

High-Density Lipoprotein Subfractions – What the Clinicians Need to Know

Angela Pirillo^{a, b} Giuseppe Danilo Norata^{c, d} Alberico Luigi Catapano^{b, c}

^aCenter for the Study of Atherosclerosis, Bassini Hospital, Cinisello Balsamo, ^bIRCCS Multimedica, and ^cDepartment of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milan, Italy; ^dThe Blizard Institute, Centre for Diabetes, Barts and The London School of Medicine and Dentistry, Queen Mary University, London, UK

Key Words

High-density lipoprotein · High-density lipoprotein function · High-density lipoprotein subpopulations · Coronary heart disease

Abstract

Although the inverse relationship between plasma levels of high-density lipoprotein (HDL) and cardiovascular disease has been largely demonstrated, many observations have suggested that the assessment of HDL functionality might be more informative than a simple measurement of HDL-cholesterol plasma levels. HDLs are a class of structurally and functionally heterogeneous particles; in atherosclerosis-related diseases, changes in HDL subfraction levels and functions are frequently observed. Circulating levels of large HDL particles are decreased in dyslipidaemic conditions, while levels of small dense HDL particles are increased in patients with coronary heart disease. Furthermore, specific genetic defects in proteins involved in HDL metabolism significantly impact the distribution of HDL subpopulations. Finally, many drugs used for dyslipidaemia induce changes in HDL subfractions strictly related to cardiovascular disease. Although several methods exist to evaluate HDL subclass levels, most of them are not easily applicable in clinical practice, due to the costs and high variability. However, the possibility to measure the levels of specific HDL subfractions in patients with atherosclerosis-related diseases might help to better define their cardiovascular risk.

Background

The inverse relationship between plasma levels of high-density lipoprotein (HDL) and cardiovascular disease has been extensively described [1, 2]. However, several lines of evidence have indicated that HDL functionality can be impaired under pathological conditions [3, 4], suggesting that the assessment of HDL function might be more relevant than that of HDL-cholesterol (HDL-C) plasma levels.

HDLs are a class of structurally and functionally heterogeneous particles [5]. In atherosclerosis-related diseases, variations in HDL subfraction levels and functions are frequently observed, suggesting that large HDL particles are inversely associated while small HDL particles are positively associated with cardiovascular disease [5].

These considerations, supported by several studies, indicate that beside the measurement of standard lipids, including HDL-C levels, the measurement of specific HDL subfractions might help to better evaluate the risk of cardiovascular events.

HDL Classification, Metabolism and Functions

HDLs are a class of heterogeneous lipoproteins [5]; their heterogeneity is attributable to a different content of apolipoproteins, lipids and enzymes and to the remodelling of HDL particles by lipolytic enzymes, lipid trans-

Copyright © 2013 S. Karger AG, Basel

KARGER

E-Mail karger@karger.com
www.karger.com/crd

© 2013 S. Karger AG, Basel
0008-6312/13/1242-0116\$38.00/0

Prof. Alberico L. Catapano
Department of Pharmacological Sciences, University of Milan
Via Balzaretti, 9
IT-20133 Milan (Italy)
E-Mail alberico.catapano@unimi.it

Table 1. HDL subclasses

Shape
Discoidal
Spherical
Density (ultracentrifugation)
HDL2
HDL3
Size (non-denaturing gel electrophoresis)
HDL2b
HDL2a
HDL3a
HDL3b
HDL3c
Charge (2-dimensional electrophoresis)
Pre- β -particles:
Pre- β_1 (HDL3, LpA-I)
Pre- β_2 (LpA-I)
Pre- β_3 (LpA-I)
Pre- β_4 (LpA-I)
α -Particles:
Very small discoidal α_4 (HDL3, LpA-I)
Small spherical α_3 (HDL3, LpA-I:A-II)
Medium spherical α_2 (HDL3, LpA-I:A-II)
Large spherical α_1 (HDL2, LpA-I)
Pre- α particles:
Pre- α_1 (LpA-I)
Pre- α_2 (LpA-I)
Pre- α_3 (LpA-I)
Pre- α_4 (LpA-I)
Composition (immunoaffinity)
LpA-I (prominent components of both HDL2 and HDL3)
Large LpA-I
Medium LpA-I
Small LpA-I
LpA-I
A-II (most found in HDL3)

porters and by lipid and apolipoprotein exchange with other circulating lipoproteins and tissues. Different HDL subpopulations carry distinct and specific proteins or lipids, suggesting distinct and characteristic functions [5].

Several HDL subpopulations can be obtained using various separation techniques; the comparison between particles obtained with different procedures is not easy, as each subclass may contain particles with different characteristics (table 1).

HDL can be classified on the basis of density, resulting in the large buoyant HDL2 and the small dense HDL3, which can be further subfractionated into 5 distinct subpopulations (HDL2b, HDL2a, HDL3a, HDL3b and HDL3c; table 1) [5]. Alternatively, HDLs can be separated on the basis of their electrophoretic mobility in pre- β -

particles, α -particles and pre- α -particles [5]. HDLs can also be classified according to their main apolipoprotein content [5] into particles containing only apolipoprotein A-I (apoA-I, LpA-I) or both apoA-I and apoA-II (LpA-I/A-II; table 1).

After synthesis in the liver and intestine, apoA-I is secreted and interacts with cells, acquiring phospholipids (PL) and free cholesterol (FC), thus generating discoidal pre- β -HDL (fig. 1). These nascent particles interact with peripheral tissues further acquiring FC, which is then esterified by lecithin-cholesterol acyltransferase (LCAT); the hydrophobic cholesteryl esters (CE) move into the centre of the particles, resulting in the conversion into spherical lipoproteins (α -HDL), the major type of circulating HDL. Small α -HDLs may further enlarge by acquiring more FC from peripheral cells; LCAT-mediated esterification of FC generates large spherical HDL2 particles that contain a lipid core composed of CE and triglyceride (TG). Phospholipid transfer protein (PLTP) and cholesteryl ester transfer protein (CETP) contribute to the HDL remodelling. Spherical HDL can be remodelled by lipases resulting in the reduction in HDL size, the formation of lipid-poor HDL particles and the release of lipid-free apoA-I, which can restart the lipidation cycle.

The heterogeneity of HDLs is also reflected in their functions, since different subpopulations play distinct roles. HDLs possess several anti-atherogenic functions. The best characterized activity of HDL is reverse cholesterol transport, the process by which excess cholesterol is transported from the peripheral tissues to the liver for excretion. Different HDL subpopulations interact with different cellular receptors to remove excess cholesterol from cells [5]. Besides, HDLs, and in particular small dense HDL3, exert anti-inflammatory and anti-oxidant activities [5]. HDLs have a protective effect on vascular endothelium, are anti-thrombotic and anti-infectious [5] and play a role in the modulation of immune responses [6] and the control of glucose homeostasis [7].

HDL Subpopulation and Cardiovascular Disease

The relationship between circulating levels of HDL subpopulations and protection against cardiovascular disease is still unclear; nevertheless, it is widely established that HDL metabolism and subfraction distribution are altered under dyslipidaemic conditions and, except for slight differences, decrease in large HDL2 and

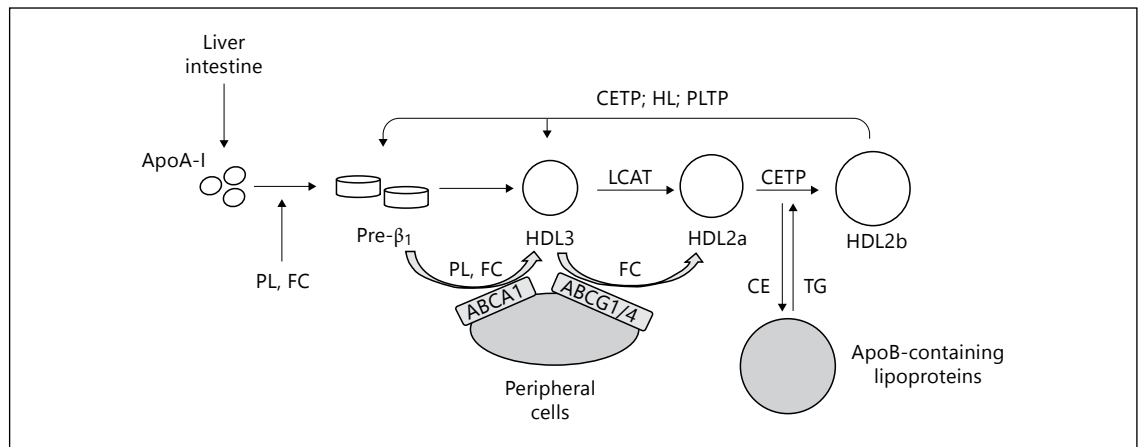


Fig. 1. HDL metabolism. ApoA-I secreted by the liver and intestine associates with PL and FC to form discoidal nascent pre- β -HDLs, which acquire further PL and FC from peripheral tissues. LCAT esterifies FC into cholesteryl ester (CE), which moves into the core of the particle converting discoidal to spherical HDL particles. CETP mediates the exchange of HDL cholesteryl ester onto apoB-containing lipoproteins in exchange for TG. HDL is also digested

by hepatic lipase (HL), which hydrolyzes HDL PL and TG, releasing smaller HDL and lipid-poor apoA-I that can restart the cycle. CETP and phospholipid transfer protein contribute to the generation of small pre- β ₁-particles from large α -particles. ABCA1 = ATP-binding cassette transporter A1; ABCG1/4 = ATP-binding cassette transporter G1/4.

increase in small HDL3 and pre- β ₁-HDLs are commonly observed in dyslipidaemia [8]. Several observations have suggested that HDL subpopulation levels are better predictors of coronary heart disease (CHD) than HDL-C levels.

HDL2 and HDL3

Several studies have shown an inverse relationship between HDL-C levels and coronary artery disease (CAD) risk, but different HDL subfractions may exhibit distinct protective activities. In fact, HDL2 levels, in particular HDL2b subfraction, are lower in patients with CAD compared to healthy subjects [9] and inversely related to both disease severity and progression of coronary lesions [10]. Based on several observations, the HDL2-C level appears to be more predictive of CHD risk than HDL-C or HDL3-C (table 2) [9, 11].

In dyslipidaemic subjects, a significant reduction in large HDL2b and a rise in small pre- β ₁-HDLs have been observed, compared to subjects with desirable plasma lipid levels [12]. Accordingly, average HDL particle size was reduced in women with CAD compared to healthy subjects [13]; patients with acute ischemic stroke had significantly smaller HDL size, more HDL3 and less HDL2b particles [14], and in myocardial infarction survivors, HDL2b was significantly lower than in controls and inversely correlated with body mass index, smoking, TG and low-density lipoprotein-cholesterol (LDL-C) levels

[15]. A similar reduction in large HDL2 particles and an increase in small particles were also observed in non-obese type 2 diabetics [16], in overweight and obese subjects [17–19], in subjects with metabolic syndrome [20] and in CHD subjects with diabetes when compared to CHD subjects without diabetes [21].

Pre- β -Particles

Pre- β -particles, which represent only a small portion of HDL, play a key role in the reverse cholesterol transport process; however, CHD patients show higher levels of pre- β -particles compared to healthy subjects [22–24], due to defective activity of enzymes involved in the HDL maturation cycle or to enhanced enzymatic remodelling of α -HDL induced by high plasma TG levels (table 2) [17, 25, 26].

Pre- β -HDL levels are increased in patients with CAD or ischemic heart disease even when excluding dyslipidaemic conditions [23, 27]; furthermore, the mean pre- β -HDL level is significantly higher in subjects with unstable angina pectoris than in patients with stable CAD [27]. A similar direct association of pre- β -HDL with CHD and myocardial infarction was found even after adjustment for established risk factors [22]. In diabetics, the pre- β -HDL concentration was higher compared to non-diabetic subjects and positively correlated with carotid intima media thickness [28]. Also, postmenopausal women with established CAD had higher levels of pre- β -

Table 2. HDL subpopulations in cardiovascular disease

HDL subclasses	Reference
HDL2, HDL3	
↓HDL2 in CAD patients	[9]
HDL2 negatively correlate with disease severity and lesion progression	[10]
Small HDL particles associate with adverse cardiometabolic risk profile	[77]
↓HDL2 in dyslipidemic subjects	[12]
Women with CAD: ↓average HDL particle size	[13]
↑HDL3 and ↓HDL2b levels in subjects with acute ischemic stroke	[14]
HDL2b: myocardial infarction survivors < controls	[15]
↓HDL2b levels in non-obese type 2 patients	[16]
↓HDL2 and ↑HDL3 in overweight and obese patients	[17–19]
↑HDL3 and ↓HDL2 in subjects with metabolic syndrome	[20]
↑HDL3 and ↓HDL2 in CHD subjects with diabetes compared to CHD subjects without diabetes	[21]
Pre-β-HDL	
CAD patients > controls	[27]
Unstable angina pectoris > stable CAD	
Ischemic heart disease patients > no ischemic heart disease	[23]
Positive association with CHD and myocardial infarction	[22]
Positive correlation with intima media thickness in type 2 diabetic patients	[28]
Postmenopausal CAD women > postmenopausal women without CAD	[29]
Positive correlation with total cholesterol levels	[78]
Higher levels in overweight and obese patients	[19]
α-HDL	
↓α ₁ and pre-α and ↑α ₃ particles in CHD patients	[31, 33]
Negative correlation between α ₁ levels and coronary stenosis	[30]
Low α ₁ -particles predict recurrence of cardiovascular events	[32]
↑1 mg/dl α ₁ -particle reduced odds of CHD by 26%	[31]
↑1 mg/dl HDL-C reduced odds of CHD by 2%	
LpA-I, LpA-I:A-II	
↓LpA-I and LpA-I:A-II in patients with established CAD	[34]
↓LpA-I and LpA-I:A-II in patients with incident CHD	[35]
↓Large LpA-I in subjects with symptomatic CAD	[36]

HDL and lower levels of large α-particles than control women, confirming that the profile distribution of HDL subpopulations could be more informative than HDL-C concentrations in the assessment of cardiovascular risk [29].

α-Particles

Large α-particles are inversely associated with cardiovascular disease [30–32]; in fact, CHD patients have significantly lower concentrations of large α₁- and pre-α-particles compared to control subjects, while small pre-β₁ and α₃-subfractions are considerably higher (table 2) [31, 33]. Similarly, a significant negative correlation between changes in α₁-HDL particle concentration and coronary stenosis has been reported [30]. In CHD patients with low HDL-C and normal LDL-C levels, a low α₁-particle level was the most significant parameter able to predict recurrence of cardiovascular events

[32]. Accordingly, the Framingham Offspring Study revealed that 1 mg/dl increase in the α₁-particle level reduced odds of CHD by 26%, while 1 mg/dl increase in HDL-C only reduced odds of CHD by 2% [31].

LpA-I and LpA-I:A-II

Both LpA-I and LpA-I:A-II levels were reduced in subjects with angiographically established CAD compared to control subjects [34] and in patients with incident CHD compared to subjects free of CHD (table 2) [35]. In men with symptomatic CAD, the proportion of large LpA-I, the most effective in promoting cholesterol efflux, was lower compared to subjects with no symptoms of CAD [36]. LpA-I is considered anti-atherogenic, due to its greater ability to promote cholesterol efflux, and an increase in LpA-I/LpA-I:A-II ratio seems to have a protective role. However, other studies did not support this hypothesis [37], a discrepancy that may be once more re-

lated to the heterogeneity of LpA-I and LpA-I:A-II subpopulations (table 1). The inverse correlations between large and small particles suggest that when one particle level increases the other decreases within the same LpA-I or LpA-I:A-II subclasses, resulting in unchanged LpA-I or LpA-I:A-II levels but in structurally and physiologically different HDL particles.

Effects of Genetics on HDL Subpopulations

A large number of proteins, enzymes and receptors are involved in the metabolism of HDL; mutations in the genes encoding for these proteins have been associated not only with changes in HDL-C levels but also with alterations in HDL structure, as is the case for CETP and LCAT.

Hyperalphalipoproteinaemia caused by CETP deficiency has been associated with striking changes in lipoprotein profile and composition [38]. HDL particles from subjects with complete CETP deficiency have an abnormal size and exhibit relevant changes in the apoprotein content [38], and their functionality is a matter of debate. HDL2 from subjects with CETP mutations more efficiently promoted cholesterol efflux compared to control HDL2 [39–41]. A case of complete CETP deficiency resulted in extremely elevated HDL-C and apoA-I levels, with a significant increase in both LpA-I and LpA-I:A-II levels, a high pre- β -particle content and HDL2 larger than normal [42]. The subject had no atherosclerotic plaques in carotid and femoral arteries; however, it is difficult to relate this finding with the HDL phenotype determined by CETP deficiency. Overall, CETP deficiency was associated with altered composition. Whether the same occurs with pharmacological CETP inhibitors is still under investigation.

LCAT mutations are associated with multiple alterations in HDL structure and particle distribution, with a selective depletion of large α -particles and a predominance of small pre- β -HDLs [43], due to their inability to mature into spherical HDLs caused by LCAT deficiency. Alterations in HDL particle distribution, and particularly the very high content of pre- β -HDL, resulted in an increased capacity of LCAT-deficient serum to promote cholesterol efflux [43]. This might explain why, despite reduced HDL-C levels, LCAT deficiency does not remarkably increase preclinical atherosclerosis and could even be protective for human arteries [43].

Other mutations are associated with altered HDL subpopulation profile (decrease in large α -particles or preva-

lence of small particles), as well as with altered antioxidant/anti-inflammatory properties [44].

Although mutations in genes predisposing to low HDL levels result in altered HDL subfraction composition, the connection between these alterations and increased cardiovascular damage is still elusive and deserves further investigation.

Dysfunctional HDL under Pathologic Conditions

In addition to changes in the levels of the different subclasses, alterations in the structure and, as a consequence, in the functions of HDL subpopulations can be observed. In fact, under pathological conditions, including chronic inflammation, oxidative stress, dyslipidaemia, diabetes and atherosclerosis, HDL particles can undergo significant composition and structure modifications thus resulting in 'dysfunctional HDL', which not only exhibit reduced anti-atherogenic properties, but can also acquire pro-atherogenic characteristics [45]. Subjects with metabolic syndrome show altered anti-atherogenic properties of HDL, due to changes in the activity of HDL-associated enzymes [20]; HDL3 from type 2 diabetics display a significantly reduced anti-oxidant activity, due to altered composition of this subfraction linked to oxidative stress, glycaemia and hypertriglyceridemia [46]. Both HDL2 and HDL3 from familial hypercholesterolemic (FH) subjects display a reduced ability to mediate cholesterol efflux compared to lipoproteins from normolipidemic subjects [47]. Furthermore, TG-rich HDL3 from FH patients have reduced the anti-inflammatory activity and capacity to promote reverse cholesterol transport compared with HDL3 derived from FH patients with low serum TG levels, confirming that TG enrichment of HDL particles confers pro-atherogenic characteristics [48].

In addition, several enzymatic and non-enzymatic modifications of HDL have been described that can induce structural and functional changes in lipids and apoproteins of HDL, thus impairing its atheroprotective role [49–52]. Dysfunctional HDL can be either the result of a disease or the cause of the disorder.

HDL as a Therapeutic Target: Effect on HDL Subpopulations

The enzymes involved in HDL metabolism respond to changes in the metabolic status and are modulated not only following alterations in HDL levels but also respond

to changes in TG-rich lipoproteins and their lipid composition. As a consequence, drugs modulating fatty acids and cholesterol availability impact both HDL metabolism and subclass composition.

CETP is a key enzyme involved in HDL maturation, and its inhibition was associated with the enhancement of HDL capacity to promote FC efflux and to mediate the selective hepatic uptake of cholesteryl esters, due to the increase in large HDL2 [53–55]. Despite CETP inhibitors representing the most potent class of molecules to increase HDL-C levels, the possibility that in the long term the increase in large HDL2, but not in small HDL3, could limit the beneficial impact of these drugs is debated. Of note, out of four CETP inhibitors in clinical development, torcetrapib was stopped because it was associated with an increased risk of death from any cause and a higher major cardiovascular event rate, probably for an off-target effect on blood pressure and aldosterone levels [56]. Trial with dalcetrapib, that increased HDL-C levels (by increasing both HDL2 and HDL3 subfractions) without affecting LDL-C and TG levels [57], despite its safety, was suspended for futility (http://www.roche.com/media/media_releases/med-cor-2012-05-07.htm) due to lack of efficacy on clinical endpoints, as no effects on endothelial function, atherosclerosis progression or arterial wall inflammation were observed [56]. Data on the effects of the two CETP inhibitors still in development, anacetrapib and evacetrapib (which, beside HDL-C increase, also induce a decrease in LDL-C), which did not show negative effects on blood pressure or aldosterone levels [56], on HDL subclass composition and how this might translate into beneficial effects on CAD are awaited, representing the last chance for CETP inhibition to be considered as a useful strategy for CVD risk reduction.

Niacin is the most effective HDL-C-increasing drug available in clinical practice which also acts by lowering other plasma lipids, including TG and LDL-C. Some studies have shown therapeutic efficacy of niacin in reducing cardiovascular risk or in stabilizing or regressing atherosclerosis [58]. Niacin, in combination with lovastatin, was more effective in increasing the proportion of the cardioprotective HDL2b subclass, compared with statin monotherapies [59], and also in selectively increasing LpA-I particle concentration in subjects with low HDL-C [60]. In a small group of diabetics with low pre- β -HDL, niacin treatment was associated with a further reduction in pre- β -HDL, as the drug promoted pre- β -particle maturation and improved reverse cholesterol transport in diabetes [61]. A similar effect was reported

with atorvastatin which was shown to reduce plasma pre- β -particle formation in a patient with type 2 diabetes [62]. It is conceivable that, as increased pre- β -HDL levels could be a marker of impaired HDL maturation, a reduction in pre- β -HDL by niacin or statins reflects a beneficial cardiovascular effect associated with a more rapid metabolism of such particles into mature α -HDL, contributing to an efficient reverse cholesterol transport pathway. Simvastatin-niacin therapy significantly increased the large α_1 -HDL particles [30]; this increase was significantly associated with less progression of coronary stenosis even after adjusting for traditional risk factors.

Despite these cardiovascular benefits, the AIM-HIGH trial, which compared the combination niacin/simvastatin with simvastatin alone, was stopped for lack of efficacy (<http://www.nih.gov/news/health/may2011/nhlbi-26.htm>), as niacin, despite an increase in HDL-C levels, failed to determine an incremental clinical benefit in CVD patients with LDL-C values at target [58]. In addition, during the follow-up period, a small and unexplained approximately 2-fold increase in ischemic stroke rate was observed in subjects treated with the high dose of niacin. However, this trial had many limitations which, together with the early study termination, reduce its possible interpretation [63]. The much larger HPS2-THRIVE trial (<http://clinicaltrials.gov/ct2/show/NCT00461630>), designed to assess the effect of adding niacin to a statin therapy, will probably give a definitive conclusion on niacin add-on therapy, which AIM-HIGH was not designed to be.

Treatment with fibrates, including fenofibrate and bezafibrate, was associated with increased pre- β_1 -HDL levels [64, 65], as these drugs promote the conversion of large HDL2 into pre- β_1 -HDL by increasing hepatic lipase activity, increase very-low-density lipoprotein catabolism and induce hepatic apoA-I production. Fibrates were ineffective in reducing the vascular event risk, with the exception of subgroups of patients with atherogenic dyslipidaemia, characterized by high TG and low HDL-C [66], suggesting that these drugs should be recommended only for specific subgroups of dyslipidaemic patients.

Several studies have reported a significant association between omega-3 polyunsaturated fatty acid (PUFA) intake and reduction in cardiovascular risk [67]. Omega-3 PUFA supplementation was associated with an improved anti-atherogenic profile of HDL [68], characterized by an increase in large HDL2 and a decrease in small HDL3. Of note, such beneficial HDL subclass changes

were observed even with the small dose of omega-3 PUFA provided by daily consumption of fatty fish or below the average dose of purified PUFA preparations [68]. Despite these positive effects on the HDL subpopulation profile, a recent study in patients at high cardiovascular risk showed that daily supplementation of omega-3 PUFA did not reduce the rate of cardiovascular events [69], a finding confirmed by two meta-analyses [70, 71], suggesting that there is no evidence to support the use of omega-3 supplementation in secondary prevention.

Overall, available data on the effects of lipid-related drugs on HDL subclasses indicate that an increase in mature large HDL reflects the beneficial effects of these compounds on HDL maturation and activity. The exception is represented by fibrates which, due to their peculiar mechanism of action, work at different levels in the life cycle of HDL, promoting both maturation and activity but also the late stage associated with catabolism, thus resulting in an increase in pre- β_1 -HDL, probably as a consequence of improved hepatic catabolic activity.

Conclusions

Although several studies have clearly shown an inverse correlation between plasma HDL-C levels and cardiovascular risk, emerging evidence suggests that the anti-atherogenic role of HDL is not simply defined by the plasma HDL-C level but rather by the function of HDL subspecies. For this reason, besides the measurement of HDL-C levels, the assessment of the HDL subpopulation distribution profile and functionality might represent the best approach to investigate the relationship between HDL and CHD and to improve CV risk prediction. However, although several methods exist to separate HDL subfractions [72, 73], most of them are not useful in clinical practice, as they are expensive, lengthy and results are often ambiguous. In addition, the National Lipid Association Biomarkers Expert Panel could not find evidence to support HDL subfraction measurement for initial clinical assessment or on-treatment management decisions in patients with low or intermediate risk, nor in patients at high risk or with established CHD who experience recurrent events despite appropriate therapy [74]; similarly, no special patient subgroups were identified in which HDL sub-fractionation should be recommended.

However, the study of HDL subclass distribution in subjects with cardiovascular disease significantly enrich-

es the comprehension of the complex relationship between HDL and CAD. It also suggests that HDL-C measurement could not be sufficient to evaluate the impact of this lipoprotein class on cardiovascular risk, as demonstrated by the use of CETP inhibitors or by the lack of increase in carotid intima media thickness in carriers of apoA-I mutation, despite very low levels of HDL-C [75], suggesting that HDL quality, i.e. subclass distribution and/or subclass functionality, could be more informative than HDL-C levels.

Raising HDL-C is considered one of the targets to reduce the residual cardiovascular risk [76]. However, despite the demonstrations that low HDL-C levels are associated with major cardiovascular events in CHD patients, even when at target with LDL-C levels, there are no demonstrations that low HDL-C causes cardiovascular events; the failure of trials investigating the role of HDL-raising therapies (http://www.roche.com/media/media_releases/med-cor-2012-05-07.htm; <http://www.nih.gov/news/health/may2011/nhlbi-26.htm>) further challenged the concept that raising HDL-C levels will always translate into risk reduction. Furthermore, as pharmacological interventions able to change the HDL subpopulation profile can also induce changes in HDL-C levels, HDL particle number and/or other lipid parameters such as TG or LDL-C, it is difficult to distinguish the effects related to changes in HDL-C and/or specific subfractions from the effects related to a general improvement in the lipid profile. Finally, there are no prospective studies demonstrating that a pharmacological intervention aimed at shifting the HDL profile to a more favourable condition is superior to traditional lipid-lowering therapies in reducing cardiovascular risk. Until reliable results from clinical trials will be available, we should withhold the final word on the usefulness of HDL subfraction determination in clinical practice.

Conflict of Interest

A.L.C. is a member of the speaker bureau for Merck, AstraZeneca, Sanofi, Genzyme and Sigma-Tau and is on the advisory board of Merck, Astrazeneca, Sanofi, Genzyme and Aegerion.

References

- 1 Barter P, Gotto AM, LaRosa JC, Maroni J, Szarek M, Grundy SM, Kastelein JJ, Bittner V, Fruchart JC: HDL cholesterol, very low levels of LDL cholesterol, and cardiovascular events. *N Engl J Med* 2007;357:1301–1310.
- 2 Gordon DJ, Probstfield JL, Garrison RJ, Neaton JD, Castelli WP, Knoke JD, Jacobs DR Jr, Bangdiwala S, Tyroler HA: High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation* 1989;79:8–15.
- 3 Smith JD: Dysfunctional HDL as a diagnostic and therapeutic target. *Arterioscler Thromb Vasc Biol* 2010;30:151–155.
- 4 Ansell BJ, Fonarow GC, Fogelman AM: The paradox of dysfunctional high-density lipoprotein. *Curr Opin Lipidol* 2007;18:427–434.
- 5 Camont L, Chapman MJ, Kontush A: Biological activities of HDL subpopulations and their relevance to cardiovascular disease. *Trends Mol Med* 2011;17:594–603.
- 6 Norata GD, Pirillo A, Ammirati E, Catapano AL: Emerging role of high density lipoproteins as a player in the immune system. *Atherosclerosis* 2012;220:11–21.
- 7 Drew BG, Rye KA, Duffy SJ, Barter P, Kingwell BA: The emerging role of HDL in glucose metabolism. *Nat Rev Endocrinol* 2012;8:237–245.
- 8 Tian L, Fu M: The relationship between high density lipoprotein subclass profile and plasma lipids concentrations. *Lipids Health Dis* 2010;9:118.
- 9 Drexel H, Amann FW, Rentsch K, Neuenchwander C, Luethy A, Khan SI, Follath F: Relation of the level of high-density lipoprotein subfractions to the presence and extent of coronary artery disease. *Am J Cardiol* 1992;70:436–440.
- 10 Johansson J, Carlson LA, Landou C, Hamsten A: High density lipoproteins and coronary atherosclerosis. A strong inverse relation with the largest particles is confined to normotriglyceridemic patients. *Arterioscler Thromb* 1991;11:174–182.
- 11 Lamarche B, Moorjani S, Cantin B, Dagenais GR, Lupien PJ, Despres JP: Associations of HDL2 and HDL3 subfractions with ischemic heart disease in men. Prospective results from the Quebec Cardiovascular Study. *Arterioscler Thromb Vasc Biol* 1997;17:1098–1105.
- 12 Yang Y, Yan B, Fu M, Xu Y, Tian Y: Relationship between plasma lipid concentrations and HDL subclasses. *Clin Chim Acta* 2005;354:49–58.
- 13 Blackburn P, Lemieux I, Lamarche B, Bergeron J, Perron P, Tremblay G, Gaudet D, Despres JP: Angiographically-assessed coronary artery disease associates with HDL particle size in women. *Atherosclerosis* 2012;223:359–364.
- 14 Zeljkovic A, Vekic J, Spasojevic-Kalimanovska V, Jelic-Ivanovic Z, Bogavac-Stanojevic N, Gulan B, Spasic S: LDL and HDL subclasses in acute ischemic stroke: prediction of risk and short-term mortality. *Atherosclerosis* 2010;210:548–554.
- 15 Mueller O, Chang E, Deng D, Franz T, Jing D, Kincaid R, Konigshofer Y, Kratzmeier M, McNulty M, Qian H, Schneider J, Schulte H, Seedorf U, Tian X, Van Cleve M, Yang D, Assmann G: PROCAM Study: risk prediction for myocardial infarction using microfluidic high-density lipoprotein (HDL) subfractionation is independent of HDL cholesterol. *Clin Chem Lab Med* 2008;46:490–498.
- 16 Xian X, Ma Y, Yang DD, Huang W, Wang Y, Mueller O, Chang E, Konigshofer Y, Van-Cleve M, Yang J, Liu G: Reduced high-density lipoprotein 2b in non-obese type 2 diabetic patients analysed by a microfluidic chip method in a case-control study. *Biomarkers* 2009;14:619–623.
- 17 Tian L, Xu Y, Fu M, Peng T, Liu Y, Long S: The impact of plasma triglyceride and apolipoproteins concentrations on high-density lipoprotein subclasses distribution. *Lipids Health Dis* 2011;10:17.
- 18 Rosenbaum D, Hansel B, Bonnefont-Rousselot D, Bittar R, Girerd X, Giral P, Bruckert E: Waist circumference is a strong and independent determinant of the distribution of HDL subfractions in overweight patients with cardiovascular risk factors. *Diab Vasc Dis Res* 2012;9:153–159.
- 19 Tian L, Jia L, Mingde F, Tian Y, Xu Y, Tian H, Yang Y: Alterations of high density lipoprotein subclasses in obese subjects. *Lipids* 2006;41:789–796.
- 20 Lagos KG, Filippatos TD, Tsimihodimos V, Gazi IF, Rizos C, Tselepis AD, Mikhailidis DP, Elisaf MS: Alterations in the high density lipoprotein phenotype and HDL-associated enzymes in subjects with metabolic syndrome. *Lipids* 2009;44:9–16.
- 21 Tian L, Long S, Li C, Liu Y, Chen Y, Zeng Z, Fu M: High-density lipoprotein subclass and particle size in coronary heart disease patients with or without diabetes. *Lipids Health Dis* 2012;11:54.
- 22 Guey LT, Pullinger CR, Ishida BY, O'Connor PM, Zellner C, Francone OL, Laramie JM, Naya-Vigne JM, Siradze KA, Deedwania P, Redberg RF, Frost PH, Seymour AB, Kane JP, Malloy MJ: Relation of increased prebeta-1 high-density lipoprotein levels to risk of coronary heart disease. *Am J Cardiol* 2011;108:360–366.
- 23 Sethi AA, Sampson M, Warnick R, Muniz N, Vaisman B, Nordestgaard BG, Tybjaerg-Hansen A, Remaley AT: High pre-beta1 HDL concentrations and low lecithin: cholesterol acyltransferase activities are strong positive risk markers for ischemic heart disease and independent of HDL-cholesterol. *Clin Chem* 2010;56:1128–1137.
- 24 Suzuki M, Wada H, Maeda S, Saito K, Minatoguchi S, Seishima M: Increased plasma lipid-poor apolipoprotein A-I in patients with coronary artery disease. *Clin Chem* 2005;51:132–137.
- 25 Borggreve SE, De Vries R, Dullaart RP: Alterations in high-density lipoprotein metabolism and reverse cholesterol transport in insulin resistance and type 2 diabetes mellitus: role of lipolytic enzymes, lecithin:cholesterol acyltransferase and lipid transfer proteins. *Eur J Clin Invest* 2003;33:1051–1069.
- 26 Rye KA, Barter PJ: Formation and metabolism of prebeta-migrating, lipid-poor apolipoprotein A-I. *Arterioscler Thromb Vasc Biol* 2004;24:421–428.
- 27 Tashiro J, Miyazaki O, Nakamura Y, Miyazaki A, Fukamachi I, Bujo H, Saito Y: Plasma pre beta1-HDL level is elevated in unstable angina pectoris. *Atherosclerosis* 2009;204:595–600.
- 28 Hirayama S, Miida T, Miyazaki O, Aizawa Y: Pre beta1-HDL concentration is a predictor of carotid atherosclerosis in type 2 diabetic patients. *Diabetes Care* 2007;30:1289–1291.
- 29 Lamon-Fava S, Herrington DM, Reboussin DM, Sherman M, Horvath KV, Cupples LA, White C, Demissie S, Schaefer EJ, Asztalos BF: Plasma levels of HDL subpopulations and remnant lipoproteins predict the extent of angiographically-defined coronary artery disease in postmenopausal women. *Arterioscler Thromb Vasc Biol* 2008;28:575–579.
- 30 Asztalos BF, Batista M, Horvath KV, Cox CE, Dallal GE, Morse JS, Brown GB, Schaefer EJ: Change in alpha1 HDL concentration predicts progression in coronary artery stenosis. *Arterioscler Thromb Vasc Biol* 2003;23:847–852.
- 31 Asztalos BF, Cupples LA, Demissie S, Horvath KV, Cox CE, Batista MC, Schaefer EJ: High-density lipoprotein subpopulation profile and coronary heart disease prevalence in male participants of the Framingham Offspring Study. *Arterioscler Thromb Vasc Biol* 2004;24:2181–2187.
- 32 Asztalos BF, Collins D, Cupples LA, Demissie S, Horvath KV, Bloomfield HE, Robins SJ, Schaefer EJ: Value of high-density lipoprotein (HDL) subpopulations in predicting recurrent cardiovascular events in the Veterans Affairs HDL Intervention Trial. *Arterioscler Thromb Vasc Biol* 2005;25:2185–2191.
- 33 Asztalos BF, Roheim PS, Milani RL, Lefevre M, McNamara JR, Horvath KV, Schaefer EJ: Distribution of ApoA-I-containing HDL subpopulations in patients with coronary heart disease. *Arterioscler Thromb Vasc Biol* 2000;20:2670–2676.
- 34 O'Brien T, Nguyen TT, Hallaway BJ, Hodge D, Bailey K, Holmes D, Kottke BA: The role of lipoprotein A-I and lipoprotein A-I/A-II in predicting coronary artery disease. *Arterioscler Thromb Vasc Biol* 1995;15:228–231.

- 35 Luc G, Bard JM, Ferrieres J, Evans A, Amouyel P, Arveiler D, Fruchart JC, Ducimetiere P: Value of HDL cholesterol, apolipoprotein A-I, lipoprotein A-I, and lipoprotein A-I/A-II in prediction of coronary heart disease: the PRIME Study. *Prospective Epidemiological Study of Myocardial Infarction. Arterioscler Thromb Vasc Biol* 2002;22:1155–1161.
- 36 Decossin C, Castro G, Derudas B, Bertrand M, Fruchart JC, Fievet C: Subclasses of LpA-I in coronary artery disease: distribution and cholesterol efflux ability. *Eur J Clin Invest* 1997;27:299–307.
- 37 Asztalos BF, Demissie S, Cupples LA, Collins D, Cox CE, Horvath KV, Bloomfield HE, Robins SJ, Schaefer EJ: LpA-I, LpA-I:A-II HDL and CHD-risk: The Framingham Offspring Study and the Veterans Affairs HDL Intervention Trial. *Atherosclerosis* 2006;188:59–67.
- 38 Arai T, Tsukada T, Murase T, Matsumoto K: Particle size analysis of high density lipoproteins in patients with genetic cholesteryl ester transfer protein deficiency. *Clin Chim Acta* 2000;301:103–117.
- 39 Matsuura F, Wang N, Chen W, Jiang XC, Tall AR: HDL from CETP-deficient subjects shows enhanced ability to promote cholesterol efflux from macrophages in an apoE- and ABCG1-dependent pathway. *J Clin Invest* 2006;116:1435–1442.
- 40 Miwa K, Inazu A, Kawashiri M, Nohara A, Higashikata T, Kobayashi J, Koizumi J, Nakajima K, Nakano T, Niimi M, Mabuchi H, Yamagishi M: Cholesterol efflux from J774 macrophages and Fu5AH hepatoma cells to serum is preserved in CETP-deficient patients. *Clin Chim Acta* 2009;402:19–24.
- 41 Plengpanich W, Le Goff W, Poolsuk S, Julia Z, Guerin M, Khovidhunkit W: CETP deficiency due to a novel mutation in the CETP gene promoter and its effect on cholesterol efflux and selective uptake into hepatocytes. *Atherosclerosis* 2011;216:370–373.
- 42 Calabresi L, Nilsson P, Pinotti E, Gomaschi M, Favari E, Adorni MP, Bernini F, Sirtori CR, Calandra S, Franceschini G, Tarugi P: A novel homozygous mutation in CETP gene as a cause of CETP deficiency in a Caucasian kindred. *Atherosclerosis* 2009;205:506–511.
- 43 Calabresi L, Simonelli S, Gomaschi M, Franceschini G: Genetic lecithin: cholesterol acyltransferase deficiency and cardiovascular disease. *Atherosclerosis* 2012;222:299–306.
- 44 Daniil G, Phedonos AA, Holleboom AG, Motazacker MM, Argyri L, Kuivenhoven JA, Chroni A: Characterization of antioxidant/anti-inflammatory properties and apoA-I-containing subpopulations of HDL from family subjects with monogenic low HDL disorders. *Clin Chim Acta* 2011;412:1213–1220.
- 45 Kontush A, Chapman MJ: Functionally defective high-density lipoprotein: a new therapeutic target at the crossroads of dyslipidemia, inflammation, and atherosclerosis. *Pharmacol Rev* 2006;58:342–374.
- 46 Nobecourt E, Jacqueminet S, Hansel B, Chanterie S, Grimaldi A, Chapman MJ, Kontush A: Defective antioxidative activity of small dense HDL3 particles in type 2 diabetes: relationship to elevated oxidative stress and hyperglycaemia. *Diabetologia* 2005;48:529–538.
- 47 Bellanger N, Orsoni A, Julia Z, Fournier N, Frisdal E, Duchene E, Bruckert E, Carrie A, Bonnefont-Rousselot D, Pirault J, Saint-Charles F, Chapman MJ, Lesnik P, Le Goff W, Guerin M: Atheroprotective reverse cholesterol transport pathway is defective in familial hypercholesterolemia. *Arterioscler Thromb Vasc Biol* 2011;31:1675–1681.
- 48 Ottestad IO, Halvorsen B, Balstad TR, Otterdal K, Borge GI, Brosstad F, Myhre AM, Ose L, Nenseter MS, Holven KB: Triglyceride-rich HDL3 from patients with familial hypercholesterolemia are less able to inhibit cytokine release or to promote cholesterol efflux. *J Nutr* 2006;136:877–881.
- 49 Norata GD, Pirillo A, Catapano AL: Modified HDL: biological and physiopathological consequences. *Nutr Metab Cardiovasc Dis* 2006;16:371–386.
- 50 Pirillo A, Uboldi P, Bolego C, Kuhn H, Catapano AL: The 15-lipoxygenase-modified high density lipoproteins 3 fail to inhibit the TNF-alpha-induced inflammatory response in human endothelial cells. *J Immunol* 2008;181:2821–2830.
- 51 Pirillo A, Uboldi P, Kuhn H, Catapano AL: 15-Lipoxygenase-mediated modification of high-density lipoproteins impairs SR-BI- and ABCA1-dependent cholesterol efflux from macrophages. *Biochim Biophys Acta* 2006;1761:292–300.
- 52 Pirillo A, Uboldi P, Ferri N, Corsini A, Kuhn H, Catapano AL: Upregulation of lectin-like oxidized low density lipoprotein receptor 1 (LOX-1) expression in human endothelial cells by modified high density lipoproteins. *Biochem Biophys Res Commun* 2012;428:230–233.
- 53 Catalano G, Julia Z, Frisdal E, Védie B, Fournier N, Le Goff W, Chapman MJ, Guerin M: Torcetrapib differentially modulates the biological activities of HDL2 and HDL3 particles in the reverse cholesterol transport pathway. *Arterioscler Thromb Vasc Biol* 2009;29:268–275.
- 54 Yvan-Charvet L, Matsuura F, Wang N, Bamberg MJ, Nguyen T, Rinninger F, Jiang XC, Shear CL, Tall AR: Inhibition of cholesteryl ester transfer protein by torcetrapib modestly increases macrophage cholesterol efflux to HDL. *Arterioscler Thromb Vasc Biol* 2007;27:1132–1138.
- 55 Bellanger N, Julia Z, Villard EF, El Khoury P, Duchene E, Chapman MJ, Fournier N, Le Goff W, Guerin M: Functionality of postprandial larger HDL2 particles is enhanced following CETP inhibition therapy. *Atherosclerosis* 2012;221:160–168.
- 56 Duivenvoorden R, Fayad ZA: Safety of CETP inhibition. *Curr Opin Lipidol* 2012;23:518–524.
- 57 Shinkai H: Cholesteryl ester transfer-protein modulator and inhibitors and their potential for the treatment of cardiovascular diseases. *Vasc Health Risk Manag* 2012;8:323–331.
- 58 Michos ED, Sibley CT, Baer JT, Blaha MJ, Blumenthal RS: Niacin and statin combination therapy for atherosclerosis regression and prevention of cardiovascular disease events: reconciling the AIM-HIGH (Atherothrombosis Intervention in Metabolic Syndrome With Low HDL/High Triglycerides: Impact on Global Health Outcomes) trial with previous surrogate endpoint trials. *J Am Coll Cardiol* 2012;59:2058–2064.
- 59 Bays HE, McGovern ME: Once-daily niacin extended release/lovastatin combination tablet has more favorable effects on lipoprotein particle size and subclass distribution than atorvastatin and simvastatin. *Prev Cardiol* 2003;6:179–188.
- 60 Sakai T, Kamanna VS, Kashyap ML: Niacin, but not gemfibrozil, selectively increases LP-AI, a cardioprotective subfraction of HDL, in patients with low HDL cholesterol. *Arterioscler Thromb Vasc Biol* 2001;21:1783–1789.
- 61 Pan J, Shilian P, Ishida B, Wu X, Kane JP, Malloy MJ, Charles MA: Effect of niacin on pre-beta-1 high-density lipoprotein levels in diabetes. *Metabolism* 2011;60:292–297.
- 62 Dall'Agna-Thie GM, van Tol A, Dullaart RP: Plasma pre beta-HDL formation is decreased by atorvastatin treatment in type 2 diabetes mellitus: role of phospholipid transfer protein. *Biochim Biophys Acta* 2009;1791:714–718.
- 63 Brinton EA: Search and rescue for hypotheses surviving AIM-HIGH, the niacin therapy earthquake: still problematic after the primary publication. *J Clin Lipidol* 2012;6:312–317.
- 64 Filippatos TD, Liberopoulos EN, Kostapanos M, Gazi IF, Papavasiliou EC, Kiortsis DN, Tselepis AD, Elisaf MS: The effects of orlistat and fenofibrate, alone or in combination, on high-density lipoprotein subfractions and pre-beta1-HDL levels in obese patients with metabolic syndrome. *Diabetes Obes Metab* 2008;10:476–483.
- 65 Miida T, Sakai K, Ozaki K, Nakamura Y, Yamaguchi T, Tsuda T, Kashiwa T, Murakami T, Inano K, Okada M: Bezafibrate increases prebeta 1-HDL at the expense of HDL2b in hypertriglyceridemia. *Arterioscler Thromb Vasc Biol* 2000;20:2428–2433.
- 66 Lee M, Saver JL, Towfighi A, Chow J, Ovbiagele B: Efficacy of fibrates for cardiovascular risk reduction in persons with atherogenic dyslipidemia: a meta-analysis. *Atherosclerosis* 2011;217:492–498.
- 67 Marik PE, Varon J: Omega-3 dietary supplements and the risk of cardiovascular events: a systematic review. *Clin Cardiol* 2009;32:365–372.

- 68 Burillo E, Martin-Fuentes P, Mateo-Gallego R, Baila-Rueda L, Cenarro A, Ros E, Civeira F: Omega-3 fatty acids and HDL. How do they work in the prevention of cardiovascular disease? *Curr Vasc Pharmacol* 2012;10:432–441.
- 69 Bosch J, Gerstein HC, Dagenais GR, Diaz R, Dyal L, Jung H, Maggiono AP, Probstfield J, Ramachandran A, Riddle MC, Ryden LE, Yusuf S: n-3 fatty acids and cardiovascular outcomes in patients with dysglycemia. *N Engl J Med* 2012;367:309–318.
- 70 Rizos EC, Ntzani EE, Bika E, Kostapanos MS, Elisaf MS: Association between omega-3 fatty acid supplementation and risk of major cardiovascular disease events: a systematic review and meta-analysis. *JAMA* 2012;308:1024–1033.
- 71 Kwak SM, Myung SK, Lee YJ, Seo HG: Efficacy of omega-3 fatty acid supplements (eicosapentaenoic acid and docosahexaenoic acid) in the secondary prevention of cardiovascular disease: a meta-analysis of randomized, double-blind, placebo-controlled trials. *Arch Intern Med* 2012;172:686–694.
- 72 Guo ZG, Li C, Zhong JK, Tu Y, Xie D: Laboratory investigation of dysfunctional HDL. *Chem Phys Lipids* 2012;165:32–37.
- 73 Yang DH, Konigshofer Y, Dike J, Chang E, Mueller O: Performance characteristics of a microfluidic Lab-on-Chip electrophoresis system for high-density lipoprotein (HDL) subfraction separation and measurement. *Clin Chem Lab Med* 2010;48:883–889.
- 74 Davidson MH, Ballantyne CM, Jacobson TA, Bittner VA, Braun LT, Brown AS, Brown WV, Cromwell WC, Goldberg RB, McKenney JM, Remaley AT, Sniderman AD, Toth PP, Tsimikas S, Ziajka PE, Maki KC, Dicklin MR: Clinical utility of inflammatory markers and advanced lipoprotein testing: advice from an expert panel of lipid specialists. *J Clin Lipidol* 2011;5:338–367.
- 75 Sirtori CR, Calabresi L, Franceschini G, Baldassarre D, Amato M, Johansson J, Salvetti M, Monteduro C, Zulli R, Muiesan ML, Agabiti-Rosei E: Cardiovascular status of carriers of the apolipoprotein A-I(Milano) mutant: the Limone sul Garda study. *Circulation* 2001;103:1949–1954.
- 76 Sampson UK, Fazio S, Linton MF: Residual cardiovascular risk despite optimal LDL cholesterol reduction with statins: the evidence, etiology, and therapeutic challenges. *Curr Atheroscler Rep* 2012;14:1–10.
- 77 Arsenault BJ, Lemieux I, Despres JP, Gagnon P, Wareham NJ, Stroes ES, Kastelein JJ, Khaw KT, Boekholdt SM: HDL particle size and the risk of coronary heart disease in apparently healthy men and women: the EPIC-Norfolk prospective population study. *Atherosclerosis* 2009;206:276–281.
- 78 Tian L, Long S, Fu M, Liu Y, Xu Y, Jia L: Characteristics of high-density lipoprotein subclasses distribution for subjects with desirable total cholesterol levels. *Lipids Health Dis* 2011;10:64.