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Lipid Club Letter

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Editorial

Dear Colleagues and Friends,

As you will see, the presentation of the Lipid Club Letter has undergone some changes but its content remains the same and we hope that it will continue to come up to your expectations.

In addition, in order to update the list of our readers and their details, a reply card has already been sent to you. In case you did not reply the first time, a new reply card is appended to the present issue.

The routes followed by the ideas and projects of our Minister for Health, particularly with regard to amending the reimbursement criteria for antilipemic medications, are not easily understood. But we are told the conclusion is at hand!

The present issue is entirely devoted to high-density lipoproteins. The subject was discussed at a meeting of the Belgian Lipid Club. The article highlights the uncertainties which persist regarding the correct technique for assessing HDLs and the use of this parameter in clinical practice.

In the name of the Belgian Lipid Club, I send you my best wishes for the year 2006.

Prof. F.R. Heller
President

HDL in Practice

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1. Introduction

High-density lipoprotein (HDL) comprises a heterogeneous population of protein-rich particles that are the densest and smallest in size among the human plasma lipoproteins [1]. There is general acceptance now of the inverse association between HDL-cholesterol (HDL-C) and the risk of developing coronary heart disease (CHD). This has been established from epidemiologic and prospective studies; it is a relationship that persists after adjustment for other risk factors and is consistent with protective mechanisms demonstrated in animal studies. Atheroprotective and less atheroprotective HDL subclasses have been described. Guidelines for primary and secondary prevention have been developed by task forces of the NCEP (National Cholesterol Education Programme) and EAS (European Atherosclerosis Society), and have been accepted by the relevant European Societies. Consequently, the measurement of HDL-C in characterizing risk for CHD and managing treatment of dyslipidemia has become increasingly common in clinical laboratories. Until recently, laboratory methods for HDL-C measurement, adapted from research techniques, were timeconsuming and labour-intensive. More recently developed homogeneous assays have enabled direct, fully automated measurement of HDL-C, without the need for sample pretreatment to separate non-HDL particles. Although homogeneous assays show improved accuracy and precision in normal serum, discrepant results occur in samples with altered lipoprotein characteristics. Hypertriglyceridemia is an important interference factor. A novel approach is nuclear magnetic resonance (NMR) spectroscopy, which allows rapid and reliable analysis of lipoprotein subclass profiles and may improve the identification of individuals at increased CHD risk.

2. HDL definition

Classically, HDL particles are defined as the fraction having a density between 1.063 and 1.21 kg/l obtained by preparative ultracentrifugation [2]. Traditionally, the *alpha-migrating lipoprotein fraction* in electrophoresis is referred to as HDL. However, in practice this and the HDL fraction separated by other methods may not be exactly equivalent. HDLs are therefore defined in terms of the analytical procedure used to isolate them and comprise a heterogeneous population of particles that differ in density, size, shape, electrophoretic mobility, or in lipid and apolipoprotein composition **(Table 1)**. At first three main subclasses were isolated and referred to as pre-beta HDL, HDL2, and HDL3. However, modern high-resolution procedures can fractionate many more subpopulations, depending on the analytical technique used for separation [3].

Apolipoproteins A-I (apo A-I) and A-II are the major proteins of HDL. Two A-I-containing HDL subfractions have been described, *LpA-I* and *LpA-I/A-II*, which are themselves heterogeneous with respect to size and density [4,5]. Small quantities of apo A-IV, C-I, C-II, C-III, D, and E are also found. HDLs do not contain apo B, the major protein in LDL, IDL, VLDL and Lp(a). Of particular concern for HDL-C measurement is one of the larger

Table 1. Characteristics of major human HDL subclasses

Property	Pre-beta HDL	HDL2	HDL3
Electrophoretic mobility	Pre-β	α	α
Density (g/ml)	>1.210	1.063-1.125	1.125-1.210
Shape	Discoidal	Spherical	Spherical
Subpopulations	Pre-β_1 , Pre-β_2 , Pre-β_3	HDL_{2a} , HDL_{2b}	HDL_{3a} , HDL_{3b} , HDL_{3c}
Major apolipoproteins	A-I	A-I (predominant), A-II	A-I, A-II
Major lipids	Phospholipids, free cholesterol	Phospholipids, free cholesterol, cholesteryl esters, triglycerides	Phospholipids, free cholesterol, cholesteryl esters, triglycerides

HDL subclasses enriched with apo E. The amounts found of this subclass vary and may or may not be included with HDL-C, causing significant biases among different HDL-C methods.

3. HDL metabolism

The interconversion of HDL particles is shown in **Fig. 1**. Apo AI interacts with phospholipids and free (unesterified) cholesterol from the *ATP-binding cassette transporter AI* (ABCA1) of peripheral cells to form a small precursor particle (nascent discoidal HDL) with pre-beta migration on electrophoresis. In the circulation, additional phospholipids, cholesterol, and apolipoproteins are transferred to pre-beta HDL, converting them to larger, spherical HDL3 particles. Cholesterol is esterified by the action of *lecithin cholesterol acyltransferase* (LCAT) and transferred to the hydrophobic core to generate large HDL2 particles [6,7].

The cholesteryl esters are exchanged with triglycerides (TG) from apo B-containing lipoproteins by the action of the *cholesterol ester transfer protein* (CETP), causing the formation of TG-rich HDL2 particles, which deliver the remaining cholesteryl esters to the hepatocytes. These processes constitute the *reverse cholesterol transport* mechanism by which cellular and lipoprotein cholesterol is delivered back to the liver, a process that is considered to contribute to the cardioprotective role of HDL [8-10]. However, patients with Tangier disease have extremely low HDL-C concentrations and severe failure of reverse cholesterol transport due to ABCA1 gene mutation, although they do not necessarily have premature atherosclerosis.

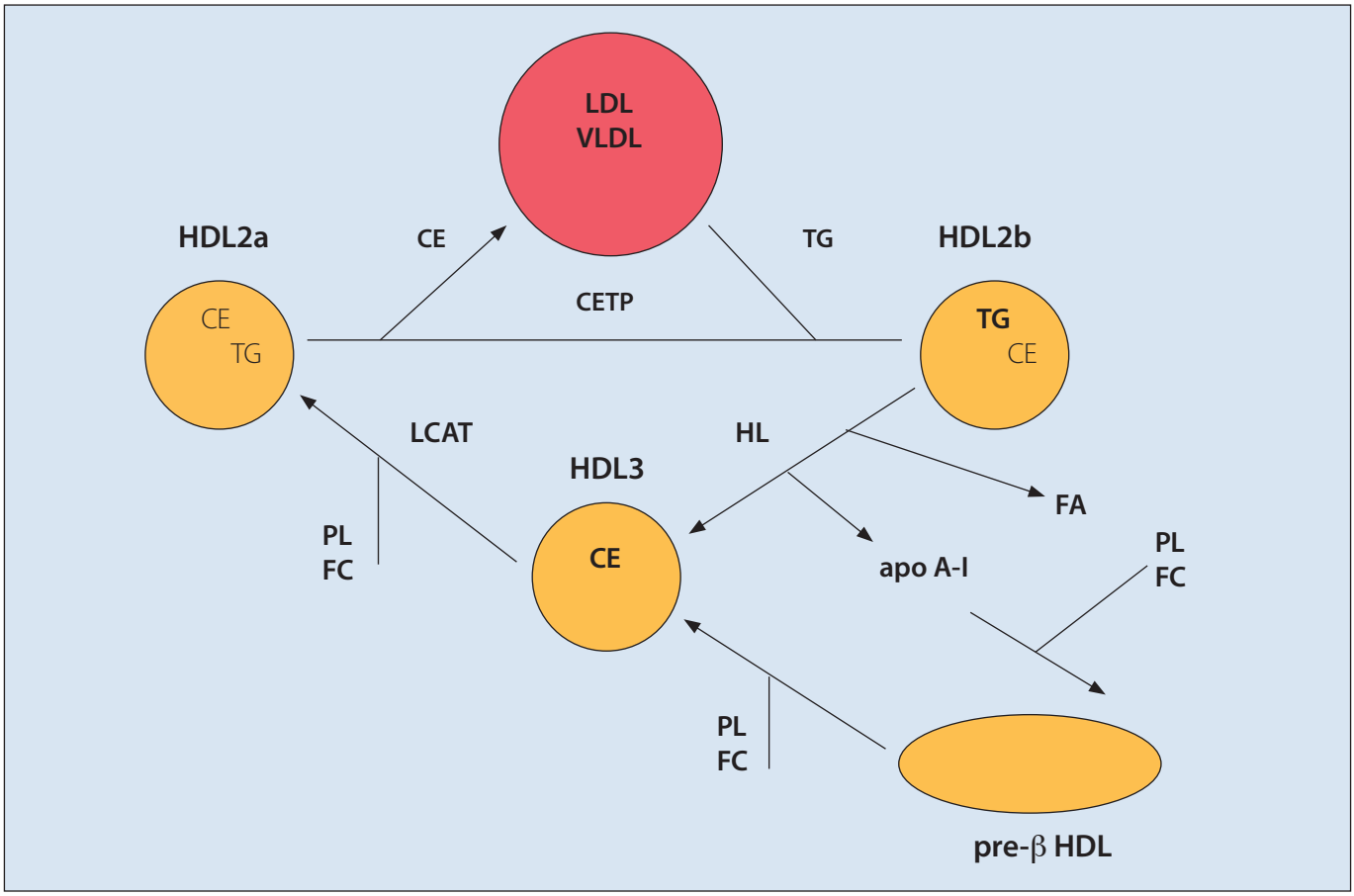
Several other protective functions of HDL have been proposed. For example, HDLs have the ability to inhibit oxidation of LDL. This is achieved both by antioxidants, such as paraoxonase, which are transported by HDL, and by apolipoproteins, including apo A-I and apo A-II, which have intrinsic anti-oxidant properties [11]. Other presumed protective roles of HDL are breaking the inflammatory cycle initiated by LDLs, inhibiting the expression of adhesion molecules by endothelial cells, improving endothelial vasomotor function, and anti-thrombotic effects [12,13].

4. HDL-C in clinical practice

Although it is recognized that low HDL-C concentration is a strong, independent risk factor for atherosclerosis, it has not received nearly the same attention as that given to LDL-C [14]. Nevertheless, the *Framingham Heart Study* demonstrated that HDL-C is a stronger predictor of risk of CHD than LDL-C (15,16). The risk of CHD increases 2-4% for every 1 mg/dl decrease in HDL-C. In the Helsinki Heart Study, in those subjects with high LDL- C (Fredrickson Type IIa hyperlipidemia), the factor with the highest predictive value for CHD was low HDL-C, not

Fig.1. The interconversion between HDL subclasses.

Abbreviations are: ABCA1, ATP-binding cassette transporter AI; CE, cholesteryl esters; CETP, cholesterol ester transfer protein; FA, fatty acids; FC, free (unesterified) cholesterol; HL, hepatic lipase; LCAT, lecithin cholesterol acyltransferase; PL, phospholipids; TG, triglycerides.



the severity of the increase in LDL-C [17,18]. The *Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial* (VA-HIT), in which subjects were randomised to gemfibrozil or placebo, suggested that therapy aimed at raising HDL-C could reduce the incidence of cardiovascular events [19]. In this clinical trial, the increase in HDL-C was the only on-treatment lipid alteration that significantly correlated with the reduction in CHD risk [20]. Fibrates and niacin reduced the risk of major coronary events by 25-27% by raising HDL-C in randomized controlled trials [21].

Some studies have identified associations of HDL particle size with CHD risk [22-26]. The hope is that a single HDL subpopulation might emerge as the *"good HDL particle"*, the HDL responsible for protection from CHD. Of the HDL subclasses, the largest (HDL2b, HDL2a and HDL3a) show the expected inverse correlation with disease incidence and severity, whereas the smaller HDL3b and HDL3c subclasses show a positive association. Thus, for the same reason that total cholesterol (TC) is often an unreliable indicator of CHD risk, HDL-C levels might not accurately predict the degree of CHD protection.

Clinical practice guidelines

The National Institutes of Health (NIH)-sponsored *National Cholesterol Education Programme* (NCEP) was successful in bringing HDL-C to the attention of the public during the 1990's. The consequence has been that most current guidelines formally recognize low HDL-C as an independent risk factor. Low HDL- C concentration is also recognized as part of the *atherogenic dyslipidemia* or *"lipid triad"* (low HDL, small, dense LDL, high TG) and as part of the cluster of features of the *Metabolic Syndrome*. However, HDL is still not considered as a primary target of therapy in most guidelines. Much of primary and secondary prevention effort is primarily based on risk as defined by LDL-C. Treatment is aimed at reducing LDL-C, and the success of therapies is evaluated by their impact on LDL-C.

The 1993 NCEP guidelines were the first to recommend the addition of HDL-C measurement to initial TC and LDL-C screening, designate high HDL-C as a negative CHD risk factor, and recommend use of HDL-C to guide the choice of therapy (27). Medical decision points were given for HDL-C with values <35 mg/dl considered "high risk", equivalent to non-lipid risk factors such as smoking or hypertension, and values ≥60 mg/dl considered "protective", thereby compensating for the presence of a positive risk factor.

Subsequent consensus statements in 1997 from the American Heart Association (AHA) and American College of Cardiologists (ACC) for primary [28] and secondary prevention [29] reinforced the enhanced role of HDL-C.

In 2001, the NCEP increased the high-risk medical decision point to <40 mg/dl and proposed the following guidelines on HDL-C management [30]:

- **HDL-C >60 mg/dl counts as a "negative" risk factor: its presence removes one risk factor from the total count.**
- **Treatment of low HDL-C (<40 mg/dl):**
  - **First reach LDL goal, then intensify therapeutic lifestyle changes (weight management, physical activity, giving up smoking).**
  - **If TG <200 mg/dl (isolated low HDL) in CHD or CHD equivalent, consider nicotinic acid or fibrate.**

In 2004, Grundy et al. [31] proposed some modified NCEP treatment algorithms:

- **In high-risk patients, the LDL-C goal is <100 mg/dl, but LDL-C goal <70 mg/dl is a therapeutic option when the risk is *very high* (e.g., HDL-C <40 mg/ dl).**
- **When a high-risk patient has high TG or low HDL-C, the addition of fibrate or nicotinic acid to LDL-lowering therapy can be considered.**
- **Any person at high or moderate risk who has life-style-related risk factors (obesity, physical inactivity, high TG, low HDL-C, or metabolic syndrome) is a candidate for therapeutic lifestyle changes to modify these risk factors regardless of LDL-C.**

The guidelines of the European Atherosclerosis Society (EAS), the European Society of Cardiology (ESC), and the European Society of Hypertension (ESH), in association with other international consensus panels, were published by *European Task Forces* in 1994, 1998 and 2003. The Third Joint Task Force proposed [32]:

- **"**

  No specific treatment goals for HDL-C, but that HDL- C concentration be used as a marker of increased risk".
- **Subjects with HDL-C <40 mg/dl (men) or <46 mg/dl (women) should be assigned to a higher risk category.**
- **"HDL-C should also be used to guide the choice of therapy".**

The Task Force allows the use of the ratio TC/HDL-C as an alternative indicator of risk based on the "SCORE" model and risk charts. A TC/HDL-C ratio >5 indicates increased risk.

HDL-C in the statin era

Most of the epidemiological evidence linking HDL-C to CHD predates the widespread use of statins to reduce LDL-C. Analysis of extensive statin trials, however, indicates that the strong and independent relationship between HDL-C and CHD risk persists despite the therapeutic effects of statins, and that HDL- C concentrations in statin-treated patients are significantly related to major residual cardiovascular events that are not prevented by aggressive lowering of LDL-C [33]. These observations, combined with the evidence that the infusion of recombinant apoA-I Milano (a mutant form of apoA-I whose carriers are protected from atherosclerosis) significantly reduces coronary atheroma volume in humans [34], provided a major stimulus leading to the discovery of novel therapeutic approaches to increasing the plasma concentration of HDL, including the use of statins, CETP inhibitors, oral apoA-I mimetics, and dual peroxisome proliferator-activated receptor (PPAR) α/γ ago-

nists currently under development [12,35,36]. Recently (2004), the European Consensus Panel on HDL-C suggested that the high residual risk among statin-treated patients indicates the need to modify other major components of the atherogenic lipid profile incl. low HDL [37].

5. HDL-C in laboratory practice

The dissemination of the NCEP and EAS guidelines has led to increased HDL- C measurements by clinical laboratories. Initially, highly sophisticated techniques such as ultracentrifugation, electrophoresis, and liquid chromatography systems were used to separate and quantify lipoproteins, including HDL and its subclasses **(Table 2)**. Although highly useful in lipoprotein and apolipoprotein research, these methods have disadvantages for use in high-work-load clinical laboratories because they are expensive, labour-intensive, time-consuming, and require technical expertise.

Table 2. Methods for separation and quantification of HDL-C and HDL subclasses.

- **Density gradient ultracentrifugation**
  - Equilibrium (isopycnic) ultracentrifugation
  - Rate zonal ultracentrifugation
- **Electrophoresis**
  - Zone electrophoresis
  - Gradient polyacrylamide gel electrophoresis
  - Isoelectric focusing
  - Isotachoforesis
  - Twodimensional electrophoresis
  - Capillary electrophoresis
- **Liquid chromatography**
  - Size exclusion chromatography
  - (Immuno-)affinity chromatography
- **Chemical precipitation**
  - Heparin-MnCl2
  - Dextran sulfate MgCl2
  - Phosphotungstate MgCl2 (PTA)
  - Polyethylene glycol (PEG)
- **Homogeneous (direct) HDL-C assays**
- **Proton nuclear magnetic resonance (NMR) spectroscopy**

Burstein et al. pioneered the chemical precipitation methods to separate lipoproteins [38]. Certain polyanions, such as heparin, dextran sulfate, or phosphotungstic acid, can selectively aggregate apo B-containing lipoproteins, leaving HDL in solution. The insoluble non-HDL lipoproteins are then sedimented by centrifugation, and cholesterol analysis of the isolated supernatant is a measure of HDL-C. However, major errors arise from incomplete precipitation of apo B-containing lipoproteins and interference from increased TGs, which yield turbid (cloudy) supernates contaminated with non-HDL lipoproteins, causing an overassessment of HDL-C.

The fact of clinical laboratories having to cope with increasing workloads while reducing costs, as well as the suboptimal precision associated with precipitation methods, has created a demand for total automation. Recent breakthroughs are the *"homogeneous"* or *"direct" HDL-C* methods capable of full automation that do not require sample pretreatment and separation, eliminating the manual pipetting and centrifugation steps [39]. Direct HDL-C methods are multi-reagent assays based on chemical or immunological blocking of non-HDL particles without precipitation, followed by determination of cholesterol directly in the treated serum sample. Many innovative homogeneous reagents have been introduced by various manufacturers and rapidly adopted by clinical laboratories [39].

Analytical guidelines

Accurate HDL-C measurements are critical for appropriate classification of patients based on the cut-points. In 1995, the *NCEP Working Group on Lipoprotein Measurement* published guidelines for HDL-C, including analytical performance goals for total error and corresponding precision and accuracy [40]. Reliable and accurate HDL-C measurement is important, not only for the correct identification and treatment of patients at high CHD risk, but also for the calculation of LDL-C using the Friedewald formula: LDL-C = TC - (HDL-C + TG/5) (41). Inaccurate measurement of HDL-C leads to substantial misclassification because the risk associations of HDL-C and LDL-C are opposite and the error is reciprocal; erroneously decreased HDL-C leads to erroneously increased LDL- C.

The physiological variation observed for HDL-C (7.5%) is similar to TC and LDL-C, and accounts for 69% of the total variance of HDL-C. For this reason, a patient's HDL-C concentration cannot be reliably established from a single measurement. Many factors strongly affect HDL-C values, including lifestyle factors, inflammatory conditions, hormones and other medications, genetic factors, and pre-analytical factors. The NCEP Working Group has provided recommendations to minimize the sources of variation **(Table 3)**.

In normal serum, homogeneous HDL-C assays generally meet the NCEP analytical criteria. However, discrepant results are observed in samples from patients with dyslipidemia, particularly type III hyperlipoproteinemia, or other conditions with altered? lipoprotein distribution and composition, such as advanced age, diabetes, liver and kidney disease. Some but not all direct HDL-C assays include the apo E-containing HDL particles, which account for 10% of total HDL-C, consistent with the bias observed in comparison studies. High TGs may interfere with any of the methods, regardless of whether the TGs are in chylomicrons or VLDL. Monoclonal paraproteins give discrepant results in some samples. Effects due to anticoagulants, especially EDTA, as well as prolonged storage and freezing-and-thawing have been reported. Furthermore, not all HDL-C methods have been standardized or validated by comparison with the *CDC Reference Method* or the *Designated Comparison Method* [39].

Lipoprotein subclass profiles

A novel approach is *proton nuclear magnetic resonance (NMR) spectroscopy*, which simultaneously provides the concentration of VLDL, LDL and HDL subclasses without physical fractionation of the plasma, and results in rapid and reproducible

Table 3. Major causes of discrepancies in HDL-C test results

Type of discrepancy	Recommendations for identifying or minimizing discrepancies
Biological variation	Two to three serial specimens, at least 1 wk apart, within 2 months. Results should be averaged. Information on patient lifestyle and medical condition (physical activity, diet, alcohol, smoking, obesity, pregnancy, disease, medication). Patients should maintain their usual diet and stable weight for 2 wks, and avoid strenuous exercise, before blood samples are taken. Avoid measurements within 8-12 wks after acute myocardial infarction, acute trauma, surgery, acute infection or inflammation, or pregnancy.
Pre-analytical factors	Standardized blood sampling (12 h fasting, sitting position). Remove serum or plasma from cells within 3 h of venipuncture. Determine effects of sample anticoagulant (Li heparin, EDTA), sample storage, freezing and thawing. Perform HDL separations within 1-2 days of collection. Specimens can be stored at 4°C for 3 days, at -20°C for 1 month, and at -50 °C for 1-2 years. Eliminate errors in specimen identification.
Method-related differences	Comparison with CDC Reference Method, or Designated Comparison Method. NCEP goals for bias (?5% from true reference value), imprecision (SD ≤17 mg/l at <42 mg/dl; CV≤4% at ≥42 mg/dl) and total error (≤13% of the true value).
Assay interferences	Determine interferences of triglycerides, monoclonal paraproteins, bilirubin, hemoglobin. Method validation in atypical specimens (dyslipidemia, diabetes, liver and renal disease).

measurements [42]. NMR spectroscopy-derived particle size determinations have yielded important information about the atherogenicity of lipoprotein profiles. CHD severity has been positively associated with levels of small HDL particles and inversely associated with intermediate-size HDL particles, independently of standard lipid measurements [43,44]. Individuals with exceptional longevity have been found to have significantly larger HDL and LDL particle sizes [45].

6. Apo A-I: a new breakthrough?

Like HDL itself, its major protein apo A-I is inversely correlated to atherosclerosis and is generally believed to represent the number of anti-atherogenic lipid particles, although the LpA-I subfraction of HDL has been shown to be more anti-atherogenic than the LpA-I/A-II particles [46].

As for apo B, apo A-I measurements have been proposed to predict cardiovascular risk [47]. In the Apolipoprotein related MOrtality RISk (AMORIS) study, the apoB/apoA-I ratio was strongly related to cardiovascular risk even after adjustment for age, TC, and TG [48]. These results were confirmed by, among other studies, the INTERHEART and AFCAPS/TexCAPS studies, where after adjustment for non-lipid risk factors the apo B/apo A-I ratio was the best discriminator [49,50]. Furthermore, the AFCAPS/TexCAPS study proved that under statin treatment, the apoB/apoA-I ratio predicted cardiovascular risk while on-treatment LDL-C did not.

The Canadian guidelines (2003) were among the first to propose that apo B and apo A-I should be introduced into medical practice as an alternative to LDL-C and HDL-C to assess CHD risk and guide therapy [51]. Calculated LDL-C has technical limitations as does directly measured LDL-C and HDL-C. In contrast, apo B and apo A-I measurements are precise, easily automated, not expensive and are internationally standardized.

7. Current status and recommendations

A consensus is growing that HDL-C might be a *"missing link"* in *cardiovascular risk reduction*. Almost all international guidelines recognize low HDL-C as an independent marker of increased risk for CHD, and consider the use of the ratio TC/HDL-C instead of using TC for risk estimation. Although some clinical trials suggest that raising HDL-C may reduce risk, further studies are needed, and only a few guidelines mention HDL-C as an element in the treatment of dyslipidemia [52]. The lack of comprehensive data highlights the need to emphasize the

importance of continued research for future generations. In fact, the mechanisms by which HDL provides protection have not been fully elucidated nor have the characteristics of desirable HDL subpopulations (those whose absence results in increased CHD risk).

At present, we cannot recommend a particular method as providing the best measurement of HDL-related risk of CHD. HDLs are complex and heterogeneous particles with overlapping properties that have not been fully characterized, and the various separation methods can obtain different populations of particles with varying associations with CHD risk. Numerous problems in laboratory techniques for measuring HDL-C remain unresolved. The homogeneous HDL-C assays represent a remarkable technological breakthrough and have replaced the traditional precipitation methods in most laboratories. Although homogeneous assays reduce operating costs while improving workflow and facilitate achieving the NCEP analytical performance goals, areas of concern still exist. The positive bias due to high TGs is especially important, particularly at HDL-C concentrations <40 mg/dl, which makes it difficult to identify a low HDL-C concentration and consequently an increased CHD risk. Laboratories having lipid clinics with a high proportion of specimens with atypical lipoprotein characteristics could produce impaired results, which might disorient treatment decisions. Therefore, before the popular homogeneous assays can be recommended for such laboratories, they must be evaluated thoroughly in atypical samples. Laboratories involved in lipid research and clinical trials should also consider introducing lipoprotein subclass profiles using promising novel techniques such as proton NMR spectroscopy, which may improve CHD risk assessment.

8. Conclusion

The development of a reliable and accurate assay for HDL-C, as well as the publication of coherent guidelines for the management of low HDL-C, are critical factors in the identification and treatment of patients at high CHD risk. Further studies are needed to determine whether apoA-I, and particularly the apoB/apoA-I ratio, could represent an alternative in estimating CHD risk and guiding therapy, enabling the analytical limitations of HDL-C to be avoided.

References

1. Rifai N. Lipoproteins and apolipoproteins. Composition, metabolism, and association with coronary disease. Arch Pathol Lab Med 1986;110:694-701.
2. Havel RJ, Eder HA, Bragdon JH. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. J Clin Invest 1955;34:1345-1353.
3. Blanche PJ et al. Characterization of human high-density lipoproteins by gradient gel electrophoresis. Biochim Biophys Acta 1981;665:408-419.
4. Cheung MC, Albers JJ. Characterization of lipoprotein particles isolated by immunoaffinity chromatography. Particles containing A-I and A-II and particles containing A-I but no A-II. J Biol Chem 1984;259:12201-12209.
5. Duriez P, Fruchard JC. High-density lipoprotein subclasses and apolipoprotein A-I. Clin Chim Acta 1999;286:97-114.
6. Young SG, Fielding CJ. The ABCs of cholesterol efflux. Nat Genet 1999;22:316-338.
7. Rye K-A, Barter PJ. Formation and metabolism of prebeta-migrating, lipid-poor apolipoprotein A-I. Arterioscler Thromb Vasc Biol 2004;24:421-428.
8. Lewis GF, Rader DJ. New insights into the regulation of HDL metabolism and reverse cholesterol transport. Circ Res 2005;96:1221-1232.
9. Assman G, Gotto AM Jr. HDL cholesterol and protective factors in atherosclerosis. Circulation 2004;109:III-8-III-14.
10. Von Eckardstein A, Nofer JR, Assmann G. High density lipoproteins and arteriosclerosis: role of cholesterol efflux and reverse cholesterol transport. Arterioscler Thromb Vasc Biol 2001;21:13-27.
11. Mertens A, Holvoet P. Oxidized LDL and HDL: antagonists in atherothrombosis. FASEB Journal 2001; 15: 2073-2084.
12. Barter P. The inflammation:lipoprotein cycle. Atheroscler Suppl 2005;6:15-20.
13. Bisoendial RJ et al. Restoration of endothelial function by increasing high-density lipoprotein in subjects with isolated low-density lipoprotein. Circulation 2003;107:2944-2948.
14. Gordon DJ, Rifkind BM. High-density lipoproteins: the clinical implications of recent studies. N Engl J Med 1989;321:1311-1316.
15. Gordon T et al. High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. Am J Med 1977;62:707-714.
16. Castelli WP et al. Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham Study. JAMA 1986;256:2835-2838.
17. Manninen V et al. Predictive value for coronary heart disease of baseline high-density and low-density lipoprotein cholesterol among Fredrickson type IIa subjects in the Helsinki Heart Study. Am J Cardiol 1990;66:24A-27A.
18. Manninen V et al. Joint effects of serum triglyceride and LDL cholesterol and HDL cholesterol concentrations on coronary heart disease risk in the Helsinki Heart Study. Implications for treatment. Circulation 1992;85:37-45.
19. Rubins HB et al. Gemfibrozil for the secondary prevention of coronary heart disease in men with low levels of high-density lipoprotein cholesterol. Veterans Affair High-Density Lipoprotein Cholesterol Intervention Trial. N Engl J Med 1999;341:410-418.
20. Robins SJ et al. Veterans Affair HDL Intervention Trial. Relation of gemfibrozil treatment and lipid levels with major coronary events: VA-HIT: a randomised controlled trial. JAMA 2001;28:1585-1591.
21. Birjmohun RS et al. Efficacy and safety of high-density lipoprotein cholesterol-increasing compounds. J Am Coll Cardiol 2005;45:185-197.
22. Cheung MC et al. Altered particle size distribution of apolipoprotein A-I containing lipoproteins in subjects with coronary artery disease. J Lipid Res 1991;32:383-394.
23. Salonen JT et al. HDL, HDL2, and HDL3 subfractions and the risk of acute myocardial infarction. A prospective population study in eastern Finnish men. Circulation 1991;84:129-139.
24. Miller NE. Associations of high density lipoprotein subclasses and apolipoproteins with ischemic heart disease and coronary atherosclerosis. Am Heart J 1987;113:589-597.
25. Sweetnam PM et al. Associations of HDL2 and HDL3 cholesterol subfractions with the development of ischemic heart disease in British men. Circulation 1994; 90: 769–774.
26. Tailleux A, Fruchart JC. HDL heterogeneity and atherosclerosis. Crit Rev Clin Lab Sci 1996;33:163-201.
27. The Expert Panel. Summary of the second report of the National Cholesterol Education Program expert panel on detection, evaluation, and treatment of high blood cholesterol in adults. JAMA 1993;269:2329-2331.
28. American Heart Association.. Consensus panel statement: guide to primary prevention of cardiovascular diseases. Circulation 1997;95:2329-2331.
29. Ryan TJ et al. ACC/AHA guidelines for the management of patients with acute myocardial infarction. A report of the American College of Cardiology/American Heart Association Task Force on Practice Guideline (Committee on Management of Acute Myocardial Infarction). J Am Coll Cardiol 1996;28:1328-1428.
30. The Expert Panel. 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.
31. Grundy SM et al. Implications of Recent Clinical Trials for the National Cholesterol Education Program Adult Treatment Panel III Guidelines. Circulation 2004;110:227-239.
32. De Backer G et al. European guidelines on cardiovascular disease prevention in clinical practice. Third Joint Task Force of European and other Societies on Cardiovascular Disease Prevention in Clinical Practice. Eur Heart J 2003;24:1601-1610.
33. Alsheikh-Ali AA, Kuvin JT, Karas RH. High-density lipoprotein cholesterol in the cardiovascular equation: Does the "Good" still count? Atherosclerosis 2005;180:217-223.
34. Nissen SE et al. Effect of recombinant ApoA-I Milano on coronary atherosclerosis in patients with acute coronary syndromes: a randomised controlled trial. JAMA 2003;290:2292-2300.
35. Jones PH et al. The STELLAR Study Group. Comparison of the efficacy and safety of rosuvastatin versus atorvastatin, simvastatin and pravastatin across doses (STELLAR trial). Am J Cardiol 2003;92:152-160.
36. Brousseau ME et al. Effect of an inhibitor of cholesteryl ester transfer protein on HDL cholesterol. N Engl J Med 2004;350:1505-1515.
37. Chapman MJ et al. Raising high-density lipoprotein cholesterol with reduction of cardiovascular risk: the role of nicotinic acid – a position paper developed by the European Consensus Panel on HDL-C. Curr Med Res Opin 2004;20:1253-1268.
38. Burstein M, Scholnick HR, Morfin R. Rapid method for the isolation of lipoproteins for human serum by precipitation with polyanions. J Lipid Res 1970;11:583-595.
39. Warnick GR, Nauck M, Rifai N. Evolution of methods for measurement of HDL-cholesterol: from ultracentrifugation to homogeneous assays. Clin Chem 2001;47:1579-1596.
40. Warnick GR, Wood PD for the National Cholesterol Education Program Working Group on Lipoprotein Measurement. National Cholesterol Education Program recommendations for measurement of high-density lipoprotein cholesterol: executive summary. Clin Chem 1995;41:1427-1433.
41. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-501.
42. Otvos JD et al. Development of a proton nuclear magnetic resonance spectroscopic method for determining plasma lipoprotein concentrations and subspecies from a single, rapid measurement. Clin Chem 1992;38:1632-1638.
43. Freedman DS et al. Relation of lipoprotein subclasses as measured by proton nuclear magnetic resonance spectroscopy to coronary artery disease. Arterioscler Thromb Vasc Biol 1998;18:1046-1053.
44. Freedman DS et al. Sex and age differences in lipoprotein subclasses measured by nuclear magnetic resonance spectroscopy: the Framingham Study. Clin Chem 2004;50:1189-1200.
45. Barzilai N et al. Unique lipoprotein phenotype and genotype associated with exceptional longevity. JAMA 2003;290:2030-2040.
46. Asztalos BF et al. Distribution of ApoA-I-containing HDL subpopulations in patients with coronary heart disease. Arterioscler Thromb Vasc Biol 2000; 20: 2670–2676.
47. Walldius G, Jungner I. Apolipoprotein B and apolipoprotein A-I: risk indicators of coronary heart disease and targets for lipid-modifying therapy. J Intern Med 2004;255:188-205.
48. Walldius G et al. High apolipoprotein B, low apolipoprotein A-I, and improvement in the prediction of fatal myocardial infarction (AMORIS study): a prospective study. Lancet 2001;358:2026-2033.
49. Yusuf S et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet 2004;364:937-52.
50. Gotto AM Jr. et al. Relation between baseline and on-treatment lipid parameters and first acute major coronary events in the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS). Circulation 1999;99:2517-22.
51. Genest J et al. Recommendations for the management and treatment of dyslipidemia and the prevention of cardiovascular disease: summary of the 2003 update. Can Med Assoc J 2003;169:921-924.
52. Devroey D et al. A review of the treatment guidelines on the management of low levels of high-density lipoprotein cholesterol. Cardiology 2004;102:61-66.

ORBITA Awards2006

Instructions for the participants

Profile

Participation is open to researchers, not older than 40 years, conducting research in the field of atherosclerosis in a Belgian hospital or research center.

Submission

The subject must be related to the field of atherosclerosis.
The summary will include not more than 15 pages, exclusive of the figures and references.
It should include a one-page abstract and must be accompanied by a curriculum vitae.
The summary must be send by mail or e-mail before February 17, 2006.

Jury

The scientific jury, composed of a prominent panel of Belgian Specialists, will meet to select the 3 best abstracts.
The 3 authors will be invited to present their work orally (15 presentation in English) during the ***ORBITA Symposium on Saturday March 18, 2006*** (from 9.00 to 12.30 a.m.)
The announcement of the winner will be made by the Chairman at the end of the symposium.

Prizes

First prize:	5.000,-
Second prize:	3.000,-
Third prize:	1.500,-

Each of the 3 winners will also be offered a course in «Public Speaking» (value of 800,-)
Each participants will receive a book voucher for medical literature (value of 125,-)

DEADLINE for SUBMISSION : February 17, 2006

For additionnal information, please contact Kathleen Commers, Medical Advisor
Tel.: 02/554.66.89 or e-mail: kathleen.commers@pfizer.com

To send your work with curriculum vitae, please contact Carine Decoster
Tel.: 02/554.67.51 or e-mail: carine.decoster@pfizer.com

Pfizer Global Pharmaceuticals
Pleinlaan, 17 Boulevard de la Plaine
B-1050 Brussel-Bruxelles

Pfizer

MSD & Schering-Plough Belgian Lipid Club Awards

Now is the time of the two biannual Lipidology prizes in a total amount of €6,500, offered by the Belgian Lipid Club, with the support of Laboratoires MSD & SCHERING-PLOUGH. The first prize will be awarded to an **article** published in the area of Lipidology (fundamental, epidemiological or clinical research) in the sum of €5,000. Publication must be in English in an internationally recognised medical journal. The second prize will be awarded to an **abstract** published in the area of Lipidology (fundamental, epidemiological or clinical research) in the sum of €1,500. Publication must have been in English in an internationally recognised medical journal.

Below, you will find the conditions for taking part and for award of the prizes.

Article 4. Candidates must be Belgian or Luxembourg nationals; there is no age limit. Writers must be in full professional practice.

Article 5. The works (articles or abstracts) that are presented may have been the subject of one or more studies carried out abroad.

Article 6. The works must be sent to the current Chairman of the Belgian Lipid Club before 1 July of the year in which the "**MSD & SCHERING-PLOUGH BELGIAN LIPID CLUB AWARDS"** are presented, viz. for the first time before 1 July 2001: the postmark shall constitute proof of adherence to this condition.

Article 7. The works presented must have been published in the two years preceding the last date for submission. Priority will be given to works that have not yet been endowed with a prize.

Article 8. The sum of the **"MSD & SCHERING-PLOUGH BELGIAN LIPID CLUB AWARDS"** will be awarded to the first writer of each work, and he shall be responsible for distributing all or part of the sum to his co-writer(s) as he sees fit. The first writer must be understood as being the person that has produced the essence of the work that is presented.

Article 9. The Jury will be made up of 7 members of the Executive of the Belgian Lipid Club and the Medical Advisers of MSD and SCHERING-PLOUGH. Each member of the jury has one deliberate vote, with the exception of the MSD and SCHERING-PLOUGH Medical Advisers, who for obvious reasons may not vote. Voting is compulsory and secret. The "MSD & SCHERING-PLOUGH BELGIAN LIPID CLUB AWARDS" must be awarded at one, single deliberative session. The voting procedure will be decided on by all the members of the Jury at that session before commencement of the discussions.

Article 10. The jury shall endeavour to make an award to at least one clinical work every two years. However, this rule may be deviated from if the works submitted in this domain have been of insufficient quality in the opinion of the Jury. If the Jury sees fit, the prize may be split between two submissions.

Article 11. There is no appeal against the decisions of the Jury, which are final. Participation in the competition implies full, complete acceptance of these rules.

Works must be sent before 1 July 2006 to

Professor F. R. HELLER
Chairman of the Belgian Lipid Club
C.H. Jolimont-Lobbes
159 Rue Ferrer
7100 HAINE ST PAUL
Tel.: 064 233 896 – Fax: 064 233 697
E-mail: heller.cs.jolimont@skynet.be

The **MSD & SCHERING-PLOUGH BELGIAN LIPID CLUB AWARDS** will be presented at an academic meeting organised during the course of 2006.