Executive Summary

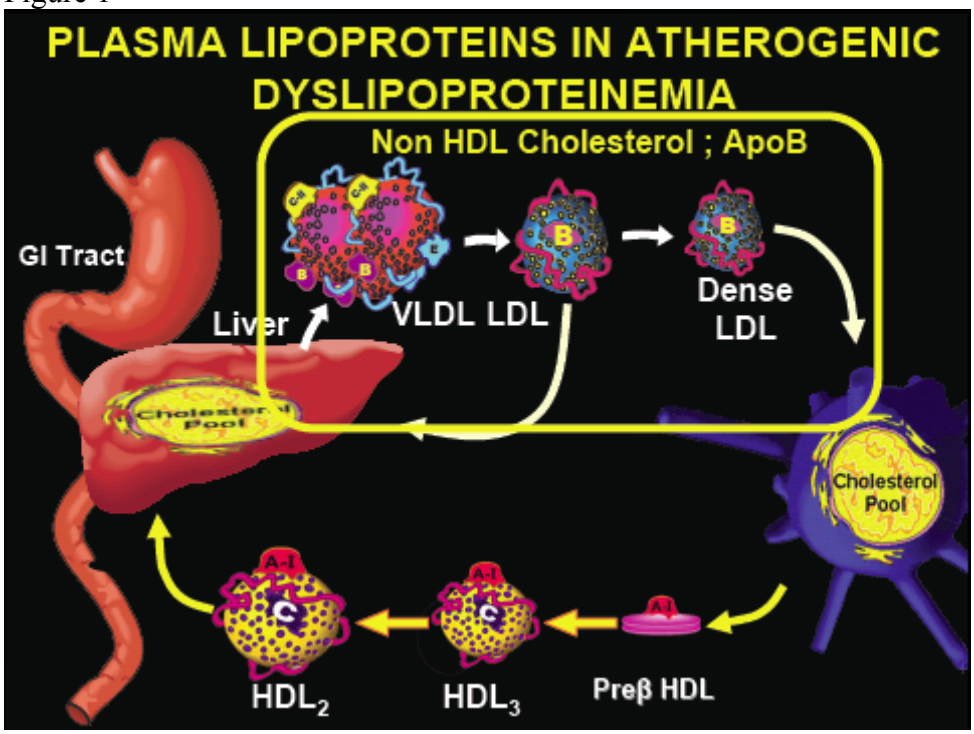
Background

For decades, cardiovascular disease (CVD) has been a major cause of death and disability in developed countries.[1] Despite a significant reduction in mortality due to CVD in recent years, it remains the leading cause of death in the United States.[2] As a result, the discovery of new biomarkers to detect CVD in patients who could benefit from medical intervention is a national priority. Public Health initiatives have focused on an increased effort in the early indication, prevention and treatment of heart attack and stroke, and in the prevention of recurrent cardiovascular events.[3,4] Guidance issued by the American Heart Association[5], the National Cholesterol Education Program's (NCEP) Adult Treatment Panel III (ATPIII)[6], and draft guidance by the National Academy of Clinical Biochemistry (NACB)[7] are available to assist clinicians in the identification of people who are asymptomatic and free of CVD but are at high risk for coronary events or stroke. Risk prediction algorithms, such as the Framingham Risk Score, are used to assess global risk for CVD. Recommended methods to assess cardiac risk include the measurement of specific risk factors such as total cholesterol, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C). Recently a number of candidate biomarkers have been introduced that may emerge as new risk factors for CVD. However, their causative nature is uncertain and their contributions to CVD are not yet as well established as factors such as dyslipidemia, high blood pressure, and smoking.[8] Among the new candidate biomarkers are subclasses of known lipid risk factors, obtained by partitioning LDL and HDL cholesterol particles by size, by density, or by particle number. The purpose of this panel meeting is to obtain expert recommendations regarding the analytical and clinical validity of lipid subfraction diagnostic assays.

Summary

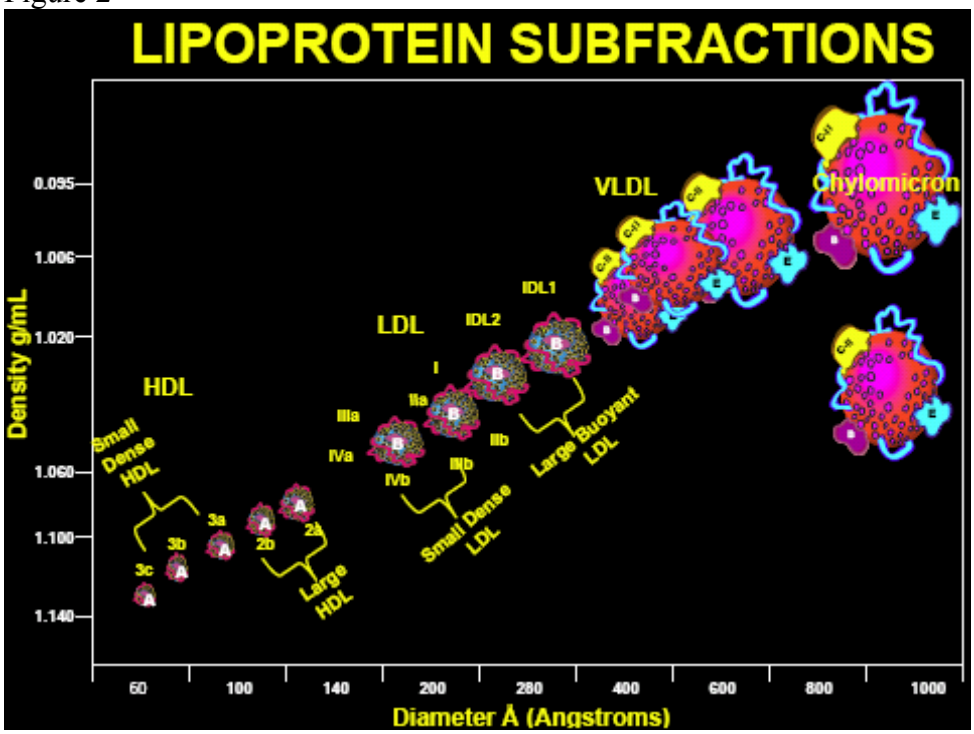
Early studies of cholesterol and lipids identified two distinct patterns of lipid profiles categorized as Profile A and B. Individuals who had profile A were said to be at low risk, while individuals whose lipid pattern fit profile B were determined to have a greater risk of CVD.[9] One factor that contributed to Profile B was non-HDL Apolipoprotein B containing cholesterol particles. These non-HDL lipid particles, later identified to be atherogenic and composed of Low Density Lipoproteins (LDL), Very Low Density Lipoproteins (VLDL) and Intermediate Density Lipoproteins (IDL), are displayed in the yellow box in Figure 1.

Figure 1



These lipoproteins are spherical particles with non-polar lipids in their core (triglycerides and cholesterol esters), more polar lipids (phospholipids and free cholesterol) near the surface, and one or more apolipoprotein molecules on their surface.[10] Later studies helped establish the presence of a variety of LDL particles of different size and density due to varying amounts of loosely bound core lipids. As a result each of the lipoproteins can be separated into a series of subfractions. Similarly, HDL particles vary in size and composition and can also be separated into subfractions (Figure 2).

Figure 2



A variety of technologies have been developed to separate and measure lipid subfractions. For example, samples may be fractionated and quantified for density, particle size, molecular weight and/or particle number. This quantitation can be accomplished by a variety of methods such as nuclear magnetic resonance (NMR) or electrophoresis.

Recent studies of subfractions have identified differences between the varying methods used for lipid subfraction testing. In one study Ensign et al.[11] compared LDL subfractions by four commercially available methods - nuclear magnetic resonance (NMR), density gradient ultracentrifugation, gradient gel electrophoresis (GGE), and tube gel electrophoresis (TGE). In their comparison, Ensign et al. identified a number of differences between these assays, including differences in nomenclature, in expected values from one assay to another, and in the identification of specific lipid subfractions. They concluded that the estimated number of LDL subfractions is method-dependent, with different methods identifying different LDL subfractions. They report a substantial heterogeneity of interpretations among the four methods with complete agreement occurring in only 8% of the samples tested. This lack of standardization does not seem to allow for comparability of the data derived from the different methodologies. This may have the potential to complicate the clinical interpretation of assay-specific results.

In a separate study, Bays and McGovern[12] provide a table comparing terminology of lipid subclasses. An adaptation of that table can be found in Table 1 below.

Table 1

Simplified Terminology of Lipoprotein Subclasses												
Segmented Gradient Gel Electrophoresis												
	Pattern B				Pattern Intermediate						Pattern A	
LDL Particles	IVb		Iva		IIIb	IIIa		IIb		IIa	I	
HDL Particles	3c			3b		3a		2a			2b	
Nuclear Magnetic Resonance												
	Pattern B										Pattern A	
LDL Particles	L1					L2					L3	
HDL Particles	H1			H2			H3			H4		H5
VLDL Particles	V1		V2			V3			V4		V5	V6
Short Single Vertical Automated with Gradient Ultracentrifugation												
	Pattern B					Pattern A/B					Pattern A	
LDL Particles	LDL4			LDL3			LDL2				LDL1	
HDL Particles	HDL3(d,c,b,a)						HDL2 (a,b,c)					
VLDL Particles	VLDL 3b			VLDL 3a			VLDL 1 + 2					

In addition to the early work establishing a correlation between LDL levels and CVD, current evidence strongly suggests that CVD risk increases with elevated triglycerides and low HDL-C levels. The ATPIII guidelines identify elevated LDL as a primary risk factor for CVD, while the combination of elevated triglycerides and low HDL-C is identified as an associated risk for CVD. Furthermore, the guidelines recommend clinical treatment of individuals at high risk based on LDL-C and triglyceride values. Yet, studies have shown that normolipidemic individuals may develop CVD despite a lack of measurable risk factors.[14-23] A number of investigators have studied specific patient populations with established CVD compared to normolipidemic non-cardiac risk individuals.[8,9,14-23] These investigators have observed an increase in the LDL subfractions (particularly small dense LDL) as total LDL increases and a marked decrease in the larger HDL particles (identified as the "most protective") HDL species, as HDL decreases. Based upon these findings, it has been suggested that elevated LDL, elevated small dense LDL subfractions, low HDL and low HDL subfractions are predictive of CVD.[13]

Although there is some evidence that lipid subfraction profiles differ between individuals with established CVD and normolipidemic individuals, it is unclear to FDA whether meaningful and reproducible diagnostic cutoffs for particle size, density and/or number can be established. Some investigators have observed that lipid subfraction reference ranges for patients at risk for CVD (as defined by NCEP) vs. normolipidemic patients have considerable overlap. An example of what we believe to be typical performance of these assays appears in a published study by Morais, et al.[24] The reference ranges for normolipidemic populations and dyslipidemic populations appear below (Table 2 and Table 3).

Table 2 Normolipidemic population

	HDL Large (mg/dL)	HDL Intermediate (mg/dL)	HDL Small (mg/dL)	HDL total (mg/dL)	Cholesterol total (mg/dL)
Range	8-43	18 – 44	0 - 12	40 - 89	110 - 199
Mean	21.7	30.4	4.3	56.5	166.2
SD	8.05	5.06	2.56	10.87	19.37
96% range	10 - 41.9	22.0 - 41.9	1.0 - 11.0	41.0 - 79.9	118.5 - 197.8
N	123	123	123	123	123

Table 3 Dyslipidemic population

	HDL Large (mg/dL)	HDL Intermediate (mg/dL)	HDL Small (mg/dL)	HDL total (mg/dL)	Cholesterol total (mg/dL)
Range	2 - 90	13 – 53	1 - 19	21 - 122	94 - 322
Mean	14.5	28.1	6.2	49.0	213.9
SD	10.3	7.01	3.05	15.54	39.17
96% range	3.8 - 37.0	16.8 - 43.2	1.0 - 12.6	27.0 - 85.8	124.6 - 299.4
N	191	191	191	191	191

The considerable overlap that was observed between the values in the normolipidemic population compared to the values in the dyslipidemic population suggests that the concentration of the lipid subfractions may not be predictably different between "at risk" and normal populations. This raises concerns regarding whether these candidate biomarkers can be used safely and effectively to, for example, predict CVD risk or determine lipid lowering therapy.

The NCEP ATPIII guidelines recognize that small LDL particles have been identified as a component of atherogenic dyslipidemia, and that some studies have suggested that some HDL subfractions may make important contributions to CVD risk assessment. The guidelines state that LDL particles are formed in large part as a response to elevated triglyceride. However, while these guidelines assert that LDL subfractions plus elevated triglyceride is associated with CHD, they also note that the ability of LDL subfractions to predict CHD independently of other risk factors is not well defined. The guideline also points out that the clinical performance of HDL subfractions has not been established. As a result of this and the ready availability of inexpensive standard methodologies, the ATPIII does not recommend the measurement of small lipid particles in routine practice.[6]

In addition, the NACB recently proposed new guidelines for the use of several new biomarkers for the assessment of CVD risk. The NACB is proposing the following three recommendations concerning lipid subclasses:[7]

1 Lipid subclasses, especially the number or concentration of small dense LDL particles have been shown to be related to the development of initial coronary heart disease events, but the data analysis of existing studies are generally not adequate to show added benefit over standard risk assessment.

[Classification/Weight of Evidence: The committee found that there is evidence and/or general agreement that measurement of lipid subclasses is not useful (and in some cases might be harmful) based on data obtained from multiple randomized clinical trials that involved large numbers of patients.]

2 There is insufficient data that measurement of lipid subclasses over time is useful to evaluate the effects of treatments.
[Classification/Weight of Evidence: The committee found that there is conflicting evidence and/or divergence of opinion about the usefulness/efficacy of these assays, with the usefulness/efficacy of the test being less well established. This conclusion was based on a consensus of opinion of the experts in the field.]

3 Several methods are available to assess lipoprotein subclasses. Standardization is needed for this technology.
[Classification/Weight of Evidence: The committee found that there is conflicting evidence and/or divergence of opinion about the usefulness/efficacy of standardization, with the weight of evidence/opinion being in favor of standardization. This conclusion was based on a consensus of opinion of the experts in the field.]

The proposed recommendations cited above and the published reports give the FDA insight regarding the currently understanding of the clinical usefulness of these types of assays and the potential strengths and weaknesses of these potential biomarkers. However, FDA's task when evaluating whether a novel assay should be cleared (or approved) is to determine whether the assay can be found substantially equivalent to existing assays (or is safe and effective for its intended use). For that purpose we focus on the analytical and clinical validity of the assay based on the specific claim(s) that are made when promoting and labeling the device. To this end, we have prepared a set of specific questions intended to obtain the panel's insight regarding the analytical and clinical validity of lipid subfraction diagnostic assays, to the extent possible given the current state of knowledge.

Questions

Based on the current state of knowledge, please provide input on the following questions:

1. Is there sufficient information available to conclude that HDL and/or LDL subfractions can be used:
    a. to assess a patient's risk of developing CVD?
    b. to diagnose dyslipidemia?
    c. to monitor treatment of dyslipidemic patients?
    d. for any other use?
2. If sufficient information is available for clinical use, should HDL and/or LDL subfractions be used:
    a. as a stand-alone test?

b. as an adjunctive test to be used with other traditional risk assessment tools (e.g., Total, HDL, and LDL cholesterol) and clinical judgment?

3. When used either as a stand-alone test or in conjunction with other lipid measurements (with values defined as non- cardiac risk by the NCEP ATPIII guidelines), will changes in treatment based upon the abnormal lipid subfractions pose an acceptable level of benefits compared to risk to the patient?
4. How would the accuracy of these subfractions be established? What is an appropriate reference method? What are appropriate acceptance criteria when comparing to the reference method?
5. How should expected values be determined for lipid subfraction assays? Is it possible to make meaningful test interpretations in cases where reference ranges for normal and "diseased" patients overlap?
6. If used (either as an adjunctive test to traditional lipid measurements or as a stand alone diagnostic) to diagnose or predict risk for dyslipidemia or atherosclerosis, does the lack of standardized nomenclature or differences in assay performance (e.g., reference ranges, precision, fractions analyzed, etc.) pose an unreasonable risk to the patient?
7. Is there a difference in the assessment of lipid subfractions based upon particle size versus particle number? If so, what are the strengths and weaknesses of each method? Please discuss.

Appendices

A: Literature Search Criteria
B: Literature Search Strategy
C: References

Appendix A

Criteria of Identification of Relevant studies

Includes evidence from:

- Peer reviewed publications
- Established and draft guidelines related to Cardiovascular Disease
- Peer reviewed presentation posters

For purposes of this review, information reviewed was limited to specific articles related to Lipids, Apolipoproteins and Lipid Subfractions.

This literature search does not include evidence from:

- Isolated single case studies
- Random experience
- Case Histories
- Letters, comments, news articles, editorials or other non-peer reviewed articles
- Unsubstantiated opinion

Appendix B

Search Strategy and Terms

Search	Most Recent Queries	Time	Result
#11	Search **HDL Subclasses** Limits: **published in the last 5 years**	11:24:36	126
#12	Select **4** document(s)	11:24:01	4
#10	Search **HDL Subclasses** Limits: **published in the last 3 years**	11:22:20	74
#9	Search **HDL Subclasses**	11:21:33	450
#7	Search **LDL Subclasses**	11:18:30	341
#5	Search **(("Lipoprotein subclass analysis"))** Limits: **published in the last 5 years**	10:57:43	111
#4	Search **(("Lipoprotein subclass analysis"))**	10:56:16	408
#3	Search **("Lipoprotein subclasses") AND (#1)"subclass determinations"**	10:53:05	0
#1	Search **"Lipoprotein subclasses"**	10:47:21	195
#0	**pubmed** clipboard		

Other articles included were derived from previously selected references, specific websites, and established and draft guideline references.

Appendix C

References

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