The Fats of Life

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Dawn I. Thiselton, PhD produced this issue of The Fats of Life.
The National Lipid Association (NLA) held its annual summer scientific sessions May 31-June 2 in Scottsdale AZ, celebrating the 10th year of this organization which has emerged as a key interface between practicing physicians and experts in managing cardiovascular disease. The NLA offers board certification in clinical lipidology which has substantially improved the level of knowledge and standards of practice in treating patients with lipid disorders. The NLA program was outstanding with focus on the underlying pathology of atherosclerosis, familial hypercholesterolemia (FH) and insulin resistance among other interesting topics.

Familial hypercholesterolemia (FH) is appropriately gaining increasing attention. There are now four mutations recognized as contributing; LDL receptor defects with about 1200 recognized single nucleotide polymorphisms (SNPs), apoB and PCSK9 gain of function defects, all of which are dominant, and a recessive defect of internalization of receptor bound LDL. The consequence is greater heterogeneity in expression of the disorder, including silent defects that can skip generations and compound heterozygotes with widely varying severity. With this developing knowledge, it is increasingly recognized that FH is more common than previously thought. When I came into the field 40 years ago FH was recognized as the most common severe genetic disease but was estimated to affect about 1 in 500 in the general population. Over the years estimates have moved higher. Last year at an NLA-sponsored consensus conference on FH, I heard 1 in 300. At this year’s meeting I heard estimates of even higher prevalence. At the same time estimates of the unidentified FH carriers have increased; from earlier estimates of 80% to this year estimates as high as 99% of those affected and at severe risk of undetected heart disease. This means that in the US there could be as many as 1 to 3 million affected individuals, many of whom, if left untreated, will likely experience heart attacks in their 20s, 30s and 40s.

Two years ago the NLA made FH a priority and besides convening a consensus conference which issued a statement published recently in Journal of Clinical Lipidology, also sponsors an educational website for patients (www.LearnYourLipids.com) and offers patient information including tear sheets and brochures. More recently a small group of motivated FH patients came together and organized the FH Foundation, which is in process of developing a patient registry to facilitate identification and follow-up of affected family members through NLA board certified physicians. The Lipoproteins and Vascular Disease Divisions (LVDD) should recognize and collaborate with these two worthy organizations in supporting efforts to identify and appropriately treat these unfortunate folks affected by FH.

Another interesting series was on insulin resistance, obesity and type 2 diabetes, which are increasingly epidemic. The statistics are frightening with 2/3 of all adults in the US overweight or obese, many of whom progress to diabetes with the consequent micro- and macrovascular consequences. Gary Taubes, a noted author of the books, “Good Calories, Bad Calories” and “Why We Get Fat,” whom I have mentioned in a previous editorial, presented compelling evidence that increasing consumption of carbohydrates is the primary culprit. A second presenter, Dr. Lustig from the University of California at San Francisco (UCSF), even more convincingly demonstrated a direct relationship between consumption of sugar, either sucrose or high fructose corn syrup, and obesity and type 2 diabetes, both within the US and throughout the world. He implicated the lipid/CVD experts who, two or three decades ago recommended cutting fat, among the key contributors to the increase in sugar consumption. To simplify a very complex story the food industry, in removing tasty and satisfying fat from prepared foods, had to add sugar to maintain palatability and consumer acceptance. Finally, Peter Havel of the University of California at Davis (UC Davis), the son of the famous lipid expert Richard Havel, who is still active but semi-retired at UCSF, explained that...
fructose is slightly worse than glucose in driving hyperlipidemia and other consequences of simple sugar consumption.

Finally, the NLA meeting saw the debut of the Foundation for Health Information and Technology (FHIT.org), my current interest. FHIT sponsors two websites: LecturePad, accessible through fhit.org provides convenient and high level education focused on physicians with online lectures, case studies, a quiz, and other content. And the Biomarker Bliki also at fhit.org, combines features of a wiki and a blog to provide dynamic and up to date information on biomarkers associated with cardiovascular and related diseases. Some chapters are already posted, others are in the pipeline and we are actively seeking topic experts now to take responsibility for additional chapters. If readers are interested please feel free to contact me.

With best regards,
Russ Warnick
Exciting Events at the Upcoming AACC 2012 Annual Meeting in Los Angeles:

I welcome you all to register for the upcoming AACC Annual Meeting in Los Angeles in July this year. Early registration ends soon so please take advantage of the discounted registration. The LVDD will hold its customary events at this meeting. We will have The Executive Committee /Membership meeting on Sunday morning which is open to all members and I invite you all to be there in person, introduce yourself and participate in the Committee proceedings, especially since we will present proposed changes to the existing ByLaws. In fact, we will be joined by Jim Faix, MD, Chair of the Division Management Group; On Monday night is the LVDD Annual Mixer/Dinner meeting featuring “Novel Advances in HDL and Cardiovascular Disease and Proteomics” featuring the 2 Award winners: Cooper Award: Dr. Alan Remaley, NIH and PBRF Award: Dr. Jay Heinecke, University of Washington. Also, the Past Chair Award will be given to Dr. Daniel Hoefner and Poster Awards will be announced at this meeting. On Tuesday night, we will have the International Lipoprotein Standardization Meeting. This is an exciting and interactive forum and will include discussions on “Discussion of methods/standardization for apoB/apoA1” as well as a discussion of “Assessment of the lipid and lipoprotein test accuracy from fresh frozen serum proficiency tests” and these will be led by Drs. Kotani, Bowen and Remaley.

Please remember that you will need to register for the Monday and Tuesday night dinner events, so please purchase your tickets to these early, since they have been sold out in the last few years. As in the past, there will be several workshops that have been developed by LVDD members or in association with LVDD.

I am also glad to report that we had our Spring LVDD meeting in conjunction with the meeting of the National Lipid Association in Scottsdale, AZ recently. This is one of our first efforts in initiating a strong and mutual partnership with them. We discussed the proposed changes to the existing bylaws and a paper that the group is writing on the association of apoB and NMR LDL-P with outcomes.

I would also like to take this opportunity to thank all of those volunteers for their generous donations and from the different companies that have supported us and made all these events possible.

Look forward to seeing you all in the Annual Meeting in July in Los Angeles.

With best wishes,
Sridevi Devaraj
Upcoming Events

AACC 2012 Annual Meeting Events of the LVDD

Dear members,

Please look out and register for the following LVDD sponsored events at the AACC 2012:

LVDD Executive Committee and Membership Meeting
Sunday, July 15, 8 am – 10 am
Venue TBD

Annual LVDD Dinner Meeting, “Novel Advances in HDL and Cardiovascular Disease and Proteomics”
Monday, July 16, 5:30 pm – 9:30 pm
Pending approval for 1.5 ACCENT® Credits
JW Marriott LA LIVE (900 W. Olympic Blvd., Los Angeles)

Featuring the two award winners:

Dr. Alan Remaley, National Institutes of Health. Winner of the 2012 Cooper Award given for outstanding contributions to service in the area of lipoproteins and vascular diseases.

Dr. Jay Heineke, University of Washington. Winner of the 2012 PBRF award.

Past Chair Award
will be given to Dr. Daniel Hoefner and Poster Awards will be announced.

International Lipoprotein Standardization Forum
Tuesday, July 17, 6 pm – 9:30 pm
JW Marriott LA LIVE (900 W. Olympic Blvd., Los Angeles)
Various homogeneous assays for LDL-cholesterol (LDL-C) using reagents based on different principles of analysis are available (Table 1). Every year, there are new developments and improvements in the measuring reagents, and measurement precision is therefore increasing. However, the accuracy of these methods has yet to be confirmed in patient samples containing increased levels of triglycerides (TG) and atypical lipoproteins, such as intermediate-density lipoprotein (IDL), lipoprotein (a), small dense LDL, lipoprotein-X (Lp-X) and lipoprotein-Y (Lp-Y) (1).

Lp-X is seen in the sera from patients with cholestasis, and is also known to accumulate in patients with lecithin-cholesterol acyltransferase (LCAT) deficiency (2). Otvos indicated that Lp-X occurs in patients more often than recognized (3). If the presence of Lp-X was not suspected, inaccurate LDL-C results may lead to incorrect cardiovascular risk assessment, and to improper choices in drug therapy and in the management of patient’s treatment.

Moreover, because the density of Lp-X is equivalent to that of LDL, in lipoprotein fractions produced by ultracentrifugation, Lp-X is included in the LDL fraction and are thus interpreted as LDL. This causes frequent discrepancies with the results produced by homogeneous assays for LDL-C, and difficulties in identifying the type of hyperlipidemia (4). Although the β-quantification (BQ) method is considered as a reference method for LDL-C, depending on its density, it can also detect cholesterol in Lp-X, lipoprotein (a), and IDL, and thus it may be an imperfect way for testing for the specificity of the homogeneous LDL-C assays. In this study, mainly by using gel filtration, we analyzed the reaction specificity of 6 homogeneous assays for LDL-C toward abnormal lipoproteins in cholestatic sera showing hyperlipidemia.

A good correlation exists between the modified BQ method and each of the homogeneous assays in sera of the hypercholesterolemic patients (average difference: -0.429-0.590 mmol/L, 2SD: 0.370-0.888 mmol/L, n=32). In sera of 24 cholestatic patients, each of the homogeneous assays, except for the liquid selective detergent method, was negatively biased compared to the modified BQ method, the magnitude of the bias increasing along with increasing concentrations of Lp-X.

Figure 1 shows the profiles for lipids and apoproteins obtained by gel filtration of the serum fraction (d=1.006-1.063 kg/L) of a patient with hepatic cirrhosis (male, 44 years old). The serum fractions isolated by ultracentrifugation showed biphasic peaks, one of which contained apo B and occupied the same location as LDL in the sera of healthy volunteers (specific gravity ranging from 1.019 to 1.063) in terms of particle size. However, this peak was identified as abnormal, as phospholipids (PL) and TG were markedly elevated when compared with the LDL of healthy volunteers. The lipid composition and content in the abnormal LDL varied with the background of the patients. Each of the homogenous assays showed decreased reactivity of cholesterol toward the abnormal LDL fraction along with increasing the ratio of PL and TG to total cholesterol (TC).

The other peak was identified as Lp-X because it appeared in a location corresponding to larger particle size when compared with LDL, and included apo C-II and apo C-III, with TC largely consisting of free cholesterol, only a small amount of TG and a large amount of PL (5). In the liquid selective detergent method and the elimination method, with increasing concentrations of Lp-X in
each of the Lp-X fraction, relative reactivity of cholesterol in the corresponding fraction increased, probably due to insufficient deletion of Lp-X by reaction reagents during the first process of the analytical procedure employed. In contrast, the selective solubilization method and the phosphate complex inhibition method were less reactive toward the Lp-X fraction. We are now planning to publish detailed experimental data and information on these findings elsewhere.

We demonstrated that the 6 homogeneous LDL-C assays showed different reaction specificity toward Lp-X and abnormal LDL in sera from cholestatic patients, depending upon their measuring principles and reaction characteristics. Little is known about how the change in lipid composition of abnormal LDL observed in cholestatic patients correlates to atherogenicity. Thus, the clinical value of measuring cholesterol in the abnormal LDL in the LDL-C assays for the cardiovascular risk prediction should be clarified in future studies.

In contrast with abnormal LDL, Lp-X may not be pro-atherogenic and be associated with renal disease in the LCAT deficiency (2). Thus, there is no clinical validity measuring cholesterol on Lp-X for cardiovascular risk prediction. The reaction specificity of each homogeneous LDL-C assay should be improved to exclude non-atherogenic Lp-X cholesterol. Our findings would facilitate accurate interpretation of the LDL-C values determined by homogeneous LDL-C assays, especially in samples including Lp-X, eventually.

References


Table 1. Schematic reaction mechanism for LDL-C assays. CM; chylomicron, VLDL; very low-density lipoprotein, LDL; low-density lipoprotein, IDL; intermediate low-density lipoprotein, HDL; high-density lipoprotein, CE; cholesterol esterase, CO; cholesterol oxidase, CD; cholesterol dehydrogenase, POD; peroxidase, 4AAP; 4-aminoantyriine, DS8mT; N, N-bis-(4-sulfobutyl)-m-toluidine, HDAOS; N-(2-hydroxy-3sulfopropyl)-3,5-dimethoxyaniline.

<table>
<thead>
<tr>
<th>1st step</th>
<th>2nd step</th>
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<tr>
<td><strong>Liquid selective detergent method</strong></td>
<td>1. Surfactant 1 + Enzymes (CE, CO) react only with CM-, VLDL- and HDL-C → Cholesterolone + Fatty acid + H₂O₂ 2. H₂O₂ + Catalase → 2H₂O + O₂ 3. LDL-C + Surfactant 2 + Enzymes (CE, CO, POD) + DS8mT → Color Development</td>
</tr>
<tr>
<td><strong>Selective solubilization method</strong></td>
<td>2. LDL are solubilized by Enzymes (CE, CO) and surfactants → Cholesterolone + Fatty acid + H₂O₂ 3. H₂O₂ + 4AAP/Peroxidase + HDAOS → Color Development</td>
</tr>
<tr>
<td><strong>Elimination method</strong></td>
<td>4. H₂O₂ + 4AAP/Peroxidase + HDAOS → Color Development (catalase is inhibited by sodium azide present in Reagent-2 at the 2nd step reaction)</td>
</tr>
<tr>
<td><strong>Enzyme selective protecting method</strong></td>
<td>5. LDL-Protecting Reagent + Deprotecting reagent → LDL</td>
</tr>
<tr>
<td><strong>Calixarene complex method</strong></td>
<td>6. LDL + Calixarene → LDL-Calixarene Soluble Complex 2. CM-, VLDL- and HDL-C + Enzymes (CE1 can not react to the LDL-Calixarene Soluble Complex CE1 from Chromobacterium viscosum) → H₂O + Catalase → H₂O</td>
</tr>
<tr>
<td><strong>Phosphate complex inhibition method</strong></td>
<td>3. LDL-Calixarene Soluble Complex + Enzymes (CE2, CD) + Hydrazine + β-NAD + deoxycholate + Cholesterolone hydrazone + β-NADH CE2 (from Pseudomonas species)</td>
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![Figure 1](image.png)

Fig. 1. Gel filtration profiles of the d = 1.006-1.03 kg/L fraction isolated from the serum of a patient with hepatic cirrhosis (male, 44 years old) using Superose 6 HR 10/300 columns. Concentrations of lipids and apoproteins were determined in each fraction obtained by gel filtration chromatography.
Assessment of cholesterol synthesis and absorption by the measurement of plasma noncholesterol sterols/stanols.

Thomas Dayspring MD, FACP, FNLA, NCMP. Director, North Jersey Institute of Menopausal Lipidology.

OVERVIEW

Sterols are hormone precursor molecules and essential components of cell membranes in animals (zoosterols) and plants (phytosterols). The predominant zoosterol is cholesterol. Sterols that have structural similarity to cholesterol are also referred to as noncholesterol sterols. The human diet includes many exogenous sterols from plants, (sitosterol, campesterol, stigmasterol, etc.) and animals (cholesterol), shellfish sources (desmosterol, fucosterol) and yeast sources. Phytosterols are similar in structure to cholesterol but their subtle structural differences minimize their absorption compared to cholesterol. Stanols are simply saturated sterols (Δ5 saturation) and the stanol version of cholesterol is called cholestanol which is a cholesterol produced metabolite produced in the liver and intestine.

People vary in their cholesterol balance – the amount of cholesterol they synthesize and absorb. Cholesterol is essential for life, as it is a crucial membrane molecule and the precursor of steroid hormones, vitamin D, and bile acids. In contrast, phytosterols serve no physiologic function in humans, are not readily absorbed and cannot be synthesized. However, phytosterol products are widely used therapeutically as part of lifestyle changes to lower cholesterol levels and manage dyslipidemia, a major risk factor for atherosclerosis and coronary heart disease (CHD). Cholesterol precursor sterols serve as biomarkers of cholesterol synthesis and phytosterols serve as biomarkers of cholesterol absorption because humans with perfect physiology absorb very few phytosterols/stanols. Testing for plasma sterol levels will provide information on whether a patient is more of an absorber, a synthesizer, both or neither. This information helps the physician personalize and better adjudicate cardiovascular risk, hence plan a more effective lipoprotein treatment regimen. Sterol analysis can also show the effects of drugs on cholesterol balance i.e., whether statin monotherapy is suppressing cholesterol synthesis at the cost of undesirably increasing sterol absorption.

INTRODUCTION

Biochemistry

A phenol is a compound containing a hydroxy group (-OH) and a 6 membered aromatic ring. A polyphenol is a multiphenol compound typically found in plants. A sterane is a class of 4 cyclic or aromatic triterpenes (6 isoprene units arranged as tetracyclic rings) which make up the core of sterols and steroids. A sterol (Fig.1) is simply a sterane with a hydroxy group at the third position of the A ring; the systematic names contain either the prefix hydroxy- or the suffix -ol, e.g., 3-hydroxycholesterol.

![Figure 1. Molecular structure of a sterane; carbon rings are numbered A, B, C, D from left to right.](image)

or cholesterol, sitosterol, etc. Because of the polar hydroxy group at one side of the core molecule and a nonpolar aliphatic side chain on the other (C17), sterols are amphipathic molecules. Depending on the structural position of the C17 aliphatic chain, different sterol or stanol isomers, termed α and β exist (i.e. 17α or β cholestanol). Sterols are essential components of cell membranes in animals (zoosterols) and plants (phytosterols). Zoosterols or
phytosterols that have structural similarity to cholesterol are also referred to as noncholesterol sterols.\textsuperscript{1,2}

Cholesterol can be absorbed from the gut lumen into enterocytes via membrane sterol influx transporters and then packaged into lipoproteins that enter plasma, or synthesized \textit{de novo} by virtually every cell in the body (Fig. 2). The nonpolar hydrocarbon tail and polar hydroxy group convey critical amphipathic properties that allow proper alignment in lipid membrane mono- and bilayers.\textsuperscript{3}

Figure 2. Molecular structure of 3-hydroxy cholesterol.

3-hydroxy cholesterol is also called free or unesterified cholesterol (UC) and it is the active form of cholesterol which can be converted to other sterols such as hormones or bile acids. The majority of cholesterol present in humans exists as an inactive ester, namely cholesteryl ester (CE), a nonpolar or hydrophobic molecule which serves as the storage or transportation form of cholesterol (Fig. 3). Esterification can occur in cells catalyzed by acyl-cholesterol acyl transferase (ACAT) or within lipoproteins catalyzed by lecithin cholesterol acyl-transferase (LCAT) which transfer the acyl group from the sn-2 position of a nearby phospholipid. Esterification changes the amphipathic cholesterol to a hydrophobic CE, which moves from the surface (exposure to aqueous plasma) of the lipoprotein to the very lipophilic core of the particle. Phytosterols are poor substrates for human ACAT (part of the reason they are so poorly absorbed) and LCAT. Thus any phytosterols that find themselves in lipoproteins, because of their amphipathic character, will exist on the particle surface, not the core.

The human diet includes many exogenous sterols from plants, (sitosterol, campesterol, stigmasterol, etc.) and animals (cholesterol), shellfish sources (desmosterol, fucosterol) and yeast sources. Plant or phytosterols, of which there are over 40, are similar in structure to cholesterol but have methyl, ethyl or other groups in their alipathic side chains (Fig. 4).

Figure 4. Molecular structure of sterols and stanols.

These differences minimize their absorption compared to cholesterol. Sitosterol represents 80\% of noncholesterol sterols in the diet.\textsuperscript{4,5} Stanols are simply saturated sterols, where the \(\Delta 5\) double bond present in the sterol is hydrogenated, further impairing absorption via enterocyte membrane sterol transporters (Fig. 4).\textsuperscript{1,6} The stanol version of
Cholesterol, a cholesterol metabolite, is called cholestanol, and that of sitosterol, sitostanol (available as a commercial product). If plant sterols and stanols are commercially esterified (combined with fatty acids) they can be incorporated into margarines or other food products. Although numerous esterified phytosterols are available, currently sitostanol is the only existing commercial stanol (known as a Benecol®).

Cholesterol is synthesized from acetate and acetoacetyl-CoA in a complex 37-step process, utilizing multiple enzymes (Fig. 5). Some of the intermediary sterols in the synthetic chain are squalene, lathosterol and desmosterol (Fig. 6), measurements of which can serve as a marker of cholesterol synthesis.

Physiology

The human diet includes UC, CE, phytosterols and to a lesser degree some stanols. Intestinal esterolases convert some of the ingested CE into UC. Intestinal microbes can also convert (hydrogenate) some cholesterol to cholestanol. However, after a meal the vast majority of the UC in the jejunum is of biliary origin. All of the lipids in the gut lumen are collectively organized and emulsified by lecithin (phosphocholine), a phospholipid in biliary secretions. The lipids are then surrounded by amphipathic bile acids into mixed biliary micelles which consist of collections of UC, phytosterols, stanols, phospholipids, mono-acylglycerols, and fatty acids. The micelles "ferry" these lipids to the epithelium of the intestinal microvilli. Once there, fatty acids are absorbed through the lipid cell membranes into enterocytes by passive diffusion or membrane-located fatty acid transport proteins.

The unesterified sterols (but not stanols) in the micelles are internalized into the enterocyte via a sterol permease (a protein involved with absorption of sterols) called the Niemann Pick C1 Like 1 (NPC1L1) protein (Fig. 7).
NPC1L1 is expressed in both the brush border of the intestinal epithelium and at the hepatobiliary cell junction. NPC1L1 is not involved with fatty acid absorption. Most humans absorb about 50% of the sterols in the gut, but some people are hyperabsorbers (absorbing as much as 60-80%) or hypoabsorbers (absorbing only ~20-40%). NPC1L1 expressed at the hepatobiliary interface facilitates re-entry of biliary UC back into the liver. Cholesteryl ester cannot pass through NPC1L1 and thus is not absorbed unless it is de-esterified.

Once the sterols gain entry into the enterocytes, several pathways exist for their utilization or disposal:

1) Unesterified cholesterol (but not phytosterols) is a substrate for acyl-cholesterol acyl transferase 2 (ACAT2) which leads to the production of the hydrophobic molecule CE. Cholesteryl ester and enterocyte-produced triglycerides (TG), with the aid of microsomal TG transfer protein (MTP), are lipidated to apolipoprotein B48 (apoB48), resulting in chylomicron production (Fig.7).

2) Unesterified cholesterol mixes with phospholipids to form the chylomicron surface. UC can also be effluxed via ATP binding cassette transporters A1 (ABCA1) into apolipoprotein A-I (apoA-I) or prebeta HDLs (Fig. 7). ApoE can also serve as an UC acceptor.

3) Excess UC and most phytosterols are effluxed back to the gut lumen via ABCG5 and ABCG8 (Fig. 7). Any phytosterols that reach the liver are rapidly effluxed into the bile, via ABCG5 and ABCG8 at the hepatobiliary junction, for delivery to the gut and fecal elimination. If there is reduced expression of hepatic ABCG5/G8, the amphipathic phytosterols will be incorporated into the surface of very low density lipoproteins (VLDLs) and ultimately LDLs where they can gain arterial entry and promote atherogenesis.

4) The inability to be esterified by ACAT promotes phytosterol return to the gut lumen and markedly restricts their systemic absorption. If there are defects in the sterol efflux transporters ABCG5 and/or ABCG8 expression, phytosterols are not rapidly returned to the gut lumen and can be incorporated into the surface layers of chylomicrons and, via ABCA1, into enterocyte lipidated HDLs.
Thus evolution, by making phytosterols poor substrates for ACAT2, and via placement of ABCG5 and ABCG8 in enterocytes and hepatocytes, has gone to great lengths to keep phytosterols out of the human body, leading to speculation that these molecules are certainly not needed and might be toxic. Indeed, homozygous absence of these sterol efflux proteins results in the disease sitosterolemia or phytosterolemia which is associated with anemia, xanthomata and premature atherosclerosis. It is speculated that if noncholesterol sterols (which cannot be esterified) gain entry into the arterial wall, they are more susceptible to oxidation than CE and facilitate foam cell formation. These facts should make a clinician think twice before recommending dietary phytosterol supplementation as an adjunctive cholesterol-lowering therapy. The 2001 European Guidelines (EAS/ECS) recommend close monitoring when such phytosterol products are prescribed.

The sterol absorption terminology is very confusing but it is crucial that it be understood. When clinicians speak of a substance being absorbed, they typically visualize such molecules moving from the gut lumen into the plasma. However, when experts refer to sterol absorption in the intestinal enterocyte they are referring to the process whereby the sterol enters the enterocyte by traversing the luminal cell membrane, not to whether it reaches the plasma inside a chylomicron. The enterocyte-internalized (absorbed) sterol may or may not make it into the plasma because the enterocyte has the ability, using membrane protein efflux transporters (ABCG5 and ABCG8), to return sterol molecules to the gut lumen. Therefore not all absorbed sterols necessarily enter the plasma or lymphatic system. Entry of sterols into the systemic circulation is a result of the interplay between sterol influx (NPC1L1), sterol esterification by ACAT2, and sterol efflux transporters. Sterol homeostasis is rendered even more complex by the presence of these same transporters at the hepatobiliary interface. In summary, both enterocytes and hepatocytes can respectively absorb sterols from the gut lumen (into enterocytes) and bile (into hepatocytes) or export sterols from enterocytes or hepatocytes into the gut lumen or bile.

The rate at which persons absorb cholesterol is variable and depends on the many cellular nuclear transcription factors that regulate cholesterol homeostasis, including the sterol regulatory element binding proteins (SREBPs), liver X receptors (LXRs), farnesoid receptors, and peroxisome proliferator-activated receptors (PPARs) alpha and delta. If the body needs cholesterol there will be an increase in both cholesterol synthesis and absorption: In both the liver and intestine there will be upregulation of NPC1L1 and down-regulation of ABCG5 and ABCG8. On the contrary, in cholesterol overload situations the opposite will occur, namely a down-regulation of NPC1L1 and up-regulation of ABCG5 and ABCG8.

**CLINICAL INTERPRETATION**

Cholesterol measurements do not provide information regarding its origin, i.e., whether an elevated cholesterol level is the result of increased absorption, increased endogenous synthesis, or decreased clearance. Because humans with perfect physiology absorb very few phytosterols or stanols, their assay in blood serves as a biomarker of intestinal absorption. An elevated plasma level of sitosterol or campesterol indicates a hyperabsorption state and individuals with phytosterolemia have marked elevations of these markers. Normal reference ranges have been developed (Table 1). Cholestanol, a cholesterol metabolite, is present in food substances, especially meats and are also made by intestinal microbes. Stanols are not readily absorbed and cholestanol has also been used for decades as a marker of sterol absorption. Also, a rare enzyme deficiency results in cerebrotendinous xanthomatosis (CTX) which causes marked elevation of cholestanol, leading to lipidosis, xanthomata and CNS neurologic abnormalities.

Sterols are extracted from the blood specimen and analyzed via liquid chromatography tandem mass spectrometry (LC-MSMS). Laboratories measuring phytosterols or cholestanol report them as absolute levels or as ratios adjusted for cholesterol. There is no expert consensus as to which method of reporting is best and some laboratories report both. Several trials including the Framingham Offspring, PROCAM, Helsinki Business Study, LURIC study,
and the Cardiovascular Risk in Young Finns Study have shown that markers of hyperabsorption (sitosterol, campesterol or cholestanol) are powerful indicators of CHD risk (Figure 8). There are also some trials such as the Dallas Heart Study that have not found an association between absorption markers and increased cardiovascular risk. In hyperabsorptive states, as the liver receives increased chylomicron delivery of phytosterols, UC and CE, there will be an LXR-mediated down-regulation in the production of HMGCoA reductase, which slows cholesterol synthesis (this can be confirmed by finding low plasma lathosterol or desmosterol levels). Indeed, with respect to markers of synthesis (lathosterol or desmosterol), either elevated levels (as in familial hypercholesterolemia [FH] or some apoE disorders) or low levels in the presence of elevated absorption markers, signify increased risk.

The DEBATE study showed that even with the metabolic syndrome, low cholesterol absorption was associated with fewer recurrent CVD events and higher survival rates in the elderly, perhaps due to a lower lifetime cholesterol exposure. We are now beginning to understand that not everyone has perfectly functioning ABCG5/8 transporters and studies have shown that noncholesterol sterols do gain systemic entry in some people. Patients with up-regulation of NPC1L1 and down-regulation of ABCG5 or ABCG8 will have elevated sterol absorption markers. Common hyperabsorptive states include patients with a strong family history of premature CHD, postmenopausal women, patients with T2DM, patients using statins, and in some, but not all studies, men with the apoE4 allele hyperabsorb cholesterol. Phytosterols have been found in carotid plaque in statin users. Such patients have severe, moderate or slightly elevated absorption markers (cholestanol, sitosterol, or campesterol). The plasma phytosterol levels are the highest in the phytosterolemia patients (complete absence of ABCG5 or ABCG8 caused by a homozygous mutation in the underlying gene). Without measuring markers of absorption clinicians have no way of knowing which patients may be
over absorbing cholesterol or noncholesterol sterols. Such knowledge will influence CV risk assessment, as well as lifestyle counseling and lipid drug recommendations.

**CLINICAL MANAGEMENT**

Some studies have shown that measurement of sterol absorption and synthesis markers has the potential to help a clinician choose effective therapies. Hyperabsorptive states, be they statin-induced or not, can be treated with the use of supplements (i.e., plant stanols) or drugs that reduce absorption (ezetimibe or fibrates). Cholesterol hypersynthetic states can be treated with lifestyle and statins (perhaps lower doses). Instances of elevated apoB or LDL-P occurring with normal absorption and synthesis markers suggests increased apoB production due to triglycerides or decreased clearance of atherogenic lipoproteins and can be treated with medications that upregulate LDL receptors (statins, ezetimibe, bile acid sequestrants) or by therapies that reduce apoB particle production.

Statins inhibit the action of HMGCoA reductase, the rate limiting enzyme of the cholesterol synthesis pathway. Plasma levels of markers of cholesterol synthesis (desmosterol, lathosterol) will be reduced by statins. Reflexively, statin-inhibition of cholesterol synthesis induces upregulation of the NPC1L1 protein which will influx UC from bile to liver and UC and phytosterols from the intestinal lumen to enterocyte. Thus statins often increase sitosterol, campesterol and cholestanol levels.

Typically clinicians prescribe statins to patients with elevated LDL-C. However if that high LDL-C is due not to increased synthesis but rather increased absorption (which results in decreased HMGCoA reductase activity), the statin will be ineffective. Indeed, as demonstrated in the 4S trial, simvastatin had no effect on CV endpoints when administered to hyperabsorbers of cholesterol (identified by increased cholestanol levels; Fig. 9).

Conceivably, using a cholesterol absorption inhibitor like ezetimibe would have helped such patients achieve LDL-C goals more effectively than the statin monotherapy. Of perhaps greater concern is the trial which showed that statin-treated patients, but not drug-naive patients, undergoing carotid endarterectomy plaque analysis showed increased plaque campesterol and decreased cholesterol (Fig. 10).
This implies that the statin-induced over-absorption of phytosterols resulted in those sterols entering plaque. Numerous trials show that the use of statin monotherapy, especially at the high doses, although reducing cholesterol, may significantly increase intestinal, biliary and plasma phytosterol levels (by upregulating NPC1L1). Of all of the statins tested atorvastatin seems to be the worst offender (Fig. 11).  

Ezetimibe (Zetia®) blocks sterol absorption from micelles by interfering with (by binding to) the NPC1L1/ AP2-clathrin complex in the intestinal epithelium. Ezetimibe typically reduces sterol absorption by about 50%. Because ezetimibe blocks the absorption of all sterols, it is approved not only to reduce cholesterol levels but also to reduce the very high noncholesterol sterol levels seen in patients with phytosterolemia (sitosterolemia). Since the vast majority of intestinal UC is of biliary, not exogenous origin, ezetimibe in effect has only a minor effect on blocking the absorption of ingested cholesterol. NPC1L1 is also expressed at the hepatobiliary interface and thus facilitates re-entry of biliary cholesterol back into the liver. Interestingly, ezetimibe monotherapy will reduce chylomicron delivery of cholesterol to the liver. Thus ezetimibe monotherapy, by inhibiting cholesterol absorption and reflexively increasing cholesterol synthesis, will reduce markers of absorption (sitosterol, campesterol, cholestanol) but increase synthesis markers (desmosterol, lathosterol). The simple solution is to use a low dose statin with the ezetimibe so that both synthesis and absorption markers will be normalized. Ezetimibe has no effect on fatty acid absorption. Fibrates are PPAR-alpha agonists and have a multitude of effects on lipid and lipoprotein levels. PPAR-alpha is one of the nuclear transcription factors that regulate expression of NPC1L1. By down-regulating NPC1L1, fibrates reduce cholesterol absorption and enhance its excretion in the stool. They have been a mainstay of treatment for those with phytosterolemia for some time. Ezetimibe interferes with NPC1L1 function. Combining fenofibrate with ezetimibe (an on-label use) results in significant inhibition of sterol absorption and enhanced lowering of both LDL-C and non-HDL-C.

If plant sterols and stanols are esterified (combined with fatty acids) they can be incorporated into margarines or other products. By competing with cholesterol for entry into biliary micelles, they reduce the amount of cholesterol that is available for internalization by NPC1L1. By themselves, or when combined with statins, ezetimibe, colesevelam or fibrates, such products can enhance LDL-C lowering. However one should never recommend phytosterols to a patient with elevated phytosterol levels and absorption markers need to be followed in such patients. 

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**Figure 11.**

![Cholesterol Synthesis Markers Rise with Ezetimibe and Fall with Atorvastatin](image-url)
REFERENCES

24. Wilund KR, Yu L, Xu F, Vega GL, Grundy SM et al. No association between plasma levels of plant


Title: The acute phase protein serum amyloid A induces lipolysis and inflammation in human adipocytes through distinct pathways.

Authors: Fatty A, Ferré P, Commans S.


Comment: The acute phase response (APR) induced during infection or inflammation, is an early and highly complex reaction of the host, which protects it from further injury. The APR is characterized by an increased resting energy expenditure, extensive protein and fat catabolism, negative nitrogen balance, hyperglycemia and hypertriglyceridemia. The alterations in lipid and glucose metabolism lead to an increased delivery of energy substrates. In adipocytes, there is a coordinated decrease in free fatty acids (FFAs) and glucose storage, in addition to an increase in FFAs mobilization. Serum Amyloid A (SAA) is one of the major acute-phase proteins predominantly produced by the liver. The circulating concentration of SAA protein is increased up to 1,000-fold within 24 to 48 h following infection/inflammation from a basal level of 5–8 microg/mL. SAA is primarily transported in the plasma by high-density lipoprotein (HDL), for which it has a high affinity. SAA is thought to be delivered by SAA-enriched HDL (saaHDL) to the sites of infection where it can prime monocytes through its cytokine-like properties. The authors hypothesized that saaHDL, through SAA, could play a major role in the alteration of adipocyte metabolism, providing a molecular link between APR or low grade inflammatory disorders and associated lipid and glucose metabolism abnormalities. They treated in vitro differentiated human adipocytes (hMADS) with saaHDL or recombinant SAA and analyzed the metabolic phenotype of the cells. According to the results obtained with SAA in hMADS, the decrease in the capacity of adipose tissue to store FFAs during APR may be the consequence of several effects of SAA on adipocytes such as 1) decrease in gene expression of transcription factors important for adipocyte storage such as PPARc2, C/EBPaand SREBP-1c; 2) decrease in gene expression of the main enzymes involved in lipogenesis like G3PDH and FAS; and 3) increase in lipolysis through activation of the ERK pathway and HSL as previously described also in other models. In addition, the activation of the NF-κB pathway by SAA led to the induction of pro-inflammatory cytokines and chemokines, as in the case of immune cells. These latter findings were replicated in mature human adipocytes freshly isolated from subcutaneous adipose tissue. In these adipocytes, a low dose of SAA (1 microg/mL) was able to confer a maximal induction of inflammation as well as a maximal repression of the transcription factors (PPARc2, C/EBPa and SREBP-1c) suggesting higher SAA receptor expression. In summary, findings of this study suggest that, besides its well-characterized role in cholesterol metabolism, SAA could be at least partly responsible for the metabolic changes of the adipose tissue during the APR. The effects observed at the molecular level could translate into a phenotype characterized by a coordinated decrease in the storage of FFAs and an increase in FFAs mobilization and at least partly explain the reduced insulin efficiency concomitant with APR. These findings also reveal that, in conditions of low grade inflammation such as obesity, SAA could participate to the metabolic phenotype characterized by adipose tissue inflammation, insulin resistance and fatty acid overflow from adipocytes. Although SAA concentration during APR can be as high as 1 mg/mL, the authors showed here that at much lower concentrations (1 to 30 microg/mL), similar to those observed in obese patients, SAA has already clear-cut effects on adipocytes. Of note, this is the first study to address the early SAA intracellular signaling pathways in human adipocytes.

Title: Red blood cells play a role in reverse cholesterol transport.

Authors: Hung KT, Berisha SZ, Ritchey BM, Santore J, Smith JD.


Comment: Reverse cholesterol transport (RCT) involves the removal of cholesterol from peripheral tissue for excretion in the feces. It is generally accepted that lipoproteins carry cholesterol in plasma throughout the body and increasing high-density lipoprotein cholesterol (HDL-C) via apolipoprotein A1 (apoA1) gene transfer in mice has been shown to increase RCT. Yet, in humans, whole blood is composed of ≈45% red blood cells (RBCs) by volume, and the cholesterol concentration in RBCs is comparable to that found in the plasma, carried by lipoproteins. RBC plasma membranes contain free cholesterol that can bidirectionally exchange with plasma lipoprotein cholesterol approaching equilibrium ex vivo in ≈6 hours, with the kinetics indicating transfer via aqueous diffusion. Despite their significant carrying capacity for cholesterol, the role that RBCs may play in RCT has not been previously addressed. This prompted the authors to determine whether red blood cells (RBCs)
can contribute to RCT. They performed a series of studies in apoAI-deficient mice where the HDL-mediated pathway of RCT is greatly diminished. RBCs carried a higher fraction of whole blood cholesterol than plasma in these mice, and as least as much of the labeled cholesterol derived from injected foam cells appeared in RBCs compared with plasma. To determine whether RBCs mediate RCT to the fecal compartment, they measured RCT in anemic and control apoAI-deficient (apoAI−/−) mice and found that anemia decreased RCT to the feces by over 35% after correcting for fecal mass. Transfusion of 3H-cholesterol-labeled RBCs led to robust delivery of the labeled cholesterol to the feces in apoAI−/− hosts. In wild-type (WT) mice, the majority of the blood cholesterol mass, as well as 3H-cholesterol derived from the injected foam cells, was found in plasma, and anemia did not significantly alter RCT to the feces after correction for fecal mass. Finding of a smaller fraction of RCT-derived fecal neutral sterols in apoAI−/− vs. WT mice supports the notion that the transfer of RBC cholesterol to hepatocytes may at least partially be independent of lipoproteins. Based on the results, the authors concluded that the RBC cholesterol pool may play a previously unknown role in mediating RCT. They proposed a model in which the free cholesterol pool in whole blood can passively equilibrate between lipoproteins and cell membranes, with RBCs providing the bulk of cell membranes. Although cholesteryl ester transfer protein (CETP) is not expressed in mice, it may contribute to cholesterol ester exchange from HDL to LDL in humans and other species where it is expressed. When RBCs pass through the liver, they can deliver their cholesterol to sinusoidal endothelial cells which in turn can pass it to hepatocytes. Whether this step is entirely passive or may be facilitated by a transporter is unknown. Alternatively, cholesterol transfer from RBCs to hepatocytes may again be mediated via exchange to lipoproteins that can then migrate into the space of Disse and deliver cholesterol to hepatocytes. Once in the hepatocyte, most of the RBC-derived free cholesterol is efficiently converted to bile acids, which along with the remaining free cholesterol is excreted into the bile and then into the intestine. It is noteworthy that, in a longitudinal study of 14,410 human subjects, anemia at baseline was associated with a significant hazard ratio of 1.41 for subsequent coronary vascular disease over a 6-year follow-up. Although the exact mechanism is unknown, findings of the current study suggest that diminished RCT in anemic subjects may play a role in this association. Low HDL-C levels are common in men, and men with 35 mg/dL have HDL-C levels closer to those seen in apoAI−/− mice (12 mg/dL) than seen in WT mice (93 mg/dL). Thus, anemia and hematocrits should not be overlooked in ongoing clinical studies of RCT and HDL function.

Title: Plasma kinetics of an LDL-like nanoemulsion and lipid transfer to HDL in subjects with glucose intolerance.

Authors: Bertato MP, Oliveira CP, Wajchenberg BL, Maranhão RC.


Comment: Cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP) promote lipid transfer among lipoprotein classes, including cholesterol, phospholipids and triglycerides. These lipid transfers are crucial for the formation and metabolism of HDL in the plasma and are part of the reverse cholesterol transport. Free cholesterol transferred to HDL undergoes esterification by lecithin:cholesterol acyltransferase (LCAT) using apoAI, which is the main HDL apolipoprotein, as a co-factor. Glucose intolerance is frequently associated with an altered plasma lipid profile and increased cardiovascular disease risk. Nonetheless, lipid metabolism is scarcely studied in normolipidemic glucose-intolerant patients. This study aimed to investigate whether insulin resistance affects the plasma kinetics of lipoprotein free and esterified cholesterol and the transfer of both cholesterol forms, phospholipids and triglycerides to HDL, even in the absence of altered plasma lipids. Glucose intolerant (GIt) patients with normal lipid plasma levels were studied to determine whether these parameters could indicate the existence of metabolic alterations. An artificial nanoemulsion that mimicked the LDL structure was used to probe the intravascular metabolism of LDL cholesterol and also as a lipid donor to HDL for the in vitro evaluation of the lipid transfer. Fourteen GIt patients and 15 control patients were studied; none of the patients had cardiovascular disease manifestations, and they were paired for age, sex, race and co-morbidities. A nanoemulsion resembling a LDL lipid composition (LDE) labeled with 14C-cholesteryl ester and 3H-free cholesterol was i.v. injected, and blood samples were collected over a 24-h period to determine the fractional clearance rate of the labels by compartmental analysis. The transfer of free and esterified cholesterol, triglycerides and phospholipids from the LDE to HDL was measured by the incubation of the LDE with plasma and radioactivity counting of the supernatant after chemical precipitation of non-HDL fractions. According to the results, the levels of LDL, non-HDL and HDL
cholesterol, triglycerides, apoA1 and apoB were equal in both groups. The $^{14}$C-esterified cholesterol fractional clearance rate was not different between GIt and control patients, but the $^{3}$H-free-cholesterol fractional clearance rate was greater in GIt patients than in control patients. The lipid transfer to HDL was equal in both groups. In conclusion, GIt patients without dyslipidemia or clinical manifestations of cardiovascular disease had normal *in vitro* transfer of lipids to HDL and removal of the LDL nanoemulsion probe from the plasma. The latter finding may account for their normal LDL cholesterol levels. Nonetheless, these patients exhibited an abnormal kinetic behavior of free cholesterol that may be associated with atherogenesis. These findings may help the understanding of the overall role of insulin resistance in the pathophysiology of cardiovascular disease.

**Title:** Influence of vitamin D supplementation on plasma lipid profiles: A meta-analysis of randomized controlled trials.

**Authors:** Wang H, Xia N, Yang Y, Peng DQ.

**Journal:** Lipids Health Dis. 2012;11(1):42.

**Comment:** Observational studies have shown that low serum levels of vitamin D are associated with an atherogenic lipid profile. Furthermore, randomized intervention trials showed a tendency towards a reduction in cardiovascular disease (CVD) risk with vitamin D supplementation, though the tendency was not statistically nonsignificant. Since vitamin D deficiency is highly prevalent across the world and vitamin D supplementation is simple, safe, and inexpensive, the deficiency of vitamin D may be a common and easily treatable risk factor for CVD prevention. The authors conducted a meta-analysis of randomized controlled trials to evaluate the effects of vitamin D supplementation on blood lipids. A systematic literature search was conducted *via* MEDLINE, Cochrane library, and EMBASE for randomized controlled clinical trials assessing the effects of vitamin D supplementation on lipids. The mean change in total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) from baseline was treated as a continuous variable. In all, 12 clinical trials consisting of 1,346 participants were included in the meta-analysis. The pooled estimate of effect for vitamin D supplementation on LDL-C was 3.23 mg/dL (95% confidence interval, 0.55 to 5.90 mg/dL) and this increase was statistically significant. Subgroup analyses by duration of intervention revealed that vitamin D treatment had a more obvious effect on LDL-C in the shorter duration studies. No statistically significant effects for vitamin D supplementation were observed for TC, HDL-C and TG (differences in means were 1.52 mg/dL (-1.42 to 4.46 mg/dL), -0.14 mg/dL (-0.99 to 0.71 mg/dL) and -1.92 mg/dL (-7.72 to 3.88 mg/dL) respectively). In summary, evidence from randomized, controlled trials indicates that vitamin D supplementation could increase LDL-C concentrations, but does not significantly affect TC, HDL-C and TG. The lipid modulating effects of vitamin D supplement should be further investigated through large-scale, randomized trials with adequate doses which can effectively elevated the active form of vitamin D in plasma and with proper population which has hyperlipidemia as an inclusion criterion.

**Title:** Waiting for the National Cholesterol Education Program Adult Treatment Panel IV Guidelines, and in the meantime, some challenges and recommendations.


**Journal:** Am J Cardiol. 2012 Apr 10. [Epub ahead of print]

**Comment:** The National Cholesterol Education Program Adult Treatment Panel (ATP) has provided education and guidance for decades on the management of hypercholesterolemia. Its third report (ATP III) was published 10 years ago, with a white paper update in 2004. There is a need for translation of more recent evidence into a revised guideline. To help address the significant challenges facing the ATP IV writing group, the authors of this article aimed to provide balanced recommendations that both build on ATP III and involve simplicity to increase the likelihood of implementation in clinical practice. To move from ATP III to ATP IV, they recommend the following:

1. **Assess risk more accurately:** Incorporation of risk assessment tools beyond the ATP Framingham risk score (FRS) into the treatment algorithm is recommended and outlined in a figure. There are a number of alternatives to assess cardiovascular risk beyond the ATP FRS. Two of the more common are the Reynolds risk score and the D’Agostino General Cardiovascular Disease Risk Profile for Use in Primary Care. Moving beyond risk factors closer to the atherosclerosis phenotype, noninvasive imaging of coronary artery calcium and carotid intima-media thickness are additional tools for risk stratification with class IIa indications (benefits exceed risks) in intermediate-risk patients. Quantifying atherosclerosis burden, coronary artery calcium provides robust risk
information and may be used to target patients expected to derive the most benefit from statin treatment. ATP guidelines should be further enhanced by introducing lifetime risk as a framework for patients who are estimated to carry low to intermediate short-term risk. More comprehensive risk assessment could enhance the efficiency of statin therapy allocation, optimize the risk/benefit ratio, and ensure that more patients who may benefit from therapy are not missed.

(2) Simplify the ATP III starting algorithm, which is overly complex: Direct linking risk with therapy as the first priority in the algorithm is recommended. Statins are an important adjunct to the first line of therapy: lifestyle changes. Potent statin therapy is warranted in most patients at high short-term risk. In other patients, lifetime risk should factor into decision making. If high, this may help motivate patients to make lifestyle changes.

(3) Prioritize statins over other drug classes: If a patient does not tolerate initial statin therapy, one can try a lower dose, an alternative dosing schedule, or a different statin before switching to another drug class. Given variability in metabolism, patients not tolerating one statin may tolerate another. ATP IV should also address toxicity concerns (e.g., diabetes) that have emerged since ATP III while emphasizing that benefits of therapy still generally outweigh risks.

(4) Relax the follow-up interval for repeat lipid testing: Some patients might prefer to return for repeat laboratory testing as early as 6 weeks, while others may find early follow-up inconvenient and, in addition to drug effect, prefer the opportunity to also see the added response to several more months of important lifestyle changes.

(5) Designate <70 mg/dl as an "ideal" low-density lipoprotein cholesterol target: In patients who have baseline LDL-C levels <70 mg/dl, aim for LDL-C lowering of ≥50%.

(6) Endorse targets beyond low-density lipoprotein cholesterol: Consideration is warranted to non-HDL-C, HDL-C, apoB, and lipoprotein particle concentrations.

(7) Refine therapeutic target levels to the equivalent population percentile: Use of "ideal" and "satisfactory" targets representing about the 10th and 40th population percentiles for LDL-C, non-HDL-C, and apoB concentrations (summarized in a table) from the National Health and Nutrition Examination Survey [NHANES] 2007 to 2008 is recommended.

(8) Remove descriptors: Removal of misleading descriptors such as "borderline high" and framing United States adult lipid levels in the context of evolutionarily normal levels is recommended. Although this reveals widespread abnormality, allocation of pharmacotherapy should be based on comprehensive risk assessment, not solely cholesterol.

(9) Make lifestyle messages simpler: Rather than nutrient percentages, emphasize a dietary pattern. Exercise is equally important and easily communicated by encouraging patients to wear pedometers, with a goal of 10,000 steps/day, etc.. In conclusion, the solutions offered in this statement represent ways to translate the totality of published reports into enhanced hyperlipidemia guidelines to better combat the devastating impact of hyperlipidemia on cardiovascular health.

Title: Evaluation of newer risk markers for coronary heart disease risk classification: a cohort study.


Comment: Clinical decision making for detection, management, and prevention of coronary heart disease (CHD) relies on accurate risk assessment. The Framingham risk score (FRS) is the most commonly used CHD risk prediction instrument in clinical settings and constitutes the basis for the Adult Treatment Panel III guidelines for cholesterol-lowering therapy. Since validation of the FRS, many coronary risk factors, also called risk markers, have been identified. Efforts are ongoing to assess the increment in risk prediction accuracy, if any, that these newer risk markers contribute to the FRS and other standard risk-scoring systems. The authors of this study assessed whether newer risk markers for CHD risk prediction and stratification improve FRS predictions. They conducted a prospective population-based study (The Rotterdam Study, Rotterdam, the Netherlands) of 5,933 asymptomatic, community-dwelling participants (mean age, 69.1 years [SD, 8.5]). Assessment included traditional CHD risk factors used in the FRS (age, sex, systolic blood pressure, treatment of hypertension, total and high-density lipoprotein cholesterol levels, smoking, and diabetes) and newer CHD risk factors (N-terminal fragment of prohormone B-type natriuretic peptide levels, von Willebrand factor antigen levels, fibrinogen levels, chronic kidney disease, leukocyte count, C-reactive protein levels, homocysteine levels, uric acid levels, coronary artery calcium [CAC] scores, carotid intima-media thickness, peripheral arterial disease, and pulse wave velocity). Adding CAC scores to the FRS improved...
the accuracy of risk predictions (c-statistic increase, 0.05 [95% CI, 0.02 to 0.06]; net reclassification index, 19.3% overall [39.3% in those at intermediate risk, by FRS]). Levels of N-terminal fragment of prohormone B-type natriuretic peptide also improved risk predictions but to a lesser extent (c-statistic increase, 0.02 [CI, 0.01 to 0.04]; net reclassification index, 7.6% overall [33.0% in those at intermediate risk, by FRS]). Improvements in predictions with other newer markers were marginal. In conclusion, among 12 CHD risk markers, improvements in FRS predictions were most statistically and clinically significant with the addition of CAC scores. Further investigation is needed to assess whether risk refinements using CAC scores lead to a meaningful change in clinical outcome. Whether to use CAC score screening as a more routine test for risk prediction requires full consideration of the financial and clinical costs of performing vs. not performing the test for both individuals and health systems. Note of caution: the findings may not be generalizable to younger and/or nonwhite populations.

Title: Complementary prediction of cardiovascular events by estimated apo- and lipoprotein concentrations in the working age population. The Health 2000 Study.


Comment: This is an interesting article using a neural network approach. Apolipoprotein A-I (apoA-I) and B (apoB) and multiple lipoprotein cardiovascular risk factors were computationally estimated with the authors’ extended Friedewald approach (EFW) from classical inputs. The classical Friedewald formula (FW) requires data on serum triglycerides (TG), serum total cholesterol (TC), and HDL-C. However, it is valid only if serum TG <4.52 mmol/L. Further, the estimated values with the FW do not represent pure LDL-C but include also a contribution of the intermediate-density lipoprotein (IDL). The EFW based on artificial neural network regression algorithms can computationally yield estimates of the apoB and apoA-I concentrations, VLDL-TG, IDL-C, LDL-C, and HDL(2)-C without the laborious determinations requiring ultracentrifugation by utilizing data on classical FW inputs. The technology is based on the ability of neural networks to learn inherent relations if appropriate training data are available. In an earlier work, the authors utilized patient cohorts with data on both classical FW parameters and actual measured parameters for the development of EFW and observed that apoB, apoB/apoA-I, and IDL-C provided better prediction of mortality in type 1 diabetics than the conventional FW parameters. At the present, apart from type 1 diabetes, at the population level, and in large epidemiological studies in working age subjects, the impact of the anti-atherogenic (HDL[2]) and the impact of EFW estimates of pro-atherogenic lipoprotein particles on non-fatal cardiovascular events and survival is not known. In the present study, the authors studied a large working age population cohort and analyzed the effect and value of the EFW measures in the prediction of incident non-fatal cardiovascular events and long-term cardiovascular mortality. They hypothesized that EFW parameters could be utilized to predict non-fatal cardiovascular events and survival at the working age population level. For study, the working age (≤65 years, n = 5956) prospective population-based cohort (follow-up of 7.8 ± 0.9 years; 46,572 patient years, 409 non-fatal incident cardiovascular events, and 55 cardiovascular and 266 all-cause deaths) had their total serum cholesterol (TC), triglycerides (TG), and HDL-C measured. Continuous net reclassification improvement (NRI) was calculated. In Cox models adjusted with cardiovascular risk factors, EFW-HDL(2)-C (HR 0.78, 95% CI 0.67-0.91; NRI 16.5%), apoA-I (HR 0.78, 95% CI 0.69-0.89; NRI 15.2%), apoB/apoA-I (HR 1.23, 95% CI 1.08-1.40; NRI 20.6%), and VLDL-TG (HR 1.15, 95% CI 1.05-1.25; NRI 20.1%) were associated with incident non-fatal cardiovascular events and improved risk prediction compared with TC, LDL-C, or non-HDL-C. Cardiovascular deaths could be best predicted with EFW apoB (HR 1.81, 95% CI 1.18-2.77; NRI 77.3%). It was concluded that EFW approach-derived HDL(2)-C, apoA-I, apoB/apoA-I, and VLDL-TG improve prediction of non-fatal cardiovascular events, apoB improves prediction of cardiovascular mortality, and they can be utilized for risk estimation in a working age population without extra cost.

Title: On-treatment non-high-density lipoprotein cholesterol, apolipoprotein B, triglycerides, and lipid ratios in relation to residual vascular risk after treatment with potent statin therapy: JUPITER (Justification for the Use of statins in Prevention: an Intervention Trial Evaluating Rosuvastatin).

Authors: Mora S, Glynn RJ, Boekholdt SM, Nordestgaard BG, Kastelein JJ, Ridker PM.


Comment: Current guidelines focus on reducing low-density lipoprotein cholesterol (LDL-C) as the primary target of therapy, tailoring the level of optimal LDL-C reduction to the individual’s level of cardiovascular risk.
Moreover, the risk among statin-treated individuals remains high and has been termed “residual risk.” The 5-year incidence rate of a major cardiovascular disease (CVD) event occurring among statin-treated patients in randomized clinical trials is 1 in 5 (22%) for individuals with prior CVD and 1 in 10 (10%) for individuals with no prior CVD. The goal of this study was to determine whether residual risk after high-dose statin therapy for primary prevention individuals with reduced levels of LDL-C is related to on-treatment apolipoprotein B (apoB), non-high-density lipoprotein cholesterol (non-HDL-C), triglycerides (TGs), or lipid ratios, and how they compare with on-treatment LDL-C. Participants in the randomized placebo-controlled JUPITER (Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin) trial were 8,901 asymptomatic individuals (women age ≥60 years, men age >50 years) with no history of coronary heart disease, stroke, or diabetes and who had baseline LDL-C levels <130 mg/dL, high-sensitivity C-reactive protein (hsCRP) levels ≥2 mg/L, and TG concentrations <500 mg/dL. Individuals allocated to receive rosuvastatin 20 mg daily with baseline and on-treatment lipids and lipoproteins were examined in relation to the primary endpoint of incident CVD (nonfatal myocardial infarction or stroke, hospitalization for unstable angina, arterial revascularization, or cardiovascular death). Using separate multivariate Cox models, statistically significant associations of a similar magnitude with residual risk of CVD were found for on-treatment LDL-C, non-HDL-C, apoB, total cholesterol/HDL-C, LDL-C/HDL-C, and apoB/A-I. The respective adjusted standardized hazard ratios (95% confidence intervals) for each of these measures were 1.31 (1.09 to 1.56), 1.25 (1.04 to 1.50), 1.27 (1.06 to 1.53), 1.22 (1.03 to 1.44), 1.29 (1.09 to 1.52), and 1.27 (1.09 to 1.49). The overall residual risk and the risk associated with these measures decreased among participants achieving on-treatment LDL-C ≤70 mg/dL, on-treatment non-HDL-C ≤100 mg/dL, or on-treatment apoB ≤80 mg/dL. In contrast, on-treatment TGs showed no association with CVD. In conclusion, in this large randomized, primary prevention trial of nondiabetic individuals with low LDL-C and elevated hsCRP, on-treatment LDL-C was as valuable as non-HDL-C, apoB, or several ratios in predicting residual risk. Among participants achieving on-treatment concentrations of LDL-C ≤70 mg/dL, non-HDL-C ≤100 mg/dL, or apoB ≤80 mg/dL, the overall magnitude of residual risk was small, and the risk associated with these measures decreased and was no longer statistically significant. Finally, the current study does not support the routine measurement of TGs among nondiabetic individuals without significant dyslipidemia who are treated with potent statin therapy.

**Title:** Exercise intervention and inflammatory markers in coronary artery disease: a meta-analysis.

**Authors:** Swardfager W, Herrmann N, Cornish S, Mazereeuw G, Marzolini S, Sham L, Lanctôt KL.


**Comment:** Inflammatory activity plays a role in the development and progression of coronary artery disease (CAD), and exercise confers survival benefit. The authors performed a meta-analysis of changes in inflammatory biomarkers over the course of exercise interventions in patients with CAD. They searched MEDLINE, Embase, the Cochrane Collaboration, AMED, and CINAHL for studies reporting peripheral inflammatory biomarker concentrations before and after exercise interventions of ≥2 weeks in patients with CAD. Data were summarized using standard mean differences and 95% CIs. Twenty-three studies were eligible for the meta-analysis. According to the results, concentrations of C-reactive protein (CRP), interleukin 6 (IL-6), fibrinogen, and vascular cell adhesion molecule 1 (VCAM-1) were lower post-intervention. In controlled studies, follow-up concentrations of CRP and fibrinogen were lower in subjects who exercised compared with controls. Higher total cholesterol and higher total/high-density lipoprotein cholesterol at baseline were associated with greater reductions in CRP. Collectively, the evidence from this meta-analysis supports a reduction in inflammatory activity associated with exercise training in patients with CAD as indicated by lower CRP, fibrinogen, IL-6, and VCAM-1 after intervention. Associations between these biomarkers and risk of mortality emphasize the potential significance of these findings. Controlled studies strengthened this evidence, showing lower final concentrations of CRP and fibrinogen in those who undertook exercise compared with controls.

**Title:** Apolipoprotein mimetic peptides: a new approach for the treatment of asthma.

**Authors:** Yao X, Vitek MP, Remaley AT, Levine SJ.


**Comment:** New treatments are needed for severe asthmatics to improve disease control and avoid severe toxicities associated with oral corticosteroids. Besides their well known role in cardiovascular diseases, apolipoproteins are now increasingly recognized to play a role in the pathogenesis of other acute and chronic
diseases, including asthma. In this article, the authors reviewed the literature and the results of their ongoing research with a murine model of house dust mite (HDM)-induced asthma to identify steroid-unresponsive genes that might represent targets for new therapeutic approaches for severe asthma. This strategy identified apolipoprotein E (apoE) as a steroid-unresponsive gene with increased mRNA expression in the lungs of HDM-challenged mice. Furthermore, apoE functioned as an endogenous negative regulator of airway hyperreactivity (AHR) and goblet cell hyperplasia in experimental HDM-induced asthma. The ability of apoE, which is expressed by lung macrophages, to attenuate AHR, and goblet cell hyperplasia is mediated by low-density lipoprotein (LDL) receptors expressed by airway epithelial cells. Consistent with this, administration of an apoE mimetic peptide, corresponding to amino acids 130-149 of the LDL receptor-binding domain of the holo-apoE protein, significantly reduced AHR and goblet cell hyperplasia in HDM-challenged apoE(-/-) mice. These findings identified the apoE - LDL receptor pathway as a new druggable target for asthma that can be activated by administration of apoE-mimetic peptides. Similarly, apoA-I may have therapeutic potential in asthma based upon its anti-inflammatory, anti-oxidative, and anti-fibrotic properties. Furthermore, administration of apoA-I mimetic peptides has attenuated airway inflammation, airway remodeling, and airway hyperreactivity in murine models of experimental asthma. Thus, site-directed delivery of inhaled apoE or apoA-I mimetic peptides may represent novel treatment approaches that can be developed for asthma, including severe disease.
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