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Lipid measures and cardiovascular disease prediction

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Abstract. Traditional lipid measures are the cornerstone of risk assessment and treatment goals in cardiovascular prevention. Whereas the association between total, LDL-, HDL-cholesterol and cardiovascular disease risk has been generally acknowledged, the rather poor capacity to distinguish between patients who will and those who will not develop cardiovascular disease has prompted the search for further refinement of these traditional measures. A thorough understanding of lipid metabolism is mandatory to understand recent developments in this area. After a brief overview of lipid metabolism we will discuss the epidemiological data of total, LDL- and HDL-cholesterol and focus on recent advances in measurements of these lipoproteins. In addition we will discuss the role of triglycerides and the apolipoprotein B–A-I ratio on the incidence of cardiovascular disease.

Keywords: LDL-cholesterol, HDL-cholesterol, triglycerides, Apolipoprotein B–A-I Ratio, cardiovascular disease, atherosclerosis

1. Introduction

Cardiovascular (CV) risk prediction is an area of intense research in view of the persistently high morbidity and mortality due to cardiovascular causes worldwide. Early and accurate risk assessment strategies allow for targeted therapeutic strategies in cardiovascular prevention achieving highest absolute risk reduction in subjects at high risk for developing CV disease. Although attention has recently shifted towards predominantly inflammatory markers [1], lipid biomarkers are still the cornerstone of risk assessment and treatment goals in daily clinical practice.

The two major sources of plasma lipids are cholesterol and triglycerides. In cholesterol homeostasis, approximately 80% of circulating cholesterol is derived from endogenous synthesis, for which 3-hydroxy-3-methylglutaryl coenzyme A reductase is the rate-limiting step. Only a small fraction of circulating cholesterol is absorbed from the diet. Due to its lipophilic properties, cholesterol has to be transported in the blood in special particles containing both

lipids and proteins (lipoproteins). The major classes of lipoproteins are low density lipoproteins (LDL), high density lipoproteins (HDL), very low density lipoproteins (VLDL), intermediate density lipoprotein (IDL). IDL resides between VLDL and LDL and is included in the LDL-cholesterol (LDLc) measurement in clinical practice. Total cholesterol includes all cholesterol present in lipoprotein particles including LDLc, HDL-cholesterol (HDLc), lipoprotein(a), IDL-cholesterol (IDLc) and VLDL-cholesterol. Triglycerides (TG) are made up of free fatty acids linked to a glycerol backbone. TG are synthesized in intestinal and liver cells and are transported in the plasma where they deliver free fatty acids to peripheral tissues for use as energy source.

In the present review we will discuss the lipid metabolism, focusing on the relation of total cholesterol, LDLc, HDLc and the incidence of cardiovascular disease. In addition, we will discuss current insights of TG and apolipoprotein measurements and their role in predicting cardiovascular events.

2. Lipid metabolism

2.1. Apolipoprotein B pathway

Plasma TG are derived from dietary fat as well as from de novo synthesis [2]. After uptake of ‘dietary’

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fatty acids in the small intestine by the enterocyte, fatty acids are converted into TG by the enzyme acyl-CoA:diacylglycerol acyltransferase 2 (DGAT2) [3]. Whereas TG can be stored within the enterocyte in lipid droplets, the vast majority of TG are packed in apolipoprotein(apo)-B48-containing lipoprotein particles (chylomicrons) and subsequently secreted in the lymphatic system which directly drains into the systemic circulation bypassing the liver [4]. De novo lipogenesis occurs in the liver. Released fatty acids from adipose tissue, are taken up by the liver. The liver synthesizes a triglyceride-poor VLDL particle (VLDL2) [5] which can either be secreted by the hepatocyte directly or be further lipidated to form a mature, triglyceride-rich VLDL (VLDL1) [6]. Both liver secreted particles exclusively contain apoB-100-lipoproteins.

Upon secretion, all TG rich particles are lipolysed by lipoprotein lipase (LPL) present in the capillary beds of adipose tissue and skeletal muscle. A number of different proteins such as apoC-III, apoA-V, angiopoietin-like proteins ANGPTL 3 and 4 are also present in the circulation and may act as potential inhibitors of LpL. The lipolysed TG release fatty acids that can either be stored in the adipose tissue or can be used directly as energy source by peripheral tissues. The ensuing remnants of both VLDL and chylomicrons are taken up by the liver through a concerted action of LDL-related proteins (LRP0, heparin sulphate proteoglycans, both can rapidly binding apoB-48), as well as the LDL-receptor, binding predominantly apoE present on the remnant particles [3,7,8]. A minor fraction of the apoB-100-containing VLDL remnant particles is further metabolized towards an LDL particle, mediated by the concerted actions of LpL, hepatic lipase and cholesteryl ester transfer protein (CETP). It contains a core of cholesterol esters, lesser amounts of TG and is enriched in apoB-100. LDLc can be internalized by the LDL-receptor in hepatic and non-hepatic tissues. Hepatic LDLc can be converted to bile acids and secreted into the intestinal lumen. Non-hepatic tissue can use LDLc for hormone production, cell membrane synthesis or can be stored in an esterified form and used as energy source when needed.

2.2. Apolipoprotein AI pathway

Apolipoprotein A-I (apoA-I), a 243-residues containing protein, is the major constituent of HDL and is synthesized both in the liver and the intestine [9, 10]. Once secreted, it is rapidly lipidated at the cell

surface by the action of the ABCA1 transporter [11]. ABCA1 transfers phospholipids and free cholesterol to apoA-I. After this rate-limiting lipidation step of apoA-I further remodeling of the discoidal HDL particles by predominantly lecithin cholesterol acyltransferase (LCAT) results in a fully matured spherical HDL particle. SR-BI which is predominantly expressed on the liver and steroidogenic glands, then mediates the selective uptake of cholesteryl ester and other lipids from HDLc [12] (Fig. 1).

3. LDL-cholesterol

Since cholesterol cannot be metabolized, LDL particles need to be excreted from the body via LDL-receptor mediated endocytosis into the hepatocytes thereby maintaining cholesterol homeostasis. In case of LDL-c excess, these particles will also bind to scavenger receptors on arterial-wall macrophages. LDLc that is ingested by macrophages is readily oxidized, thereby triggering cytokine production and chemotaxis of inflammatory cells. LDL-loaded arterial wall macrophages transform into foam-cells, a first step in the development of atherosclerosis [13,14].

Large epidemiological surveys consistently show robust associations between high levels of LDLc and an increase in incidence of cardiovascular events. The Framingham Heart Study [15], the MultipleRisk Factor Intervention Trial (MRFIT) [16], and the Lipid Research Clinics (LRC) trial [17] were the first large cohort studies to report a direct relation between levels of LDLc (or total cholesterol) and the rate of new-onset coronary heart disease (CHD) in men and women who were initially free of CHD. Stamler et al. reported a combined analysis of cholesterol levels in three large cohort studies including younger men and the onset of new CHD. Data of three combined cohort studies, in total including 11017 men, between 18 and 39 years, was analyzed to determine the correlation of LDLc levels and the risk of CHD. CHD mortality risk was 2.15 to 3.63 times greater for individuals with unfavorable lipid profiles (serum cholesterol > 240 mg/dl) as compared to favorable lipid values (serum cholesterol < 200 mg/dl). Hypercholesterolemic men had an age adjusted absolute risk excess of CHD death of 43.6 per 1000 men in 25 years, 81.4 per 1000 in 34 years and 12.1 per 1000 men in 16 years in the different cohorts [18]. In addition to these epidemiological data more evidence for the association between LDLc and CHD came from families with familial hyper

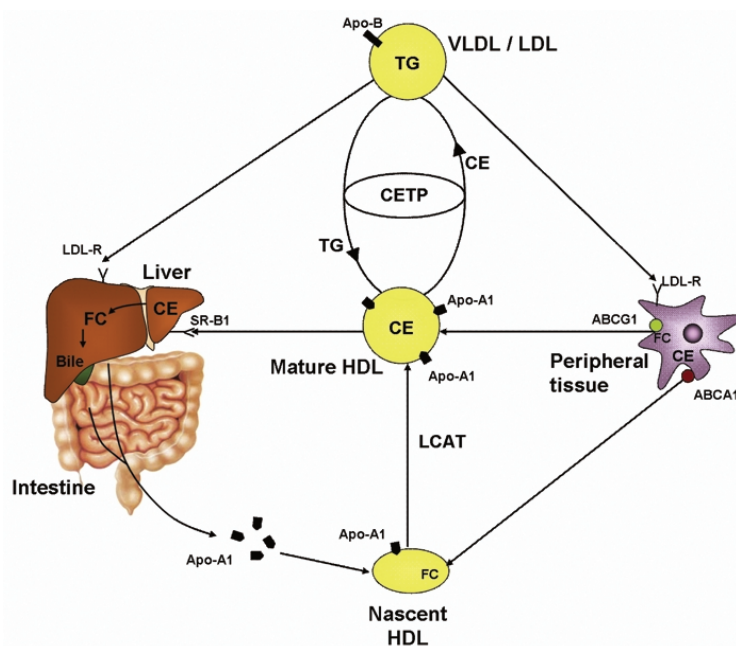


Fig. 1. Overview of lipid metabolism.

cholesterolemia. These individuals have high levels of LDLc most often due to mutations in the LDLR gene that encodes for the LDL receptor protein, which normally removes LDLc from the circulation. The incidence of CHD in these families is profoundly increased compared to controls [19]. The observation that increased levels of LDLc in cohort studies are associated with a higher incidence of CVD was further corroborated by numerous large intervention trials evaluating the impact of various LDL-lowering strategies amongst others, the HMG-CoA reductase inhibitors (statins). Statins were consistently shown to reduce CVD-risk. Although the effect of statin therapy lies beyond the scope of this review, these trials contributed enormously to our current understanding of LDLc and the risk of CVD. The Cholesterol Treatment Trialists Collaborators published a meta-analysis in the Lancet of 90056 individuals in 14 randomized statin trials. This large meta-analysis provided weighted estimates of effects on different clinical outcomes per 1.0 mmol/L reduction in LDLc. During a mean of 5 years, there were 8186 deaths, 14 348 individuals had a major vascular event. There was a 19 percent major proportional reduction in CHD deaths per mmol/L reduction in LDL cholesterol corresponding with a 12 percent proportional reduction in all cause mortality per mmol/L. This indicates that a reduction of 1.5 mmol/L in LDLc with sustained statin therapy might well be expected to

reduce the incidence of major vascular events by about one third [20].

Although LDLc is accepted to be the major risk factor in the progression of atherosclerosis, the measurement of LDLc also includes IDLc. Several studies have shown that serum IDLc concentrations are predictive of an increased incidence of CHD [21] and an increased incidence of coronary events in those with CHD, independently of other factors [22,23]. This might be of considerable importance in patients with normal total cholesterol levels and those with an elevated IDL/HDL ratio [24]. The MARS study performed analytic ultracentrifugation to determine the lipoprotein subclasses: IDLc, but not VLDLc or LDLc, was associated with the progression of carotid artery intima-media thickness [25]. Thus, IDLc may be a determinant of the atherogenic potential of LDLc. Although we know that IDLc is included in the LDLc measurement in clinical practice, we have to realize that we have no information on the LDL particle number and size. In this context, LDLc measurements alone may not suffice to determine the risk of developing CVD in all subjects. Recent research has shown that LDL particle number is related to CAD on top of Framingham risk assessment as well as after adjusting for LDLc. However this effect was abolished following adjustment for triglycerides and HDLc [26]. The benefit in daily practice seem to add little extra value at this time and currently, LDLc

is still the primary treatment target in CHD prevention guidelines [27]. However the predictive value of future cardiovascular events of LDLc is limited [28] and the search for better risk assessment lipid biomarkers and improved treatment goals is ongoing.

4. HDL-cholesterol

The protective capacity of HDLc has largely been attributed to its role in the reverse cholesterol transport (RCT), referring to the ability of HDLc to transport cholesterol from peripheral tissues back to the liver where it can be excreted into the bile. Whereas the RCT reflects a flux of cholesterol from the peripheral tissues to the liver, it should be taken into account that HDLc is a static measurement which not necessarily reflects the efficacy of the 'reverse cholesterol flux' [29]. More recently, attention has focused on a wide array of protective effects exerted by HDLc beyond its role in RCT, comprising an inhibition of inflammatory monocyte responses, platelet activation and an anti-atherogenic endothelial cell profile that is resistant to inflammation and monocyte recruitment [30]. More importantly, these other protective effects were shown to be subjected to change following exposure of the HDL to 'adverse' conditions such as hyperglycaemia and inflammatory insults [31]. The latter has led to the introduction of terms such as 'functional' versus 'non-functional' HDL particles [32–34]. Since standardized, reproducible assays to test either 'cholesterol flux' or HDL-function are not readily available, further studies are required to address the predictive value of these parameters.

With the first observational cohort studies describing the association between LDLc and CVD, the inverse relation between HDLc and the incidence of CVD was also described. In the Framingham cohort, a potent inverse relation was already observed between HDLc and the incidence of CVD in 1977. Based on these data the risk for myocardial infarction increases by about 25 percent for every 0.13 mmol/L decrease in serum HDLc in both men and women [35]. The Prospective Cardiovascular Münster (PROCAM) study determined the incidence of CAD in 4559 male participants over a 6 year follow-up period. During that period 186 study participants developed atherosclerotic CAD with 7.7 percent in the lowest tertile (< 1.01 mmol/L), 2.7 percent in the intermediate tertile and 2.6 percent in the highest tertile (> 1.24 mmol/L) [36]. Another analysis of 13,173 patients in the LIPID and CARE trials found that low

serum HDLc was a significantly stronger predictor of CHD events in patients with an LDLc < 3.2 mmol/L than \geq 3.2 mmol/L. For every 0.26 mmol/L increase in HDL, the event rate decreased by 29 percent in those with LDL < 3.2 mmol/L compared to 10 percent in those with an LDL \geq 3.2 mmol/L [37]. Thus, although high levels of HDL are more protective among lower levels of LDLc this association holds true for the entire range of LDLc levels. This was further corroborated in a recent post-hoc analysis of the Treating to New Targets (TNT) study showing that HDLc levels were predictive of major cardiovascular events in patients treated with statins, even among those with very low levels of LDLc < 1.81 mmol/L [38]. Overall, the correlation between HDLc and the incidence of CHD exceeds that of LDLc. This holds true for all LDL-levels and is independent of statin treatment.

5. Triglycerides

Various epidemiological surveys have addressed whether plasma TG are an independent risk factor for CVD. The PROCAM study involved 4849 middle-aged men who were followed up for 8 years to record the incidence of CHD events according to the risk factors present at study entry. The study showed that fasting levels of triglycerides were an independent risk factor for CHD events, independent of serum levels of HDLc or LDLc [39]. In the 8-year Copenhagen Male Study [40], a prospective study including 2906 men who were free of cardiovascular disease at baseline, the risk for ischemic heart disease was 50 percent higher in those with TG levels in the middle tertile and 120 percent higher in the upper tertile as compared to those in the lowest TG tertile after adjustment for conventional risk factors such as age, BMI, alcohol intake, smoking, physical activity, hypertension, diabetes, and LDLc and HDLc levels. In addition a number of meta-analyses have been published showing independent associations between TG levels and CHD risk [41–43]. A relative risk of 1.32 (95% CI 1.26–1.39) in men and 1.76 (95% CI 1.50–2.07) in women was originally found [43]. More recent analyses in individuals selected from the Reykjavik cohort and the Epic-Norfolk cohort revealed an odds ratio for CHD of 1.76 in the former and 1.57 in the latter when comparing individuals in the lowest tertile for TG as compared to those in the highest tertile, which was similar to data derived from another meta-analysis [42]. In line, data derived from a study of 13,953 young men for which Tirosh et al. revealed

an association between the first of 2 triglyceride measurements obtained 5 years apart with incident CHD. After an average follow-up of 10.5 years, men in the fifth quintile of triglycerides, compared with those in the lowest quintile, had a multivariable hazard ratio for CHD of 4.1 [44]. In patients with CHD triglyceride levels also related to CVD events independent of HDLc and LDLc with a hazard ratio of 1.50 [45].

Recently, the debate on fasting versus non-fasting plasma TG has emerged. Two recent publications reported a strong association between non-fasting plasma TG and CVD risk [46–49]. In the Copenhagen City Heart Study which included 19,329 men and women with an average follow up of 26 years, nonfasting TG levels were associated with increased risk of myocardial infarction, ischemic heart disease and death. An expansion of this study was recently published where they studied 33,391 inhabitants of Copenhagen (Denmark) [48]. In the Women's Health Study, which comprised 26,509 women, nonfasting TG levels were associated with the incident of cardiovascular events, independent of traditional cardiac risk factors, levels of other lipids, and markers of insulin resistance. Recently, data obtained from a prospective study in 26 330 healthy women, revealed that the association with CVD was stronger for nonfasting TG compared with fasting measurements of TG [49]. Combined, these studies suggest that nonfasting TG measurements more accurately reflects the presence of atherogenic remnant lipoproteins compared to fasting TG measurements.

6. Apolipoprotein B–A-I Ratio

To further improve CVD prediction algorithms research has focused on ApoB and apoA-I, the main structural proteins of atherogenic lipoproteins and HDL particles, respectively. ApoB levels reflect the entire spectrum of pro-atherogenic particles, including VLDLc, IDLc, and LDLc [50]. ApoB levels also provide a good measure of the number of LDL particles, which reflect the atherogenicity of LDLc [51,52]. Concomitantly, apolipoprotein A-I is more important than the HDLc content for biochemical pathways that make HDL antiatherogenic, including adenosine triphosphate binding cassette A-1-mediated cellular cholesterol efflux [53], lecithin-cholesterol acyltransferase-mediated maturation of HDL particles [54], and several antioxidative processes [55]. Besides these physiologic considerations, apolipoprotein assessment does

not require fasting blood samples [50], which greatly facilitates logistics at outpatient clinics.

Since the introduction of standardized reference materials by the International Federation of Clinical Chemistry [56,57], large studies, such as the AMORIS (Apolipoprotein-related Mortality RISK) [58] and INTERHEART [59], have unambiguously demonstrated that the apo B–apo A-I ratio is a robust risk factor for future CAD events. However, in the AMORIS study, LDLc and HDLc were indirectly estimated from total cholesterol, TG, and apoA-I values, which precluded simultaneous use of these variables in the same statistical model. In INTERHEART, LDLc and HDLc were measured directly, but these values were not incorporated in the statistical analyses. Earlier data from the Québec Cardiovascular Study showed that apoB level was associated with CAD independent of LDLc level, but apoA-I level was not associated with CAD independent of HDLc level [51]. The Prospective Epidemiological Study of Myocardial Infarction (PRIME) reported that apoA-I level was associated with CAD independent of HDLc level [60]. Data from the Atherosclerosis Risk in Communities study suggested that apoA-I and B levels no longer contributed to CAD risk prediction when considered together with traditional lipid values [61] however, the apolipoproteins were quantified by using radial immunodiffusion, a technique that has known problems with linearity and reproducibility. Overall, these studies did not address the crucial question of whether the apo B–apo A-I ratio predicts those events better than traditional lipid values do. This issue was addressed in the Women's Health Study showing no incremental value for apolipoproteins above the ratio of total cholesterol to HDLc [62]. In addition, the Framingham Heart Study showed that the apo B–apo A-I ratio for prediction of CHD is comparable with that of total cholesterol to HDLc [63]. These findings were in line with a recent study in the EPIC-NORFOLK cohort, a prospective population study among 2380 apparently healthy individuals, with a follow-up of six years showing that the apo B–apo A-I ratio is independently associated with CAD (adjusted odds ratio 1.85 (95% CI, 1.15 to 2.98) but adds little to existing measures for CAD risk assessment and prediction in the general population [64].

7. Conclusion

Traditional lipid biomarkers are still the cornerstone of risk assessment as well as treatment goals in dai-

ly clinical practice. Whereas the search for improved lipid biomarkers continues in order to improve the ability to identify subjects at increased CV-risk, the added value of 'novel' lipid biomarkers compared to traditional lipid measurements is relatively modest. In this light the addition of relative new biomarkers to the existing risk assessment algorithms have already shown to increase the predictive power to identify individuals at risk for CVD in several studies [65–67]. Currently, this field is rapidly evolving and holds great promise for future cardiovascular risk assessment strategies in clinical practice.

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