3 particles.^[27] The dal-OUTCOMES trial studied dalcetrapib, which also increases both HDL-2 and HDL-3 subfractions (measured by ultracentrifugation).^[28] dal-OUTCOMES was terminated

early for futility without any safety concerns.^[29] There are 2 other CETP inhibitors, anacetrapib and evacetrapib, that are still in phase III trials. They likely represent the last hopes on 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 small, dense LDL-P in some studies.^[30] The Diabetes Prevention Program randomized 3234 participants with impaired glucose tolerance to one of the following: metformin 850 mg twice daily, or intensive lifestyle changes (ILS) with goal of 7% weight loss through low fat diet and exercise, or placebo twice daily. Compared to placebo, metformin was found to decrease small LDL-P concentrations (711 ± 354 vs. 793 ± 394 nmol/L; $p < 0.01$) and increase large HDL-P concentrations (4.6 ± 2.7 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 SANDS (Stop Atherosclerosis in Native Diabetics Study) study evaluated the 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 70 mg/dl, non-HDL-C 100 mg/dl, and systolic blood pressure 115 mmHg) vs. standard treatment (LDL-C 100 mg/dl, non-HDL-C 130 mg/dl, and systolic blood pressure 130 mmHg). The aggressive group showed significant regression in CIMT, and it was significantly associated with decreases in LDL-C ($p < 0.005$) and non-HDL-C ($p < 0.001$). LDL-P and apo B (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 MESA (Multi-Ethnic Study of Atherosclerosis) study set out to evaluate the associations between HDL-C and HDL-P with CIMT and CHD event. ^[4] The study followed 5,598 adult participants without baseline CHD for a mean 6 years follow up. HDL-P was measured by NMR. HDL-C and HDL-P correlated with each other ($\pi = 0.69$) and LDL-P ($\pi = -0.38, -0.25$, respectively), $p < 0.05$ for all. For (1-SD) higher HDLC (15 mg/dl) or HDL-P (6.64 $\mu\text{mol/l}$), cIMT differences (95%CI) were $-26.1(-34.7, -17.4)$ and $-30.1(-38.8, -21.4)$ μm , and CHD hazard ratios (HR (95%CI)) were 0.74 (0.63, 0.88) and 0.70 (0.59, 0.82), respectively. Adjusted for each other and LDL-P, HDL-C was no longer associated with cIMT (2.3 (-9.5, 14.2) μm) or CHD (0.97(0.77, 1.22)), but HDL-P remained independently associated with cIMT ($-22.2(-33.8, -10.6)$ μm) and CHD (0.75 (0.61, 0.93)). Interactions by sex, ethnicity, diabetes and high-sensitivity C-reactive protein were not significant.

The JUPITER (Justification for the Use of statins in Prevention: an Intervention Trial Evaluating Rosuvastatin) trial evaluated alternative HDL measures such as HDL-P size and HDL-P (measured by NMR spectroscopy) as markers of residual CHD risk. The study randomized 10,866 adult participants with CHD to rosuvastatin 20 mg/d or placebo. After the first CHD event, HDL-P size, HDL-P, HDL-C and apoA-I were then measured ($n =$

234). Rosuvastatin group was associated with increases in apo A-I (2.1%; $p<0.0001$), HDL-P (3.8%; $p<0.0001$), and HDL size (1.2%; $p<0.0001$). Among the placebo group, apo A-I, and HDL-P showed similar inverse associations with CHD. Risk factor-adjusted hazard ratio and 95% CI per 1 SD are as follows: HDL-C (0.79; 0.63-0.98, $p=0.03$), apo A-I (0.75; 0.62-0.92, $p=0.004$), and HDL-P (0.81; 0.67-0.97, $p=0.02$). However among the treatment group, only HDL-P remained significantly inversely associated with CHD. Risk factor-adjusted hazard ratio, 95% CI per 1 SD, p -value are as follows: HDL-P (0.73; 0.57-0.93, $p=0.01$), HDL-C (0.82; 0.63-1.08, $p=0.16$), and apoA-I (0.86; 0.67-1.10, $p=0.22$).^[34]

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, independent of traditional lipid measurements. Lifestyle changes such as weight loss, exercise, and dietary modification correlate with improvement in advanced lipoproteins. 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.

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Table 1

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

Author	Participants	Intervention	Study Type	Subfraction Method	Results	Ref
Weight Loss and Exercise						
Mantyselka, et al.	683	Moderate weight loss	Cohort	NMR spectroscopy	Moderate weight loss: Large HDL-P ₁ ↑, small LDL-P ₁ ↓	11
Varady, et al.	60	Alternate day fasting (ADF); calorie restriction (CR); endurance exercise (EE)	Randomized, controlled	Polyacrylamide gel electrophoresis	ADF: LDL-P size↑, LDL-P ₁ ↓ CR: LDL-P size↑ EE: large HDL-P ₁ ↑	12
Bhutani, et al.	64	ADF+EE; ADF; EE	Randomized, controlled	Polyacrylamide gel electrophoresis	ADF+EE: LDL-P size↑, small LDL-P ₁ ↓ ADF: LDL-P size↑ EE: insignificant	13
Huffman, et al.	204	Aerobic exercise	Randomized, controlled	NMR spectroscopy	LDL-P ₁ ↓, LDL-P size↑, HDL-P size↑	14
Diet						
Guay, et al.	12	High fat diet; low fat diet	Randomized, double-blind, crossover	Polyacrylamide gel electrophoresis	High fat: LDL-P size↑, large LDL-P ₁ ↑, small LDL-P ₁ ↓	15
Faghini, et al.	63	High fat diet; low fat diet	Randomized, crossover	Polyacrylamide gel electrophoresis	High fat: LDL-P size↑, medium LDL-P ₁ ↑, very small LDL-P ₁ ↓, Lp(a)↓	16
LeCheminant, et al.	35	Low fat diet; low carbohydrate diet	*Quasi-experimental	NMR spectroscopy	Low carb: LDL size↑, large LDL-P ₁ ↑, large HDL-P ₁ ↑, small HDL-P ₁ ↓	17
Blesso, et al.	37	Whole eggs + low carbohydrate diet (EGG); yolk-free egg substitute + low carbohydrate diet (SUB)	Single-blind, parallel design	NMR spectroscopy	EGG: LDL-P size↓, HDL-P size↑↑ SUB: LDL-P size↓, HDL-P size↑	18
Bogl, et al.	48	Omega-3 polyunsaturated fatty acids (n-3 PUFAs) intake	Cohort twins	Polyacrylamide gel electrophoresis	Higher n-3 PUFA intake: large HDL-P ₁ ↑, small HDL-P ₁ ↓	19

* Each of the 6 clinic sites was assigned as either low carbohydrate or low fat