Winter/Spring 2013 Volume XXVII, no. 1

The Fats of Life

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Dawn L Thiselton, PhD produced this issue of *The Fats of Life*.

I have been editing and co-editing the FATS OF LIFE for longer than I care to remember—well over two decades. My predecessors as editors, Don Wiebe, who created the engaging name and also Nader Rifai, who graduated to our society journal Clinical Chemistry, set a high standard. Over the years associate editors, especially Joe McConnell, Alan Remaley, and Katsu Nakajima have been particularly helpful in providing and soliciting relevant articles. Most recently, Dawn Thiselton and Dan Hoefner have been invaluable in getting the issues out. In the early years the FATS was published in hard copy and mailed out, which was logistically highly demanding, time consuming, and expensive. Computerization and internet connections that we take for granted today have since streamlined the production and distribution process. I expect our younger audience will have difficulty imagining the additional challenges involved in creating and distributing a hardcopy newsletter. We should all take a moment and thank our computers! Nevertheless, from the beginning the biggest challenge has been obtaining content, soliciting authors to write meaty and up-to-date articles, begging, cajoling, arm-twisting, nagging about deadlines, etc.! And in parallel to the computer revolution my perception is that our lives have become busier, hence finding volunteers with time to prepare good articles becomes ever more challenging. I do appreciate and thank all of the many contributors to the success of FATS over the years.

Which brings me to a new online resource that is now available, Biomarker Bliki.org, providing the most current and up-to-date information about lab tests. This online site targets lab professionals who want to learn the latest about lab tests, initially focusing on those related to cardiovascular and cardiometabolic diseases. Some history: just about a year ago I was contemplating the fact that the latest edition of our book, Handbook of Lipoprotein Testing, came out in 2000, hence was 12 years old at that time. The Handbook has become the bible for information about lipoproteins and other cardiovascular analytes. Recognizing the substantial progress and improvements in lab testing over that period, I was aware that an update was overdue. However, as I considered the approximately two-year publication cycle, I recognized that after the time it takes to select chapter authors, make assignments, get drafts back, edit and print, much of the information finally published is already obsolete. As I pondered this dilemma a new approach came to mind. The result is the Biomarker Bliki, which has been online for several months but is now reaching a state of development that actually deserves a visit.

We all recognize the term biomarker, which actually comes out of the petroleum industry but is progressively gaining traction in our discipline. Bliki, not so obvious, combines aspects of the wiki and the blog. The idea is that subject matter experts can post chapters on relevant biomarkers, following the wiki concept and —consistent with the modern pace driven by the internet—chapters can be posted in pieces, a section at a time. Authors aren't as constrained to take the time to make chapters comprehensive and complete from the beginning, and can continually add and update the chapters, consistent with increasing knowledge and new developments. The site includes an edit trail so that readers can follow the changes made over time, and accompanying the chapters on a sidebar is a blog capability, enabling readers to pose questions, make comments and add suggestions. The blog comments are visible and sent to the chapter author who can respond and also update the chapter based on comments from readers. The bliki also has the capability for sponsored links to sources for reagents and test platforms. Thus, from one website readers can obtain up-to-date overviews of the biomarkers as well as conveniently link to commercial sources for the cited tests.

The Biomarker Bliki site together with a companion site targeting physicians, lecturepad.org, are sponsored by the Foundation for Health Improvement and Technology (fhit.org), an educational foundation organized by

Joe McConnell and myself to provide quality professional education to laboratory and medical professionals. Dr. Tom Dayspring joined the Foundation as Director of Cardiovascular Education and together with Dr. Tara Dall has contributed and organized many interesting and informative lectures for both sites. Dr. Bajet Nour developed and maintains the LecturePad platform, which includes not only lectures but also highly useful case studies and a weekly quiz.

Current Bliki chapters include:

Apolipoprotein B by John Contois and Thomas Dayspring
AtherOx by Luis Lopez
CYP2C19 Genotype by Dawn Thiselton
Electrophoresis by Phil Guadagno
Fibrinogen by David Farrell
Glycated Albumin by Chong Yuan
Homocysteine by Michael Tsai
Low Density Lipoprotein (LDL) by Mohmed Ashmaig and Thomas Dayspring
Lipoprotein (a) by Joe McConnell and Thomas Dayspring
RBC Fatty Acid Panel by William Harris
Remnant Lipoproteins by Katsuyuki Nakajima
Soluble ST2 by Alan Wu
Sterol Testing by Thomas Dayspring
Triglycerides by Thomas Cole

Several new Bliki chapters are in the pipeline and we welcome any ideas and suggestions for new chapters and authors. If any of you would like to contribute or recommend an author, please contact myself (grwarnick@fhit.org) or Jessi Thomas (jthomas@fhit.org) for more detailed information about the process. We are now in the process of rebuilding the website with plans to launch an even more versatile and functional platform this spring, designed to be more suitable for the increasingly ubiquitous mobile devices.

Again thanks to all of you have faithfully read and contributed to the FATS over these many years. I trust you will find the Biomarker Bliki and the LecturePad sites equally usefully in staying up-to-date in our rapidly evolving cardiovascular discipline.

With best regards,
Russ Warnick

Exciting Events at the Upcoming AACC 2013 Annual Meeting in Houston, TX

I welcome you all to register for the upcoming AACC Annual Meeting in Houston, TX in July-August this year. Please take advantage of the discounted early registration process. 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. On Monday night is the LVDD Annual Mixer/Dinner meeting featuring a very new and exciting presentation by Dr. Vickers from the NIH on miRNAs and Cardiovascular disease. Awards such as the Cooper/Zak/PBRF Awards will also be given and look forward to announcements regarding these in the coming months. In addition, we will introduce you to the new Chair of the Division from 2014, Dr. Kris Kulkarni from Atherotech. On Tuesday night, we will have the International Lipoprotein Standardization Meeting. This is an exciting and interactive forum and will include discussions on Development of new reference methods and standards for advanced lipoprotein testing by Dr. Vincent Delatour from France, especially since our division is supporting their BioSITrace project: Development of reference standards for lipoprotein counting. In addition, we will announce Poster Awards from the AACC LVDD posters.

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 members of the LVDD group have just published a landmark paper on the association of apoB and NMR LDL-P with outcomes. Cole TG, Contois JH, Csako G, McConnell JP, Remaley AT, Devaraj S, Hoefner D, Mallory T, Sethi A, Warnick GR. Association of Apo B and NMR LDL-P with Outcomes in 25 Clinical Studies: Assessment by the AACC Lipoprotein and Vascular Diseases Division Working Group on Best Practices. Clin Chem 2013 Feb 5 [E-pub ahead of print].

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. Please continue to generously support our efforts.

Look forward to seeing you all in the Annual Meeting in July-August in Houston, TX.

With best wishes,
Sridevi Devaraj

Cardio-Ankle Vascular Index (CAVI) and its Potential Clinical Utility as a Cardiovascular Biomarker.

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[2] Cardiopulmonary Branch, National Heart Lung and Blood Institute, National Institutes of Health, Bethesda, MD 20892-1508, USA.

Arterial stiffness is often associated with cardiovascular disease (CVD). The evaluation of arterial stiffness is, therefore, a potentially a useful way for assessing CVD risk and for monitoring therapy in lipid clinics. The cardio-ankle vascular index (CAVI), which can be non-invasively measured with the VaSera VS-1500AU device (Fukuda Denshi Co., http://www.fukuda.com/index_usa.html) is a recently developed metric for evaluating arterial stiffness and vascular function and is almost as simple as determining blood pressure [1,2].

Pulse wave velocity (PWV) is a measure of the time for the pressure wave produced by the contraction of the heart to travel down a vessel. Along with the stiffness parameter β, which is related to the elasticity of a vessel, PVW is a well-known index of arterial stiffness. The problem with the typical way PWV is measured is its significant dependence on blood pressure [3]. The aortic PWV method [4] is independent of blood pressure but is technically difficult to perform and is very user-dependent. The stiffness parameter β, which is based on the change of vascular diameter corresponding to arterial pressure, does not depend on blood pressure, but only examines the local properties of a particular segment of the artery and requires specialized ultrasonic equipment [5].

CAVI, which has both practical advantages and improved precision over some of these alternative measures of arterial stiffness, is calculated using two blood pressure cuffs placed on the upper arm and two blood pressure cuffs placed on the lower leg, while PWV is measured from the aortic valve to the ankles (Fig. 1). A small microphone is placed on the chest and two EKG leads are placed on the wrists to determine the timing of heart contraction. A notable feature of CAVI is that unlike other ways of measuring arterial stiffness it is relatively unaffected by blood pressure [2], which is an important confounding variable for cardiovascular disease. This was shown in a study in which both CAVI and brachial-ankle PWV (baPWV) were measured every hour after administrating a β_1-adrenoceptor blocker (metoprolol) in healthy humans [6]. A significant drug-induced reduction of blood pressure was found to correspond with a reduction of baPWV, whereas CAVI did not change [6]. Another attractive feature of CAVI is that the coefficients of variation of CAVI are relatively small, typically < 4%, and the procedure does not require significant training, thus making it practical to perform in a doctor's office [2].

Data from a large-scale study of healthy Japanese individuals free of cardiovascular disease showed that CAVI gradually increases with age for both men and women [7]. The CAVI score for men was on average higher than that for women in all age-stratified groups. However, both men and women revealed a similar rate of increase in CAVI of approximately 0.5 units per year [7]. Using a potential cut-off score of 8.67 yielded a sensitivity of 66.5% and a specificity of 65.8% for diagnosing coronary heart disease; thus, a CAVI score of 9.0 is commonly used for Japanese patients [8]. It will be important, however, to establish the optimum cut-off in other populations and given the close relationship between CAVI and age, it may be best to use age-dependent values.

Several cross-sectional studies have investigated the association between lipids/ lipoprotein parameters and CAVI. In one study, the CAVI score was found to be significantly correlated with LDL-cholesterol ($r = 0.26$, $P < .05$) in patients with angina [9]. The CAVI score has also been found to significantly increase with hypercholesterolemia and hypertriglyceridemia in men (of 30-69 years of age) and women (of 40-75 years) in comparison to those without CVD risk factors [7]. A significant correlation between CAVI

and oxidized Lp(a) (r = 0.38, P < .05) has also been reported among hypertensive females [10]. In addition, CAVI has been examined in well-treated patients with heterozygous familial hypercholesterolemia (FH) without clinical symptoms of CVD [11]. The mean CAVI level in these FH patients was only slightly higher (8.0) than in healthy subjects (7.5), suggesting that aggressive treatment of CVD risk factors can prevent the development of arterial stiffness in these patients [11].

To date there have been only three intervention studies using CAVI for monitoring patients with dyslipidemias. A randomized-controlled intervention study investigated the effect of eicosapentaenoic acid (EPA), an n-3 polyunsaturated fatty acid (1.8 g/day) for 3 months on CAVI in obese patients with metabolic syndrome (n = 92, mean age = 51.7 years) [12]. Compared to the control group, the EPA-treated group showed a significant reduction in mean levels of triglycerides (from 237 to 177 mg/dL *versus* from 227 to 198 mg/dL in the control, P < .01), C-reactive protein (from 1.57 to 1.16 μg/mL *versus* from 1.46 to 1.63 mg/dL in the control, P < .01), serum amyloid A-LDL complex (from 48.6 to 40.4 μg/mL *versus* from 47.5 to 51.2 mg/dL in the control, P < .01) and CAVI (from 7.87 to 7.59 *versus* from 7.76 to 7.80 in the control, P < .01). A multivariate linear regression analysis revealed that the only significant determinant for a reduction in CAVI by EPA was a reduction in serum amyloid A-LDL complex (β = 0.38, P < .05). An additional single-arm intervention study investigated the effect of ezetimibe monotherapy (10 mg/day) on CAVI for 6 months in patients with type 2 diabetes mellitus (n = 45, mean age = 64 years, HbA1c = 6.5%) [13]. After ezetimibe treatment, significant decreases in mean levels of serum LDL-C (from 160 to 123 mg/dL, P < .01) and CAVI (from 9.17 to 9.00, P < .05) were observed. Lastly, another single-arm intervention study investigated the effect of pitavastatin (2 mg/day) on CAVI for 12 months in patients with type 2 diabetes mellitus (n = 45, mean age = 66 years, HbA1c = 6.9%) [14]. After pitavastatin treatment, significant decreases in mean levels of serum LDL-C (from 166 to 127 mg/dL, P < .01), MDA-LDL (from 170 to 114 U/L, P < .01) and CAVI (from 9.54 to 8.91, P < .05) were observed. Further correlation analysis revealed that the change of CAVI was significantly and positively correlated with that of MDA-LDL (r = 0.55, P < .05) but not that of LDL-C.

In summary, CAVI is a new method for measuring arterial stiffness and is a potentially useful way to identify patients at risk for CVD and monitor the effect of lipid-lowering treatment effects. The clinical utility of measuring CAVI has, however, to be definitively established in large prospective clinical trials. The test is already widely used in Japan and the device for measuring CAVI was recently approved (December 2011) by the U.S. Food and Drug Administeration (FDA), which should facilitate future evaluation of CAVI as a CVD risk marker.

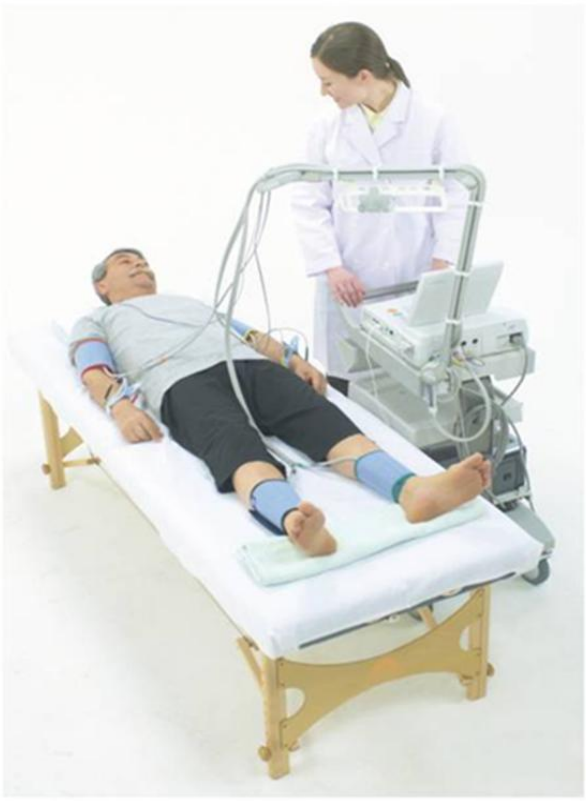

Figure 1. Scene of the CAVI measurement with the VaSera VS-1500 device.

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Assessment of cardiovascular risk by the measurement of Lp(a).

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

OVERVIEW

Lipoprotein (a) or Lp(a) is a low-density lipoprotein (LDL) enwrapped with a single molecule of apolipoprotein B-100 ($apoB_{100}$) and an apolipoprotein(a) molecule, abbreviated as apo(a). Apo(a) has structural homology with plasminogen, a fibrinolytic pro-enzyme, which contains multiple domains called kringle (named after a Scandinavian pretzel) repeats which serve as binding motifs and generate multiple apo(a) isoforms. Once synthesized in hepatocytes, apo(a) is released into plasma where it attaches to triglyceride-rich (TG-rich) $apoB_{100}$ lipoproteins to form Lp(a).

Apo(a) or Lp(a) isoform mass depends primarily on the structure (size or length and molecular weight [MW]) of one particular kringle repeat, the KIV-2 domain. This heterogeneity in the number of KIV-2 copies accounts for approximately one-half of the substantial (up to 1,000-fold) interindividual variability of Lp(a) plasma levels. Hepatocyte secretion rates are lower for large, high MW apo(a) isoforms, and as most individuals are heterozygous for two different isoforms, the smallest, low MW isoform typically predominates in the plasma of those with two distinct apo(a) alleles. Plasma Lp(a) mass is related mostly to synthesis and specifically to the MW of the specific isoforms released into plasma. In Caucasians, elevated Lp(a) levels are generally associated with low MW apo(a) isoforms, but there are ethnic variations.

A growing body of data from large clinical trials indicates that the genomic variation in Lp(a) can be used as an independent variable in relating apo(a) to cardiovascular risk. In epidemiological trials, the low MW isoforms of apo(a) or Lp(a) are associated with higher cardiovascular risk and considered more atherogenic than the larger, high MW isoforms. The physiological function of Lp(a) or apo(a) is still unknown and the mechanisms linking Lp(a) to atherogenesis and expression of cardiovascular disease end-points still undefined. Individuals without Lp(a) or with very low Lp(a) levels seem to be healthy. Thus plasma Lp(a) is certainly not vital, at least under normal environmental conditions. A role within the coagulation system seems plausible, given the high sequence homology between apo(a) and plasminogen. Lp(a) has also been linked with endothelial dysfunction. Knowledge that Lp(a) mass or Lp(a)-C is elevated affords the physician a more accurate risk assessment and to perhaps set more aggressive LDL goals.

INTRODUCTION

Biochemistry

Lipoprotein (a), abbreviated as Lp(a) and pronounced as lipoprotein "little a" is a low-density lipoprotein (LDL) which, like all lipoproteins, consists of a core of cholesteryl ester (CE) and triglyceride (TG) molecules and a surface unilayer of phospholipid and unesterified cholesterol molecules all enwrapped with a single molecule of apolipoprotein B-100 ($apoB_{100}$). Attached to the $apoB_{100}$, via a covalent disulfide bond, is a plasminogen-like glycoprotein called apoprotein (a), abbreviated as apo(a). Thus, the Lp(a) protein moiety has two components, a single copy of

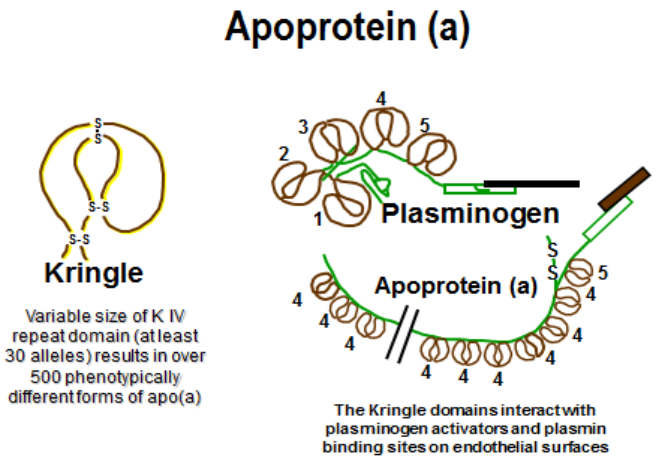


Figure 1. The structure of the kringle domain in apolipoprotein(a).

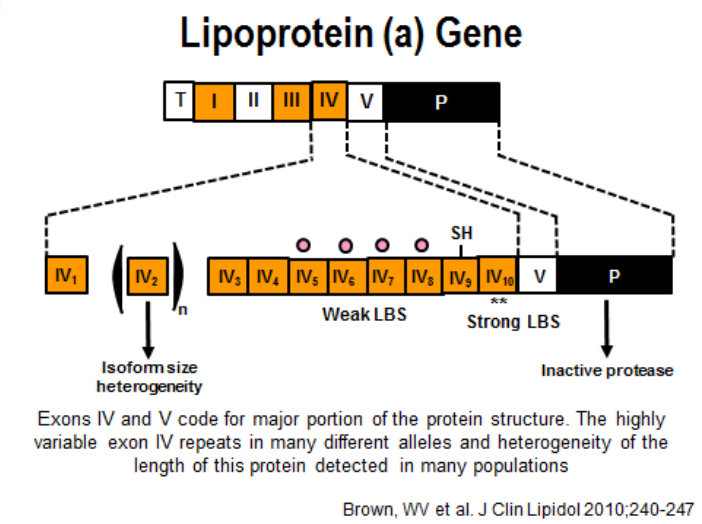


Figure 2. Structure of the apolipoprotein(a) gene (*LPA*).

$apoB_{100}$ linked to a single copy of apo(a) on a 1:1 molar basis. Although it was discovered in 1963 by Kare Berg,[1] Lp(a) and its relationship to atherogenesis has remained an enigma to clinicians and clinical chemists. Apo(a) peptides show significant size heterogeneity as a result of multiple molecular isoforms, which presents a challenge for standardization of the measurement of Lp(a) in plasma. Apo(a) has structural homology with plasminogen, a fibrinolytic pro-enzyme, which contains various multiple corresponding moieties or domains called kringles (named after a Scandinavian pretzel).[2] Kringles are tri-looped structural units of approximately 80 amino acids in length, each containing three disulfide bonds. They have a common folding pattern and serve as binding motifs.[3] Plasminogen has five kringle domains (1-5 or I-V) whereas apo(a) has two (4-5 or IV-V).

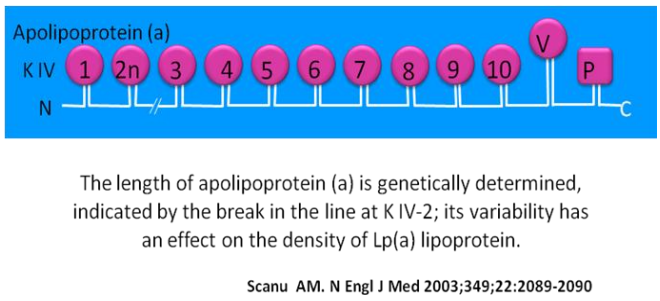


Figure 3. Kringle organization of the human Lp(a) protein.

The core structure of apo(a) kringle IV is similar to those in plasminogen whereas variations in surface residues account for differences in ligand-binding specificities between the two molecules.[4] Apo(a) contains an inactive protease domain, one kringle V, and ten different types of kringle IV motifs, referred to as KIV types 1 through 10. KIV types 1 and 3 through 10 are present as single copies, whereas KIV type 2 (KV-2) is present as multiple copies, varying in number from 3 to more than 40 copies, which explains the unique and characteristic isoform size heterogeneity in each patient.

Juxtaposition of KIV types 7 and 8 promotes proper orientation of the disulfide bond between apo(a) and apoB.[5] Apo(a) is also highly glycosylated (28% carbohydrate by weight) with a high degree of O-linked oligosaccharides, which may affect the proteolytic cleavage and hence functional capabilities of apo(a). Once synthesized in hepatocytes apo(a) is released into plasma where it attaches to triglyceride-rich (TG-rich) $apoB_{100}$ lipoproteins.

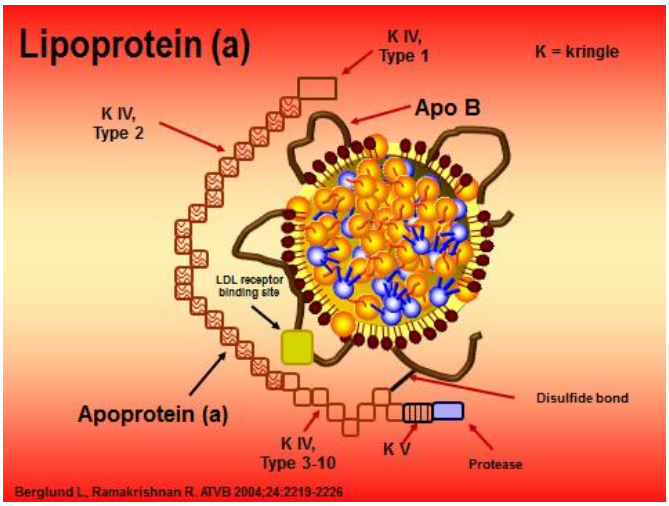


Figure 4. Diagram indicating various structural properties of Lp(a).

The heterogeneity of the apo(a) peptide is in part attributable to marked size and molecular weight (MW) differences. Unlike plasminogen which has five kringles, apo(a) consists of only two genetically determined kringles (KIV and KV). As mentioned, isoforms of KIV exist resulting in distinct apo(a) molecules and Lp(a) particles due to very different apo(a) sizes and MW. Large, high MW apo(a) isoforms have large numbers of KIV-2 repeats. Apo(a) or Lp(a) isoform mass depends on the structure (size or length and MW) of the KIV-2 domain. This heterogeneity in the number of KIV-2

copies accounts for approximately one-half of the substantial (up to 1,000-fold) inter-individual variability of Lp(a) plasma levels. Assays insensitive to isoform size are not yet widely available and thus most assays cannot directly measure molar concentrations (particle numbers) of Lp(a). Most labs reporting Lp(a) in molar concentration are simply reporting "guestimates" obtained by mathematically converting milligrams to moles using an average, not the exact apo(a) MW unique to a given patient.

Physiology

Lp(a) levels remain relatively stable over an individual's lifetime. The synthetic rate and size of the apo(a) moiety of Lp(a), which historically has been thought to control about 90% of plasma concentrations of Lp(a), is determined by the apo(a) genotype. Variation within the *LPA* gene on chromosome 6q23, which encodes apo(a), accounts for most of the variability in plasma Lp(a) concentration. It now appears that KIV-2 copy number variation and apo(a) isoform size explain approximately 30%-50% of variation in Lp(a) concentrations across multiethnic populations.[6, 7] A growing body of data from large trials is providing evidence to suggest that the genomic variation in Lp(a) can be used as an independent variable in relating apo(a) to cardiovascular risk.[7] In the Precocious Coronary Artery Disease (PROCARDIS) study, there was a strong and independent association between coronary heart disease (CHD) and two *LPA* variants, namely rs10455872, which is a noncoding intronic SNP, and rs3798220, which is a missense variant encoding an amino acid substitution (Ile4399Met) in the apo(a) protease-like domain.[7,8] Further significant associations have been reported between these two *LPA* variants and increased Lp(a) levels, and are reported to account for an additional 36% of the total variation in plasma Lp(a) levels.[7,9] Both rs10455872 and rs3798220 are considered to be the *LPA* SNPs that are most consistently associated with both plasma Lp(a) levels and CHD risk. Genetic variation at the *LPA* locus, via raised plasma Lp(a) levels, has recently been implicated in the development of aortic-valve disease.[10]

Apo(a) does not remain covalently linked to a single $apoB_{100}$ lipoprotein but reattaches at least once with another $apoB_{100}$ particle, probably newly synthesized, during its plasma metabolism.[11] This newly formed Lp(a) particle frees apo(a) as the TG-rich lipoprotein portion undergoes lipolysis and receptor-mediated clearance. The free apo(a) associates with another TG-rich $apoB_{100}$ particle. Because about 50% of TG-rich lipoproteins are converted to LDL, the second Lp(a) particle may survive catabolism.[12] A recent study demonstrated that at normal TG levels, Lp(a)-P depended mostly on Lp(a)-C; however, at high TG levels, this association was lost with the majority of the variance of Lp(a)-P related to HDL lipidation, VLDL, and TG. These results imply that Lp(a) metabolism is related to receptors and lipases primarily interacting with HDL and TG-rich lipoproteins (TRL).[13]

Hepatocyte secretion rates are lower for large, high MW apo(a) isoforms, and as most individuals are heterozygous for two different isoforms, the smallest, low MW isoform typically predominates in plasma in those with two distinct apo(a) alleles. Since apo(a) camouflages the LDL receptor binding domain on apoB, Lp(a) does not bind to the LDL receptor.[14] Lp(a) is thought to be catabolized primarily by the liver and kidney, but Lp(a) levels are minimally influenced by its clearance.[15,16] Thus, the plasma Lp(a) mass is related mostly to synthesis and specifically to the MW of the specific isoforms released into plasma.[17] Many studies reveal that small apo(a) isoform size is an independent risk factor for cardiovascular events, suggesting that laboratory analysis of Lp(a) must include apo(a) isoform size. In Caucasians, elevated Lp(a) levels are associated with low MW apo(a) isoforms in more than 80% of subjects. In blacks, elevated Lp(a) levels are distributed over a broader range of apo(a) isoform sizes, and plasma Lp(a) levels are higher in blacks within the same apo(a) isoform sizes as documented in the Dallas Heart study.[18,19]

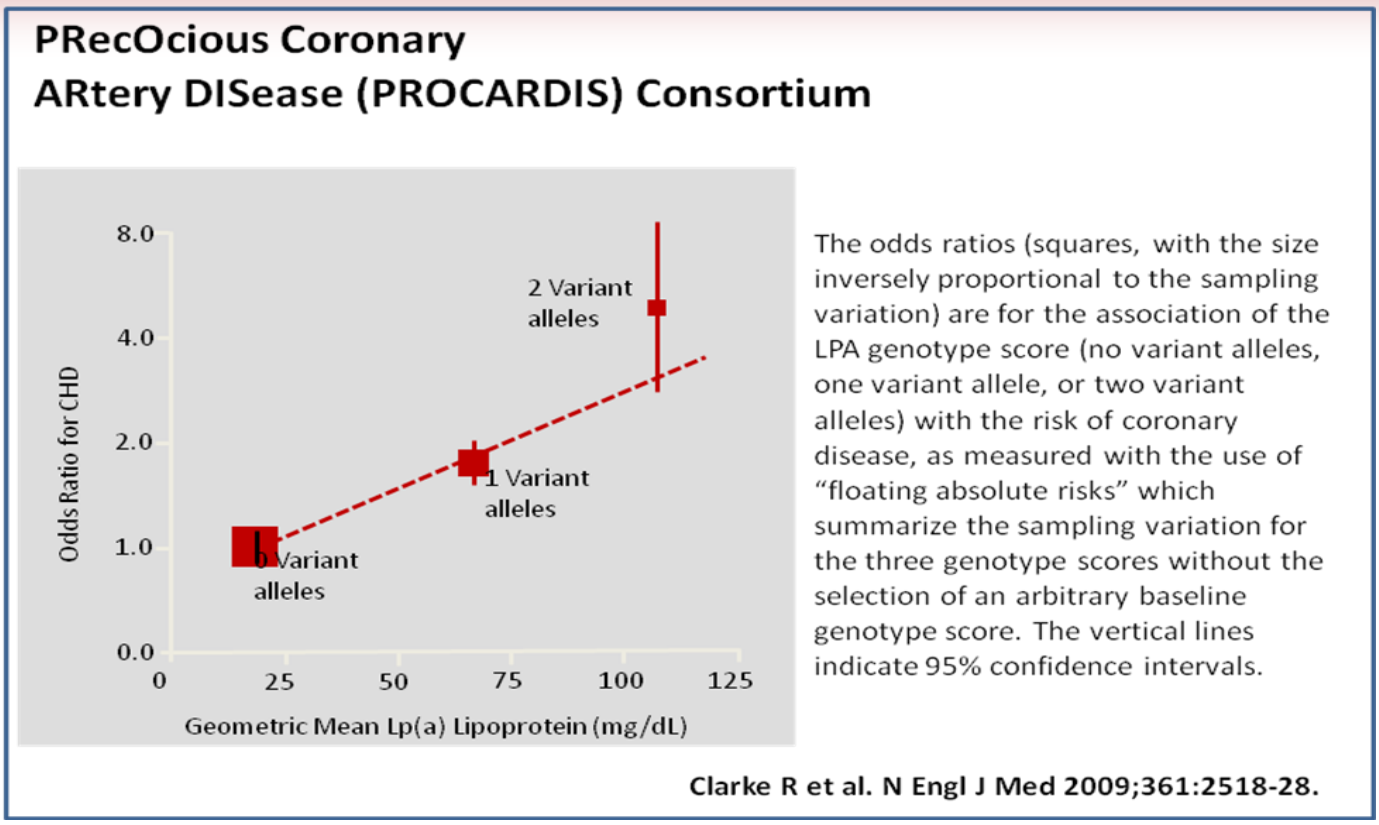


Figure 5. Association of the LPA Genotype Score with the Lp(a) lipoprotein level and the risk of coronary disease in the PROCARDIS cohort.

As mentioned above, the liver is better able to secrete the smaller apo(a) isoforms than the larger ones. Since the size and MW of the apo(a) molecule depends on how many kringle repeats are present, the less the number of KIV-2 repeats, the lower the MW and the higher the hepatic secretion rate. So paradoxically, even though small isoforms have a lower MW than the larger isoforms (which have more KIV-2 repeats), serum levels of apo(a) mass or Lp(a) will usually be higher in patients with the smaller, lower MW isoforms compared to the larger, higher MW isoforms. The total mass of multiple, smaller apo(a) peptides capable of adducting to $apoB_{100}$ particles exceeds that of fewer larger apo(a) peptides. Therefore the Lp(a) particle count or Lp(a)-P measured as Lp(a)-apoB will be much higher in those with low MW isoforms compared to a person whose apo(a) mass is related to the higher MW species. If a patient does secrete the larger, higher MW isoforms rather than small isoforms, Lp(a) mass will be high but cardiovascular risk may be lower than that suggested by the apo(a) mass measurement, i.e., there will be discordance between Lp(a)-P and Lp(a) mass. In epidemiological trials, the low MW isoforms of apo(a) or Lp(a) are associated with higher cardiovascular risk and considered more atherogenic than the larger, high MW isoforms. A systematic review by Erquo et al (2010) showed that there is a two-fold difference in the risk for myocardial infarction and stroke between Caucasian subjects with low MW (~40%) versus high MW apo(a) phenotypes (~60%) – differentiated by a cut-off of approximately 22 KIV-2 repeats.[20] Data from a recent population study of 8,720 Danish individuals suggests that either extreme Lp(a) levels or risk genotypes in *LPA* can substantially improve risk prediction for myocardial infarction and CHD.[21] Since most persons are heterozygous for large and small isoforms, apo(a) mass measurements reflect the sum of the MW of both small and large isoforms. Due to its greater hepatic release, the vast majority of apo(a) molecules in most individuals with elevated Lp(a) mass are of low MW. Therefore, the major limitation in relying on Lp(a) mass concentration testing is that Lp(a) mass does not always correlate with Lp(a)-P and risk is almost certainly more related to the number of Lp(a) particles than it is Lp(a) mass.

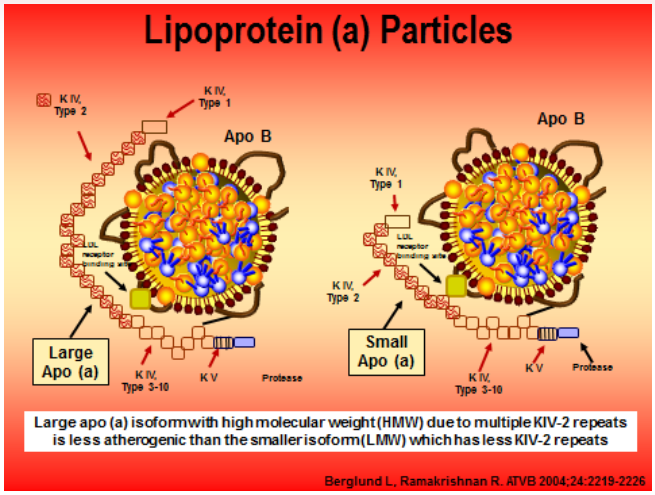


Figure 6. Two Lp(a) particles with different apo(a) sizes.

Adding to confusion in the clinical world, is the fact that many clinicians do not understand how laboratories measure and report Lp(a) concentrations. In the clinical laboratory, immunologic methods are generally used to quantify Lp(a) protein mass. Reagents for Lp(a) mass measurement are available from multiple manufacturers, and although standardization efforts are under way, currently available methods are not standardized. Difficulties in standardizing Lp(a) mass measurement arise from the variability in signals produced by different reagents due to the size polymorphisms (repeats of kringle type IV-2) of apo(a). Labs measure Lp(a) in milligrams (mg) and then, using the estimated MW of apo(a), some simply convert the mg value to a molar weight [which, if accurate, would be a measure of Lp(a) particle number]. Since the actual MW varies according to the number of amino acids in the KIV-2 kringles (which varies significantly between individuals), and because most assays are not isoform-sensitive and thus do not accurately measure the MW of KIV, one cannot not know the real MW and any conversion of milligrams to moles is subject to error. There is evidence that measuring Lp(a)-C would be a better correlate with Lp(a)-P than Lp(a) mass. For this reason, some elevations of Lp(a) mass are associated with low levels of Lp(a)-C.

Trials have clearly shown that patients with the smaller apo(a) isoforms have higher cardiovascular risk, and that this is likely related to Lp(a) particle

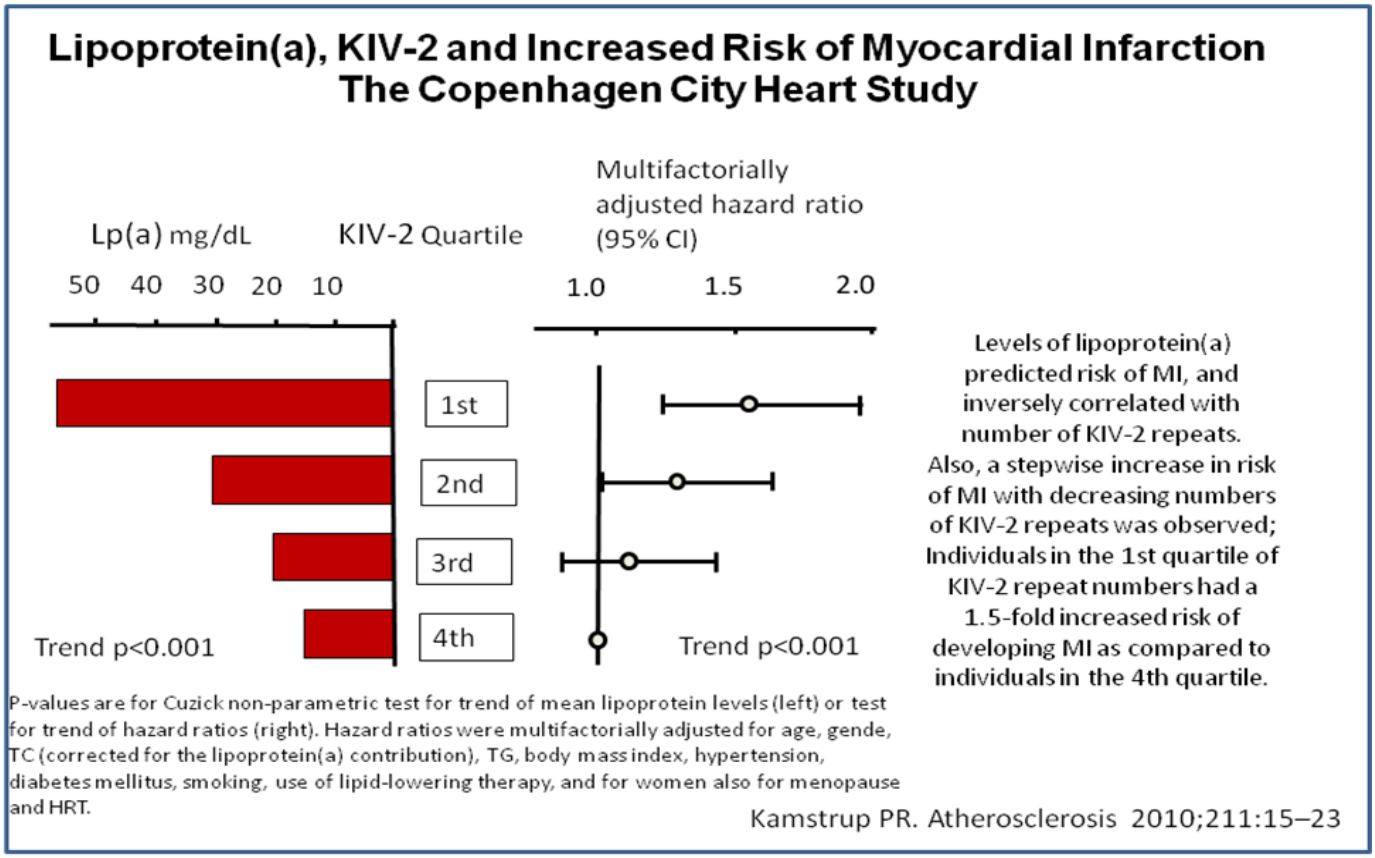


Figure 7. Mean lipoprotein(a) levels (mg/dL) and risk of myocardial infarction by quartiles of LPD kringle IV type 2 (KIV-2) repeats in the general population.

number expressed as Lp(a) apoB. Because the smaller, low MW isoforms are so readily secreted by the liver, they are associated with both high Lp(a) mass and high Lp(a)-C. One study suggests the risk of coronary atherosclerosis attributable to Lp(a) may be dependent on the concomitant elevation of LDL-cholesterol (LDL-C).[22]

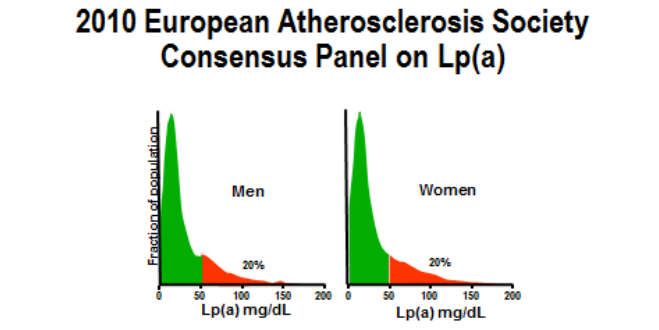


Figure 8. Typical distributions of lipoprotein(a) levels in the general population.

Lp(a) quantification can be performed by densitometric measurement of Lp(a)-C. Fig. 11 shows an actual result from such testing.

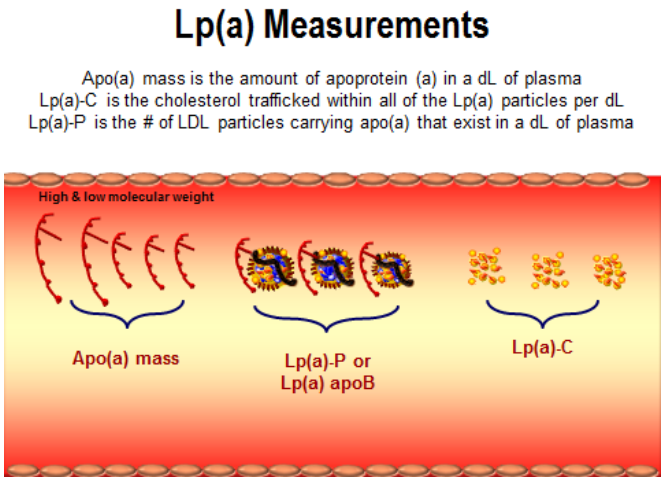


Figure 9.

As this method measures only the cholesterol contained in the Lp(a) particles and is thus not influenced by the relative size of the apo(a) molecule, it may provide a more specific assessment

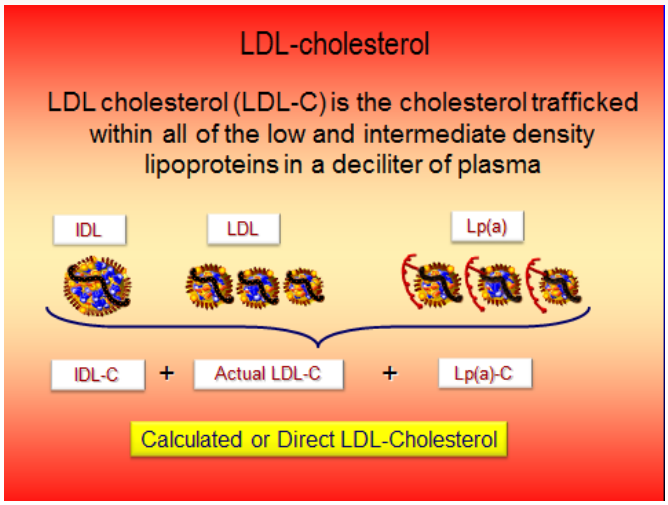


Figure 10.

of cardiovascular risk than Lp(a) mass measurement. Lp(a)-C measurement is best used in concert with Lp(a) mass determination, although it may be used as a stand-alone test for assessment of risk. Lp(a)-C is the cholesterol trafficked within all of the Lp(a) particles (regardless of particle size or apo(a) isoform) that exist in a dL of plasma. Lp(a)-C values should not be confused with Lp(a) mass values, although they are highly correlated. Lp(a)-C values will be approximately one tenth that of Lp(a) mass values, but the difference between the measures is not uniform. Lp(a) mass values are considered elevated when levels are >30 mg/dL.

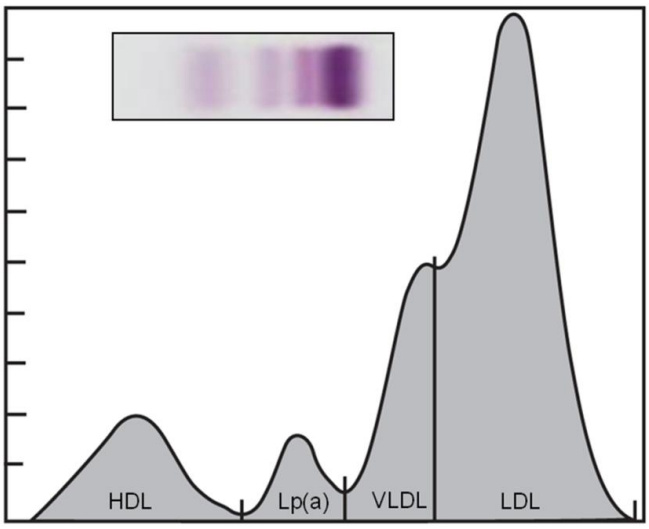


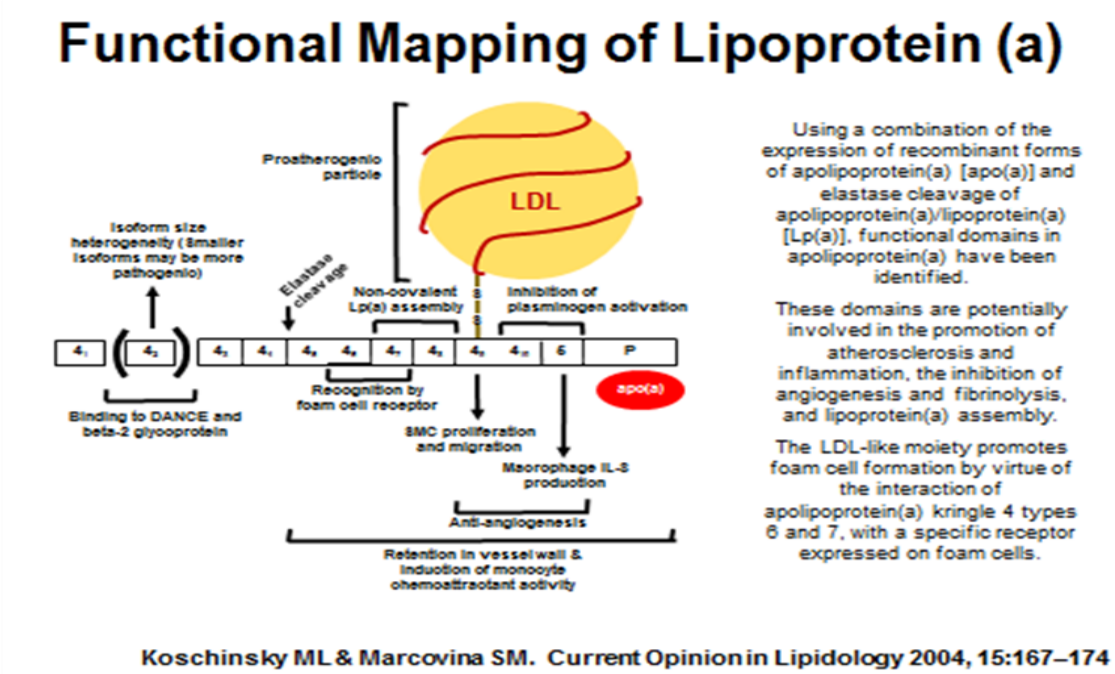
Figure 11. Densitometric measurement of Lp(a)-C.

Lp(a)-C is increased if ≥ 3 mg/dL. Since LDL-C = IDL-C + LDL-C + Lp(a)-C, Lp(a)-C is incorporated into LDL-C measurements or

calculations. Since most persons do not have any significant concentration of Lp(a), Lp(a)-C contributes minimally to LDL-C. The Mayo Clinic, in a four-year study of 504 coronary angiography patients, found that Lp(a)-C, but not Lp(a) mass, was an independent predictor of angiographic coronary artery disease (CAD) and subsequent cardiovascular events after multivariate analysis.[23] In addition to reflecting isoform size differences in apo(a), Lp(a) mass is also influenced by sequences in the apo(a) gene other than the kringle repeats, as well as to polygenic effects which vary between different ethnicities.[24]

The physiological function of Lp(a) or apo(a) is still unknown. Individuals without Lp(a) or with very low Lp(a) levels seem to be healthy. Thus plasma Lp(a) is certainly not vital, at least under normal environmental conditions. A role within the coagulation system seems plausible, given the high sequence homology between apo(a) and plasminogen.[2] Lp(a) particles accumulate in human atherosclerotic lesions, especially unstable plaques prone to rupture, but not in normal vasculature.[25] The actual mechanisms linking Lp(a) to atherogenesis and expression of cardiovascular disease end-points are still undefined. However, several plausible mechanisms have been proposed to explain the association between Lp(a) lipoprotein and initiation, progression and rupture of atherosclerotic plaque and vascular disease.[26,27] Plasminogen is the inactive form of plasmin, to which it is converted by activators such as tissue-type plasminogen activator (tPA) for subsequent fibrinolysis and breakdown of blood clots. Although structurally similar to plasminogen, the protease domain in apo(a) cannot be cleaved by plasminogen activators, rendering it catalytically inactive and potentially anti-fibrinolytic and thus thrombogenic.[28] The structural similarities between apo(a) and both plasminogen and tPA have led to the notion that apo(a) may compete with plasminogen for its binding site, leading to reduced fibrinolysis. This inhibition of plasminogen would promote proliferation of smooth-muscle cells, and as Lp(a) stimulates secretion of plasminogen activator inhibitor (PAI-1), would also lead to thrombogenesis. Yet kinetic data suggest that apo(a)/Lp(a) actually binds to the complex of plasminogen, enzyme tissue-type plasminogen activator and co-factor fibrin thereby reducing activation. Kringle V and protease domains of apo(a) as a unit can bind to fibrin. Lp(a) may also enhance coagulation by inhibiting the function of tissue factor pathway inhibitor.[29-31]

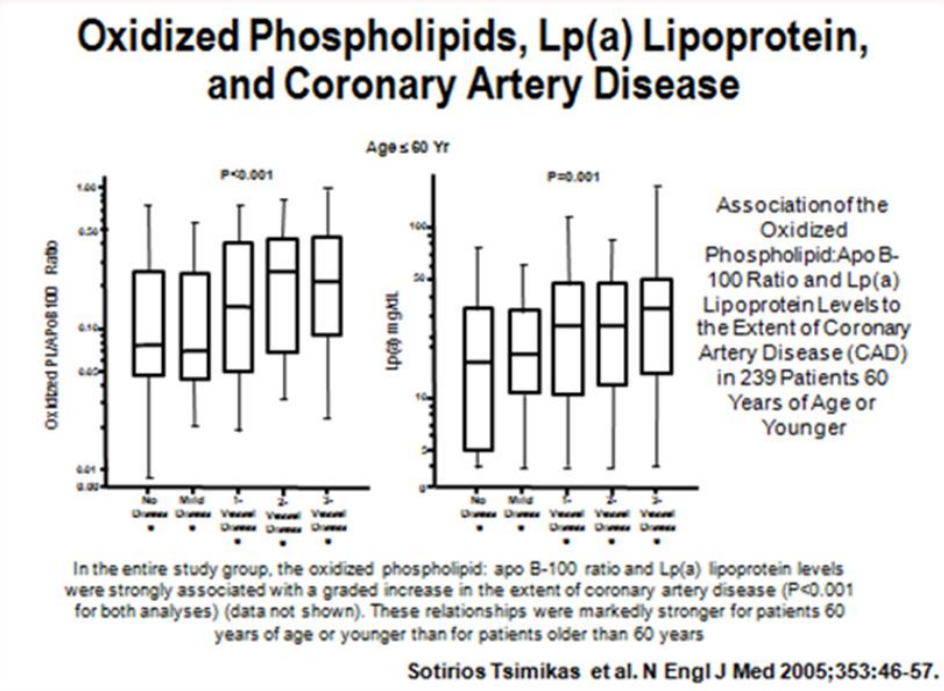
Lp(a) has also been associated with endothelial dysfunction.[32] The F2 fragment of apo(a)/Lp(a) enhances retention of Lp(a) in the intima and has sequences that upregulate the CC chemokine I-309



Koschinsky ML & Marcovina SM. Current Opinion in Lipidology 2004, 15:167–174

Figure 12

Figure 13



in endothelial cells, attenuate platelet aggregation and induce smooth muscle cell migration and proliferation. Kringle V may mediate IL-8 production. Lp(a) in atherosclerotic plaques recruits inflammatory cells through interaction with Mac-1 integrin.[33] Extensive structure-function studies have revealed important insights into the apo(a) domains that mediate Lp(a) assembly and the pathogenic effects of this lipoprotein (Fig.12).[27] Lp(a) activates monocytes, colocalizes with plaque macrophages, stimulates smooth-muscle cells, and induces inflammation.[34-38] Emerging data reveals apo(a) to be a scavenger and carrier of oxidized phospholipids, many of which are generated by the action of lipoprotein-associated phospholipase A_2 (Lp-PLA_2), elevating the relationship between apo(a) and arterial inflammation (Fig. 13). The relationship with oxidized phospholipids and Lp-PLA_2 activity is stronger in those with small (low MW) compared to larger (high MW) isoforms.[39]

CLINICAL INTERPRETATIONS

Numerous studies have established Lp(a) concentrations as an independent risk factor for cardiovascular events, including peripheral vascular disease, cerebrovascular disease, premature coronary disease, myocardial infarction, vein graft restenosis, and retinal arterial occlusion.[11,40-47] High Lp(a) predicts risk of early atherosclerosis, similar to high LDL-C, although in advanced atherosclerosis, Lp(a) is an independent risk factor. One large study of older Americans, in particular, demonstrated that elevated levels of Lp(a) lipoprotein independently predict an increased risk of stroke, death from vascular disease, and death from all causes in men.[48] There may be a relationship between Lp(a) mass and LDL-C, as some epidemiologic studies have suggested the risk of Lp(a) is worse or even limited to those with increased LDL-C [Fig. 14(a)].[47] Other trials show Lp(a) greatly increases cardiovascular event risk in patients with elevated LDL-C or low HDL-C.[49,50] Baseline Lp(a) as an independent risk factor for combined cardiovascular events is reportedly higher in women (although one large study showed that to be true only in women with very high apo(a) mass plus high LDL-C) [Fig. 14(b)][51] and in patients with a history of peripheral vascular disease. In the face of the epidemiological data, a 2011 European consensus statement declared, "Elevated Lp(a) levels associate robustly and specifically with increased cardiovascular disease (CVD) risk. The association is continuous in shape without a threshold and does not depend on high levels of LDL-C or non-HDL-cholesterol, or on the levels or presence of other cardiovascular risk factors." [52] Recent findings in the EPIC-Norfolk Study also report an association of Lp(a) risk and both coronary and peripheral artery disease that was not modified by LDL-C levels.[53]

Guidelines for cardiovascular risk screening have always suggested that elevated levels of Lp(a) are a risk marker for coronary heart disease in Caucasian populations, but not among African Americans. New data from the Atherosclerosis Risk in Communities (ARIC) Study evaluated Lp(a) and subsequent vascular risk in a biracial cohort that included 3,467 African Americans and 9,851 Caucasians. Lp(a) concentrations were significantly higher among the African American group as compared to the Caucasian group, and in both

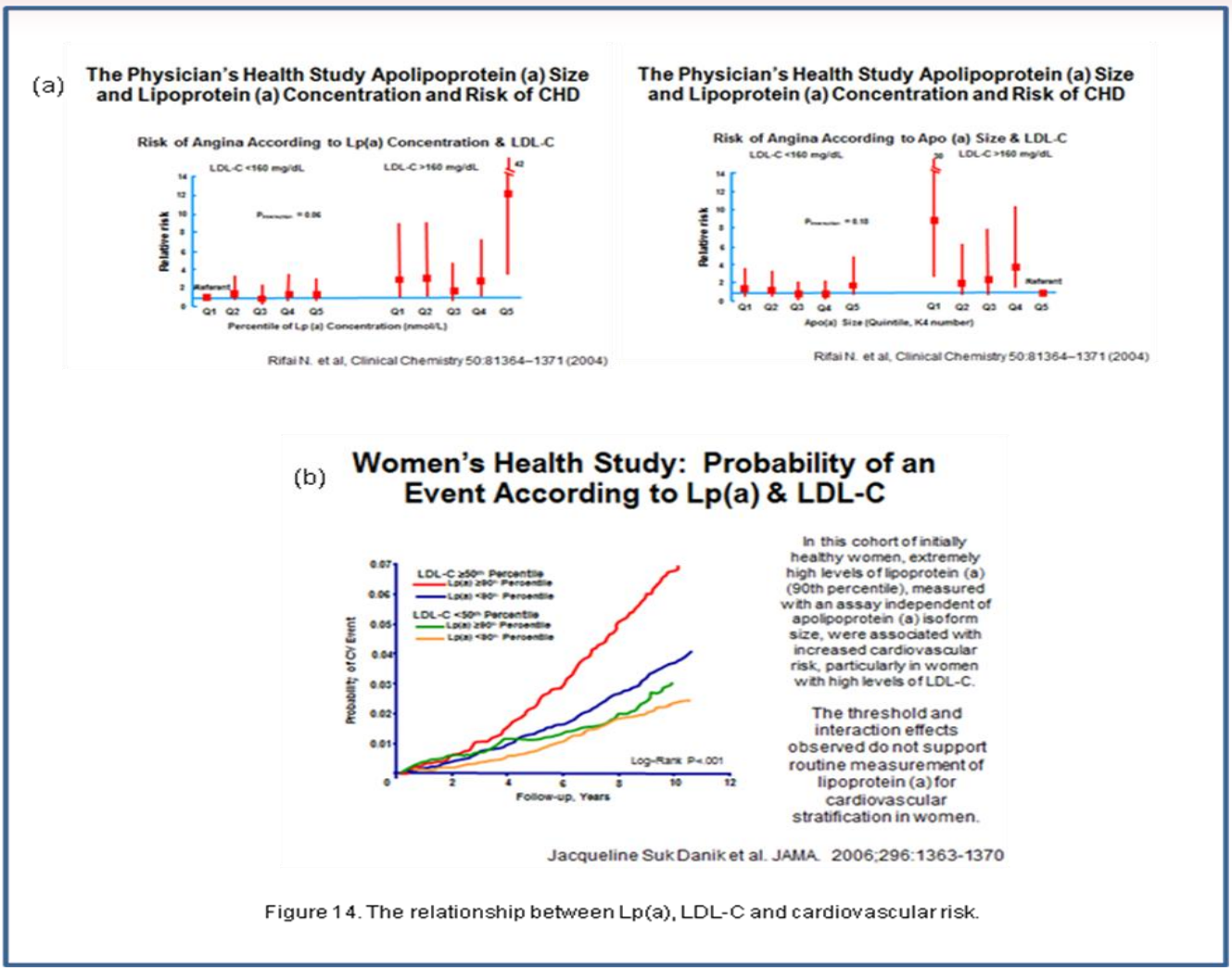
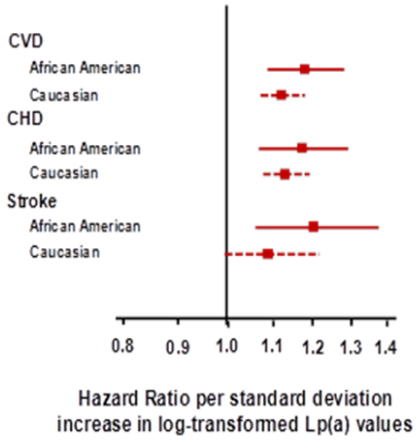


Figure 14. The relationship between Lp(a), LDL-C and cardiovascular risk.

populations, increasing Lp(a) levels tended to correlate positively with LDL-C and negatively with triglycerides. However, refuting earlier work, increasing quintiles of Lp(a) in this updated analysis were just as predictive of future CVD in the African American population as in the Caucasian population.[54]

Figure 15

Lp(a), ethnicity, and cardiovascular risk.



Lp(a) levels in ARIC were significantly higher among the black group than the white group, and in both populations, increasing Lp(a) levels tended to correlate positively with low-density lipoprotein cholesterol and negatively with triglycerides.

Increasing quintiles of Lp(a) in this updated analysis were just as predictive of future cardiovascular disease in the black population as in the white population.

Ridker PM. Circulation 2012;125(2):207-9.

TREATMENT CONSIDERATIONS

There are to date no level one evidence trials which demonstrate that lowering Lp(a) level will reduce cardiovascular benefit. One difficulty in investigating this issue is that currently available drugs that may lower Lp(a) also lower apoB, and/or beneficially modulate other lipid/lipoprotein fractions, making it impossible to predict which lipoprotein changes underlie the improvement in cardiovascular risk. Reduction in Lp(a) by hormone replacement therapy in one study (a rather weak, post-hoc analysis) or plasma apheresis has been shown to reduce cardiovascular events in post-menopausal women and limit restenosis following

angioplasty.[55,56] Niacin is the most potent Lp(a) lowering drug available (30 - 40 % in a dose dependent fashion) but there is no outcome data yet for patients thus treated: Niacin's benefit might be related to its ability to reduce apoB or modulate other lipid concentrations.[43] Statins, by upregulating LDL receptors (LDLr) enhance clearance of apoB, especially LDL particles.[57,58] Apo(a) camouflages the LDL receptor binding domain on apoB and thus LDLr cannot readily clear Lp(a) particles. Yet in persons with elevations of Lp(a) not all LDL particles have apo(a) attached and statins are able to induce clearance of non-apo(a) containing LDLs. Thus drugs that upregulate LDLr like statins, ezetimibe, and bile acid sequestrants (BAS) might be beneficial in reducing CV risk in persons with elevated Lp(a) because there would be apoB and LDL-P reductions despite no reduction in Lp(a) mass.

Table 1.

2010 European Atherosclerosis Society Consensus Panel on Lp(a)

Desirable levels for LDL-C and Lp(a) in the fasting or non-fasting state

	Patients with CVD and/or diabetes	Other patients and individuals	Highest level of evidence for treatment
LDL-C	< 2 mmol/L (<77 mg/dL)[a]	< 3 mmol/L (<116 mg/dL)[a]	Meta-analysis of RCT of statins
Lp(a)	< 80th percentile (<~50 mg/dL)[b]	< 80th percentile (<~50 mg/dL)[b]	Meta-analysis of RCT of niacin Rx [c]

[a] According to 2007 European guidelines

[b] The 80th percentile roughly corresponds to 50 mg/dL in Caucasians

[c] The evidence is for the effect of niacin treatment, not specifically for Lp(a) lowering

Nordestgaard BG et al. Eur Heart J. 2010 Dec;31(23):2844-53

Knowledge that Lp(a) mass or Lp(a)-C is elevated affords the physician a more accurate risk assessment and to perhaps set more aggressive LDL goals.[56] One might, after statin therapy, which does not reduce Lp(a) mass, select a therapy that, in addition to other effects, may reduce Lp(a) and hopefully, cardiovascular events. In terms of medications, statin or statin/ezetimibe or statin/(BAS) therapy may not decrease Lp(a) levels, but by lowering apoB, LDL-P and LDL-C may modify the cardiovascular risk associated with Lp(a). Niacin, although difficult to tolerate in the high doses needed to decrease Lp(a), does decrease Lp(a) levels and would provide additional apoB reduction while increasing HDL-C (effects of which are unknown). Despite the lack of outcome data supporting its use in persons with elevated Lp(a) mass, 2011 European Guidelines recommend the addition of niacin to statins when treating cardiovascular risk related to elevated Lp(a) (Table 1).[52]

Plasma concentrations of Lp(a) can increase in post-menopausal women, and estrogen replacement therapy will reduce them. However, data concerning the relationship between hormone therapy (HT) and Lp(a) levels are conflicting and controversial, in part because HT use in women with CVD is itself controversial and because studies that show decreases in Lp(a) levels with HT use have rarely been powered to assess the impact on CVD.[59-61] Such use should be limited to lipidologists well-versed in women's health and HT. There is no data on the use of estrogen in premenopausal women with Lp(a) issues. Brown et al. (2010) published a state of the art discussion on the nuances of treating patients with high Lp(a).[62]

ANALYTICAL APPROACHES

Apo(a) peptides show significant size heterogeneity as a result of multiple molecular isoforms, which presents a challenge for standardization of the measurement of Lp(a) in plasma. Assays insensitive to isoform size (e.g., "equimolar") are not yet widely available and thus most assays cannot directly measure molar concentrations (particle numbers) of Lp(a). Most labs reporting Lp(a) in molar concentration are simply reporting "guestimates" obtained by mathematically converting milligrams to moles using an average, not the exact apo(a) MW unique to a given patient.

Lp(a) quantification can be performed by serum electrophoresis, followed by staining and

densitometric measurement of Lp(a)-C. As this method measures only the cholesterol contained in the Lp(a) particles and is thus not influenced by the relative size of the apo(a) molecule, it provides a more specific assessment of cardiovascular risk than Lp(a) mass measurement.[23] For practicality, Lp(a)-C measurement is best used in concert with Lp(a) mass determination, although it may be used as a stand-alone test for assessment of risk. Lp(a) mass values are considered elevated when levels are >30 mg/dL. Lp(a)-C is increased if ≥ 3 mg/dL. Since LDL-C = IDL-C + LDL-C + Lp(a)-C, Lp(a)-C is incorporated into LDL-C measurements or calculations. Because most people do not have any relatively significant concentrations of Lp(a), Lp(a)-C contributes minimally to LDL-C.

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Treatment case study from the Lipid Clinic Letter: Statins and the risk of developing new-onset diabetes mellitus.

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Case: 58 y/o female with dyslipidemia – LDL 162mg/dL, Trig 168mg/dL, HDL 43mg/dL – BMI 31 Kg/m2, fasting blood sugar 111mg/dL, and family history of early heart disease. Her MD wants to put her on a statin, but she refuses because she has heard that statins cause diabetes.

With more and more studies being published on the subject, there seems little doubt that there is a definite increase in new-onset diabetes in patients placed on statins. This is not the first time that a drug has been implicated in causing diabetes, as evidenced by the experience with thiazide diuretics, beta blockers, and niacin. All drugs have side effects. The key is to evaluate the benefits of the medication and weigh them against the risks.

For statins, the benefits have been shown in multiple trials with more than 500,000 patient-years of treatment (Lancet 2010;375:742 Editorial). This benefit has lead to their inclusion in all national guidelines as a key component of both primary and secondary prevention. A recent meta-analysis of 134,537 patients showed significant reduction in CV events with statins even in patients at low risk for vascular disease (Lancet 2012; published online 5/17/12).

Let's examine some of the recent data about the risk of developing diabetes with statins and see how we can apply this to our practice.

Incidence: There have been two large **meta-analyses looking at the incidence** of developing diabetes on statins. (For the most part, the diagnosis of diabetes in these trials was made by having 2 fasting blood glucose measurements of ≥ 126mg/dL.)

1. Mills et al (QJM 2011;104:109) – looked at 17 randomized controlled trials and found the increase in risk of developing new-onset diabetes with statins was 9% (Odds Ratio 1.09, CI 1.02-1.116).

2. Sattar et al (Lancet 2010;375:735) looking at 13 randomized trials (91,140 pts) found a similar increased risk of 9% (OR 1.09, CI 1.02-1.117). This equated to one new case of diabetes per 250 patients treated for 4 years. This finding appeared to be a class effect applying to both lipophilic and hydrophilic statins. Dr. Cannon, in an accompanying editorial, states "the risk seemed to be present mostly in older patients, with no risk seen in younger patients (age<60 y/o)".

Looking at the trials included in this meta-analysis, the actual incidence of developing diabetes on statins was very low with an **absolute risk of 0.3-0.6%**, except in two studies with older individuals where it was 1.0-1.5%. The trials with the lowest occurrence of diabetes were primary prevention trials with low diabetes risk (AFCAPSTexCAPS and WOSCOPS).

Using data from the Cholesterol Treatment Trialists' Collaborators (CTT) meta-analysis of statin trials in 71,370 non-diabetic participants, Sattar et al calculated that for every 255 patients treated for 4 years, there would be one new case of diabetes, but 5.4 coronary heart disease deaths or MIs would be avoided, and nearly as many strokes and revascularizations. In other words, there was about a **9:1 ratio of benefit (reduction in CV events) to risk (development of diabetes). Their conclusion** was that "the small excess risk of incident diabetes is favorably balanced by cardiovascular benefit, implying that clinical decision making should not be changed for patients in whom statin therapy is recommended – i.e., people with existing CV disease or at medium-to-high risk of such disorders"…"however, the potentially raised diabetes risk should be taken into account if statin therapy is considered for patients with low CV risk or patient groups in which cardiovascular benefit has not been proven."

Mechanism: The mechanism of statin affect on glucose metabolism is unclear. In one study of 250 patients using 10, 20, 40, and 80 mg doses of atorvastatin followed over 2 months, there was an increase in insulin levels, reduction in insulin sensitivity, no change in fasting glucose, and a small increase in HgAIC (0.2-0.3% rise) (JACC 2010;55:1209). 50% of these patients had metabolic syndrome with an average HgAIC at baseline of 5.9-6.4% going along with the suggestion from other studies that the development of new-onset diabetes is more common in patients who are already pre-diabetic or have metabolic syndrome.

It should be noted that the 10mg dose of atorvastatin had a trend toward increased insulin levels and reduced insulin sensitivity but it was not significant compared to placebo. This raises the possibility of a dose related effect.

Predictors of new-onset diabetes in patients on statins: A meta-analysis looking at three large trials of patients on atorvastatin (TNT, IDEAL, SPARCL) with approximately 18,000 non-diabetic patients found **4 independent predictors of risk**: fasting blood sugar >100, Triglyceride >150, BMI >30, and history of hypertension (JACC 2011;57:1535). The strongest single predictor was an elevated fasting blood sugar at baseline. Those with 0-1 risk factor were at the lowest risk for developing diabetes (1-3%) while those with 3-4 risk factors were at the highest (8-25%) and this was further increased with more aggressive statin use.

If one develops statin induced diabetes, is there an associated increased CV risk such as one would expect with diabetes? This is a key question. The biggest concern would be an increase in microvascular disease since that is the target of hyperglycemia in the diabetic. For now, there is no data regarding that risk. As for an increase in macrovascular disease, the combined TNT, IDEAL, SPARCL meta-analysis found that the incidence of CV events over 5 years in patients without new-onset diabetes was 10.8%, for patients with new-onset diabetes 11.3% (not significant), and for patients with prior diabetes 17.5% (JACC 2011;57:1535).

Their conclusion: Although these results do not exclude an increased risk "which might become apparent after longer follow-up, our results suggest that the risk accompanying statin-associated diabetes might not be equivalent to the usual risk of diabetes. Patients who developed thiazide-induced new-onset diabetes in ALLHAT were also not at increased risk of a CV event" (Arch Int Med 2009;169:832).

Effect of statins on fasting glucose in the diabetic and non-diabetic patient:
Reviewing the records of 345,417 Veterans, the effect of statins on blood sugar over a two year time span was determined (J Invest Med 2009;57:495). In the non-diabetic, blood sugar rose 7mg/dL on a statin and 5mg/dL not on a statin ($p<0.0001$). In the diabetic, blood sugar rose 39mg/dL on a statin and 32mg/dL not on a statin ($p<0.0001$). Although these changes were significant in this large group, the absolute changes were very small. It would appear that one doesn't develop significant blood sugar rises in the non-diabetic with a statin, or a marked deterioration in blood sugar levels in the diabetic with a statin.

Is there a dose related effect regarding statin induced diabetes? A meta-analysis looking at 5 randomized trials comparing intensive-dose statin versus moderate-dose statin suggested an increased risk of new-onset diabetes with the intensive dose versus the moderate dose. For every 500 patients treated for 1 year, there was one new case of diabetes, but 3.2 fewer first cardiovascular events. This actually underestimates the benefit since these studies only looked at first events. It is now know that intensive statin therapy reduces multiple CV events if intensive statin therapy is continued, so the reduction in event rates would be even higher than 3.2 (JACC 2009;54:2358, AJC 2010;105:283). These benefits were seen equally in patients with recent acute coronary syndrome or patients with stable coronary disease.

Should you start a statin in patients with pre-diabetes or newly diagnosed diabetes mellitus? Dr. Naveed Sattar (at the European Association for the Study of Diabetes 2011

Meeting) said, "At the point of diagnosis, many physicians are considering diabetes as a CVD risk equivalent, which is part of the case for commencing statins, with the presumption that once a patient has moved to the threshold of diagnosis, that is the same as if they had already had a heart attack. But clearly a number of individuals who are at the threshold of diabetes diagnosis are at very low vascular risk. And if we start a statin, we will gain little benefit and in fact, we may push the individual over and above the threshold for diabetes".

The ADA recommends starting a statin in diabetics >40 y/o regardless of their LDL-C level because studies suggest that diabetes has the same risk of developing an MI as does a patient without diabetes who has already had an MI (NEJM 1998;339:229). It is likely, though, that all of these newly diagnosed diabetics don't have the same vascular risk, but rather there is a continuum with some at low risk and others at high risk.

So how can you figure in the newly diagnosed diabetic who might be at low risk versus high risk? Clearly if the patient is a high risk, they should be on a statin despite concerns about statin effects on glucose metabolism. Here are two ways that may help clarify this:

1. Presence of metabolic syndrome (or elevated triglycerides). One study looked at the prevalence of CHD in patients with or without the metabolic syndrome and with or without diabetes (Diabetes 2003;52:1210). The highest risk group was the diabetic with the metabolic syndrome (average Trig 232) with a 19.2% prevalence of CHD. Diabetics without the metabolic syndrome (average Trig 119) had a prevalence of only 7.5% which was the same as patients who had no diabetes and no metabolic syndrome (average Trig 119). Those patients with no diabetes but with the metabolic syndrome (average Trig 212) had a prevalence of 13.9%. Therefore the presence of metabolic syndrome (high Trig) in patients with diabetes marks a group at high risk. In fact, just having the metabolic syndrome marks a group at increased vascular risk. On the other hand, the absence of metabolic syndrome in the diabetic puts them in a lower risk category.

2. Coronary calcium score. The other way to risk profile the diabetic patient is to do a coronary calcium score (CCS). The recent AHA/ACC guide to the evaluation of the asymptomatic adult suggested that doing a CCS in diabetics >40y/o was a level IIA recommendation (reasonable to consider). This would clarify the patient's actual risk, and help determine whether or not they should be on a statin.

Bottom Line Summary:

1. There is a definite increased risk of developing new-onset diabetes with the use of a statin. The absolute risk, though, is small, only about 0.3-0.6%. It increases with increasing risk factors for developing statin-associated diabetes.

2. The risk factors for developing statin-associated diabetes are: fasting blood sugar>100, Triglycerides >150, BMI>30, history of hypertension, and age. The more of these risk factors that are present, the more likely the individual will develop diabetes. The best single risk predictor is an elevated fasting blood sugar.

3. The mechanism is unclear. It is a class affect, involving both lipophilic and hydrophilic statins.

4. The benefit to risk ratio greatly favors the use of statins. For every one new case of statin induced diabetes, there are approximately 9 less cardiovascular events, including non-fatal MI, coronary heart disease death, non-fatal stroke, and revascularization.

5. There is an association with increasing statin dose and increasing risk of new-onset diabetes. Once again benefit appears to outweigh risk. For every 500 patients treated for one year with intense dose statin, there is one new case of diabetes but at least 3.2 fewer cardiovascular events.

6. Not clear if this new-onset diabetes actually increases CV risk. One meta-analysis suggests minimal to no increased risk.

7. In general, the feeling is that the benefits of statin therapy far outweigh the risks in patients were statin therapy is indicated. This risk, though, should be taken into account when considering statin therapy in patients with low cardiovascular risk or in groups were cardiovascular benefit has not been proven.

Possible Ways to Reduce Likelihood of Statin Induced Diabetes:

A. Mediterranean Diet
There have been numerous studies showing that the Mediterranean diet can reduce fasting blood sugar and improve insulin resistance.

1. (Ann Int Med 2006;145:1) 772 patients with high CV risk. The group placed on Mediterranean diet had a 15mg/dL reduction in fasting glucose, and a reduction in insulin resistance.

2. (JACC 2006;48:677) The Mediterranean diet resulted in reduction in insulin resistance. (This is a good summary article of the benefits of the Mediterranean diet).

3. **(JAMA 2002;287:598)** This study looked at 120 untreated hypercholesterolemic males. Half were put on a standard low fat diet while the other half was put on a Mediterranean diet. Each subgroup was then randomized to placebo or simvastatin 20mg qd. The Mediterranean diet with placebo dropped insulin levels by 14% (p=0.02), while simvastatin with the standard diet raised it 13% (p=0.005). When simvastatin was added to a Mediterranean diet, there was no change in insulin levels.

The typical patient that develops statin-associated diabetes is not your healthy, no risk factor patient. The vast majority, if not all, are insulin resistant to start with, having elevated fasting blood sugars, or the high triglyceride-low HDL insulin resistance lipid profile, or multiple characteristics of the metabolic syndrome. The Mediterranean diet has been shown in this type of patient to improve insulin resistance, drop fasting blood sugar, lower triglycerides, raise HDL, and reduce markers of inflammation.

Therefore, **in patients at risk for developing statin induced diabetes**, it seems reasonable to put them on a **Mediterranean diet** to hopefully block the development of diabetes (as suggested by the JAMA study above), or at least blunt these statin induced metabolic effects.

B. Bile Acid Sequestrants and Statin-Induced Diabetes
Another mechanism to try and reduce or counteract statin-associated diabetes is to pair the statin with a bile acid sequestrant (BAS). The BAS have been shown to reproducibly lower HgAIC by 0.5% and so may neuter the diabetogenic effects of the statin (J Clin Lipid 2009;2:529).

The Absolute Bottom Line:
Realize that if one has a fasting blood sugar of 124, they are not considered diabetic, yet if it rises to 126 then they are classified as diabetic. Statins can cause an elevation in blood sugar above this diagnostic threshold in patients with insulin resistance, pre-diabetes, or metabolic syndrome. Statins appears unlikely to do this in patients who don't a predisposition for diabetes (i.e., insulin resistance).

Those that develop statin-associated diabetes do not suddenly become an insulin dependent diabetic, but rather just have a mild increase in blood sugar. The average rise in HgAIC in one study was 0.2-0.3%. The individuals in that study started with HgAICs of 5.9-6.4, again demonstrating that the people who develop statin induced diabetes are already pre-diabetic.
In considering whether to start a statin in someone who you suspect has insulin resistance, the patient's risk for CV disease needs to be considered. If they have vascular disease or moderate-to-high risk for vascular disease, the benefit to risk ratio is 9:1 so a statin should be started. Even though high intensity statin increases the risk of statin induced diabetes, the benefit to risk ratio is at least 3.2:1 (and probably higher) so intensive statin is indicated.

The real question is whether to start a statin in a patient with low vascular risk who has risk factors for developing statin induced diabetes,

such as elevated fasting blood sugar, elevated triglycerides, or BMI>30. In this population, the benefit versus risk ratio needs to be worked out. The presence of metabolic syndrome or a positive coronary calcium score can help clarify this risk, and make the use of statins appropriate.

Several ways to minimize the risk of statin induced diabetes are:
1. Put the patient on the Mediterranean diet.
2. Keep the statin dose low since this appears to be a dose related effect.
3. If add-on therapy is needed, consider a bile acid sequestrant.

Case: This patient is at risk for developing statin induced diabetes since she has multiple risk factors for it, specifically BMI>30, Trig >150, FBS >100, yet she also appears to be at high vascular risk with her dyslipidemia and family history. From a Framingham viewpoint she has ≥2 risk factors (age, low HDL, family history of early disease) so the recommendation would be LDL-C<130, optional <100. If knowing that, she still refuses a statin, then a coronary calcium score might be helpful. If it is zero, you could work on her therapeutic life style changes and repeat the score in 3-4 years. If it is positive, this might convince her to take statins. If she still refuses, then ezetimibe, fibrates, or bile acid sequestrants could be used in various combinations to try and get her to goal.

By Gyorgy Csako, M.D.

News About Lipoprotein(a):

Title: Genetic associations with valvular calcification and aortic stenosis.
Authors: Thanassoulis G, Campbell CY, Owens DS, Smith JG, Smith AV, Peloso GM, Kerr KF, Pechlivanis S, Budoff MJ, Harris TB, Malhotra R, O'Brien KD, Kamstrup PR, Nordestgaard BG, Tybjaerg-Hansen A, Allison MA, Aspelund T, Criqui MH, Heckbert SR, Hwang SJ, Liu Y, Sjogren M, van der Pals J, Kälsch H, Mühleisen TW, Nöthen MM, Cupples LA, Caslake M, Di Angelantonio E, Danesh J, Rotter JI, Sigurdsson S, Wong Q, Erbel R, Kathiresan S, Melander O, Gudnason V, O'Donnell CJ, Post WS; CHARGE Extracoronary Calcium Working Group.
Journal: N Engl J Med. 2013 Feb 7;368(6):503-12
Comment: Aortic stenosis is the third most prevalent form of cardiovascular disease (CVD) in the Western world, after hypertension and coronary artery disease (CAD), and is caused by calcification and hardening of the aortic valve, impeding blood flow from the heart to the rest of the body, leading to chest pain, loss of consciousness, and shortness of breath. In severe cases, patients require aortic-valve replacement. Currently, there are no medical treatments to prevent this disease, which mainly affects people over the age of 60. The authors of this international multi-center study determined genomewide associations with the presence of aortic-valve calcification (among 6942 participants) and mitral annular calcification (among 3795 participants), as detected by computed tomographic (CT) scanning. The study population for this analysis initially included persons of white European ancestry from three cohorts participating in the Cohorts for Heart and Aging Research in Genomic Epidemiology consortium (discovery population). Findings were replicated in independent cohorts of persons with either CT-detected valvular calcification or clinical aortic stenosis. The authors first looked at 2.5 million single-nucleotide polymorphisms (SNPs) among more than 6900 people of white European descent. One SNP in the lipoprotein(a) (LPA) locus (rs10455872) reached genomewide significance for the presence of aortic-valve calcification (odds ratio per allele, 2.05; $P=9.0\times10(-10)$), a finding that was replicated in additional cohorts of 700 white Europeans (German), about 2,500 African-Americans, and 2,000 Hispanic-Americans ($P<0.05$ for all comparisons). Genetically determined Lp(a) levels, as predicted by LPA genotype, were also associated with aortic-valve calcification, supporting a causal role for Lp(a). In prospective analyses, LPA genotype was associated with incident aortic stenosis (hazard ratio per allele, 1.68; 95% confidence interval [CI], 1.32 to 2.15) and aortic-valve replacement (hazard ratio, 1.54; 95% CI, 1.05 to 2.27) in a large Swedish cohort; the association with incident aortic stenosis was also replicated in an independent Danish cohort. Two SNPs (rs17659543 and rs13415097) near the proinflammatory gene IL1F9 achieved genomewide significance for mitral annular calcification ($P=1.5\times10(-8)$ and $P=1.8\times10(-8)$, respectively), but the findings were not replicated consistently. In summary, genetic variation in the LPA locus, mediated by Lp(a) levels, is associated with aortic-valve calcification across multiple ethnic groups and with incident clinical aortic stenosis. The prevalence of the SNP is about 13% in the white population and slightly less in other ethnic groups. Subjects carrying this SNP had a doubling of the risk of valve calcification on computer tomography (CT) compared with those without the variation. These results provide the first evidence for a causal relationship between Lp(a) and calcific aortic-valve disease. Although increased levels of Lp(a) have previously been associated with aortic-valve disease, prior observational studies couldn't prove that Lp(a) was a contributing factor rather than just a marker.

Title: Shared genetic risk for sclerosis of valves and vessels. [Editorial]
Authors: Dorn GW**.**
Journal: N Engl J Med 2013; 368: 569-570.
Comment: In the accompanying editorial to the above report, Dorn pointed out that the same SNP has previously been identified as a risk factor for increased Lp(a) levels and coronary artery disease (CAD). Thus the cumulative findings support the proposition that a common genetic defect in lipid metabolism underlies the pathogenesis of both atherosclerosis and aortic stenosis.

Title: Genome-wide association study of genetic determinants of LDL-c response to atorvastatin therapy: importance of Lp(a).
Authors: Deshmukh HA, Colhoun HM, Johnson T, McKeigue PM, Betteridge DJ, Durrington PN, Fuller JH, Livingstone S, Charlton-Menys V, Neil A, Poulter N, Sever P, Shields DC, Stanton AV, Chatterjee A, Hyde C, Calle RA, Demicco DA, Trompet S, Postmus I, Ford I, Jukema JW, Caulfield M, Hitman GA; on behalf of the CARDS, ASCOT, and PROSPER investigators.

Journal: J Lipid Res. 2012 May;53(5):1000-1011. [Epub 2012 Feb 24]
Comment: Statin therapy is now widely accepted for the primary and secondary prevention of cardiovascular disease (CVD) in certain patient groups. However, there is considerable variation in response to statin therapy that remains poorly understood. Two genome-wide association studies (GWAS) of statin response and several candidate gene association studies have been reported previously. From these, the only consistent finding is that variants in the *APOE* gene region are associated with variation in LDL response. The authors of the present study carried out a genome-wide association study (GWAS) of low-density lipoprotein cholesterol (LDL-C) response to statin using data from participants in the Collaborative Atorvastatin Diabetes Study (CARDS; n = 1,156), the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT; n =895), and the observational phase of ASCOT (n = 651), all of whom were prescribed atorvastatin 10 mg, to investigate genetic effects on LDL-C response to atorvastatin. Following genome-wide imputation, they combined data from the 3 studies in a meta-analysis. They found associations of LDL-C response to atorvastatin that reached genome-wide significance at rs10455872 ($P = 6.13 \times 10(-9)$) within the LPA gene and at two single nucleotide polymorphisms (SNP) within the APOE region (rs445925; $P = 2.22 \times 10(-16)$ and rs4420638; $P = 1.01 \times 10(-11)$) that are proxies for the ε2 and ε4 variants, respectively, in APOE. The novel association with the LPA SNP was replicated in the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) trial ($P = 0.009$). Using CARDS data, they further showed that atorvastatin therapy did not alter lipoprotein(a) [Lp(a)] and that Lp(a) levels accounted for all of the associations of SNPs in the LPA gene and the apparent LDL-C response levels. However, statin therapy had a similar effect in reducing cardiovascular disease (CVD) in patients in the top quartile for serum Lp(a) levels (HR = 0.60) compared with those in the lower three quartiles (HR = 0.66; $P = 0.8$ for interaction). The data emphasize that high Lp(a) levels affect the measurement of LDL-C and the clinical estimation of LDL-C response. Therefore, an apparently lower LDL-C response to statin therapy may indicate a need for measurement of Lp(a). However, statin therapy seems beneficial even in those with high Lp(a).

Title: Genetic evidence that lipoprotein(a) associates with atherosclerotic stenosis rather than venous thrombosis.
Authors: Kamstrup PR, Tybjærg-Hansen A, Nordestgaard BG.
Journal: Arterioscler Thromb Vasc Biol. 2012 Jul;32(7):1732-41. [Epub 2012 Apr 19]
Comment: The aim of this study was to determine whether lipoprotein(a) [Lp(a)], considered a causal risk factor for cardiovascular disease, primarily promotes thrombosis or atherosclerosis. Using a Mendelian randomization study design, the authors measured plasma Lp(a) and genetically elevated Lp(a) levels through the LPA kringle IV type 2 repeat genotype in 41 231 individuals. They included 2 general population studies of both venous thrombosis and combined thrombosis and atherosclerosis in coronary arteries (=myocardial infarction), and 3 case-control studies of atherosclerotic stenosis. Neither Lp(a) tertiles nor LPA kringle IV type 2 tertiles associated with the risk of venous thrombosis in general population studies (trend: P=0.12-0.76), but did each associate with risk of coronary, carotid, and femoral atherosclerotic stenosis in case-control studies (trend: P<0.001 to 0.04). Lp(a) and LPA kringle IV type 2 tertiles also associated with the risk of myocardial infarction in general population studies (trend: P<0.001 to 0.003). For doubling of Lp(a) levels, instrumental variable estimates of hazard/odds ratios were 1.02 (95% CI 0.90-1.15) and 1.04 (0.93-1.16) for venous thrombosis in the 2 general population studies, 1.12 (1.01-1.25), 1.17 (1.05-1.32), and 1.16 (1.01-1.35), respectively, for coronary, carotid, and femoral atherosclerotic stenosis in case-control studies, and 1.21 (1.10-1.33) and 1.17 (1.05-1.29) for myocardial infarction in general population studies. Findings of this study support that Lp(a) primarily promotes atherosclerotic stenosis rather than venous thrombosis.

Title: Low lipoprotein(a) concentration is associated with cancer and all-cause deaths: a population-based cohort study (The JMS Cohort Study).
Authors: Sawabe M, Tanaka N, Mieno MN, Ishikawa S, Kayaba K, Nakahara K, Matsushita S; J. M. S. Cohort Study Group.
Journal: PLoS One. 2012;7(4):e31954. [Epub 2012 Apr].
Comment: Experimental studies support the anti-neoplastic effect of apo(a), but several clinical studies have reported contradictory results. The purpose of this study was to determine whether a low lipoprotein(a) [Lp(a)] concentration is related to mortality from major causes of death, especially cancer. The subjects were 10,413 participants (4,005 men and 6,408 women) from a multi-center population-based cohort study in Japan (The Jichi Medical School cohort study). The average age at

registration was 55.0 years, and the median observation period was 4,559 days. As the estimated hazard ratio was high for both the low and very high Lp(a) levels, we defined two Lp(a) groups: a low Lp(a) group [Lp(a)<80 mg/L] and an intermediate-to-high Lp(a) group [Lp(a)≥80 mg/L]. Participants who died from malignant neoplasms (n=316), cardiovascular disease (202), or other causes (312) during the observation period were examined.
Cumulative incidence plots showed higher cumulative death rates for the low Lp(a) group than for the intermediate-to-high Lp(a) group for all-cause, cancer, and miscellaneous-cause deaths ($p<0.001$, $p=0.03$, and $p=0.03$, respectively). Cox proportional hazards analyses with the sex and age of the participants, body mass index, and smoking and drinking histories as covariates showed that a low Lp(a) level was a significant risk for all-cause, cancer, and miscellaneous-cause deaths ($p<0.001$, $p=0.003$, and $p=0.01$, respectively). The hazard ratio (95% CI) [1.48, 1.15-1.92] of a low Lp(a) level for cancer deaths was almost the same as that for a male sex (1.46, 1.00-2.13). This is the first report to describe the association between a low Lp(a) level and all-cause or cancer death, supporting the anti-neoplastic effect of Lp(a). Further epidemiological studies are needed to confirm the present findings.

News About Lipid-Altering Therapies:

As Basak *et al. (see below)* pointed out in their recent review (2012), "cardiovascular or coronary artery disease is one of the greatest plagues of modern affluent society and is linked to ~30% of all deaths each year worldwide. According to the World Health Organization (WHO), high cholesterol may cause as much as 18% of strokes and 56% of heart disease and results in ~4.4 million deaths and 40.4 million disability-adjusted life years *[www.who.int/healthinfo/statistics/bodcerebroasculardiseasestrok]*. Owing to these reasons, synthesis and/or assembly of various lipids, apolipoproteins and lipoproteins and regulation of lipid metabolism have drawn serious attention in recent decades from researchers and clinicians all over the world who are particularly engaged in finding lipid lowering drugs, primarily for the prevention and treatment of cardiovascular diseases." Albeit far from being complete, the following articles provide an insight into the current status, advances and future directions in this major area of morbidity and mortality affecting worldwide public health.

Title: LDL-cholesterol-lowering effect of a dietary supplement with plant extracts in subjects with moderate hypercholesterolemia.
Authors: Ogier N, Amiot MJ, Georgé S, Maillot M, Mallmann C, Maraninchi M, Morange S, Lescuyer JF, Peltier SL, Cardinault N.
Journal: Eur J Nutr. 2012 Apr 24. [Epub ahead of print]
Comment: Red yeast rice (RYR), sugar cane-derived policosanols (SCdP) and artichoke leaf extracts (ALEs) are currently incorporated alone or in combination into dietary supplements for their potential low-density lipoprotein cholesterol (LDL-C)-lowering effects. Yet, there is no information supporting the efficacy of this association on the reduction in LDL-C. This double-blind, randomized, parallel controlled study investigated the effects of a new dietary supplement (DS) with RYR, SCdP and ALEs on LDL-C. A total of 39 subjects, aged 21 to 55 years, with moderate hypercholesterolemia without drug treatment were assigned to two groups and then consumed either a DS containing RYR, SCdP and ALEs or a placebo over a 16-week period. Plasma concentrations of lipids [LDL-C, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triacylglycerols (TG)] and plasma levels of vitamins C and E, total polyphenols and malondialdehyde were determined at baseline and after 4, 8, 12 and 16 weeks.
LDL-C and TC were reduced by, respectively, 21.4 % (95 % CI, -13.3 to -24.9 %, $p < 0.001$) and 14.1 % (95 % CI, -10.1 to -18.0 %, $p < 0.001$) at week 16 in the DS group compared with baseline. Similar results were obtained at weeks 4, 8 and 12. TG decreased by 12.2 % after 16 weeks in the DS group (95 % CI: -24.4 to -0.1 %, $p < 0.05$). For the vitamin E/TC ratio, a difference was observed between groups at week 16 ($p < 0.05$). Other parameters were not modified. Since daily consumption of this new DS decreases LDL-C and TC, it may be a convenient aid in managing mild to moderate hypercholesterolemia.

Title: HELP apheresis in hypercholesterolemia and cardiovascular disease: efficacy and adverse events after 8,500 procedures.
Authors: Buuren FV, Kreickmann S, Horstkotte D, Kottmann T, Mellwig KP.
Journal: Clin Res Cardiol Suppl. 2012 Jun;7(Suppl 1):24-30. [Epub 2012 Mar 20]
Comment: Low density lipoprotein (LDL) apheresis is a last treatment option for hypercholesterolemic patients resistant to conservative lipid-lowering therapy. In a retrospective analysis of 8,533 heparin-induced extra-corporeal LDL precipitation apheresis treatments

(HELP), the authors evaluated the efficacy of LDL reduction, the rate of adverse events, and the progression of atherosclerosis. Between July 1992 and April 2009, they performed 8,533 HELP apheresis therapies in patients with familial hypercholesterolemia (FH). Inclusion criteria were FH with insufficient lipidological status under optimal drug therapy and diet, and at least 50 HELP therapies. Left ventricular function and valvular status was checked prior to the first apheresis therapy and at the end of the individual HELP program. Blood samples were taken directly before and after each therapy. Blood count, electrolytes, total cholesterol, LDL-cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides, lipoprotein(a) (Lp[a]), and fibrinogen were measured. Adverse events were documented weekly. *In toto*, 27 patients (19 men) with FH (age 49.2 ± 12.5 years, range 10-67 years) were evaluated. The number of HELP treatments once weekly was between 50 and 790 applications. Mean follow-up time was 7.0 ± 5.2 years (range 1.3-16.6 years). Prior to the individual apheresis program, 44.4% of the patients had a three vessel disease (VD; 25.9% two VD, 25.9% one VD) and 7.4% had a peripheral arterial occlusive disease. During the time of HELP treatment, none of the patients had a myocardial infarction; 3.7% had one percutaneous coronary intervention (PCI), 11.1% two PCI, 14.8% three PCI, 11.1% ³ four PCI. The patients received 1.2 ± 1.6 (range 0-5) PCI during follow-up time. Adverse events directly associated with HELP therapy were very rare (< 3%). Mean elimination of LDL-C was 63.49 ± 7.1%. The HELP apheresis therapy was well accepted by the patients in this program. Adverse events during HELP apheresis were rare. These data are in line with the experiences published by other authors who reported an adverse event rate of 3.6% in adults. The LDL-C/HDL-C ratio, one of the strongest predictors of premature CHD events, improved significantly during the apheresis program. In conclusion, HELP was proved to be a safe, comfortable, and highly effective treatment in which adverse events are rare. It was able to reduce the burden of atherosclerosis, with no myocardial infarction and a low coronary intervention rate, in the patient population studied.

Title: Novel LDL-oriented pharmacotherapeutical strategies.
Authors: Huang LZ, Zhu HB.
Journal: Pharmacol Res. 2012 Apr;65(4):402-10. [Epub 2012 Jan 24]

Comment: Elevated levels of low-density lipoprotein cholesterol (LDL-C) are highly correlated with increased risk of cardiovascular diseases (CVD). Thus, current guidelines have recommended progressively lower LDL-C for cholesterol treatment and CVD prevention as the primary goal of therapy. Even so, some patients in the high risk category fail to achieve recommended LDL-C targets with currently available medications. Thereby, additional pharmaceutical strategies are urgently required. In this review, the authors aimed to provide an overview of both current and emerging LDL-C lowering drugs. As for current available LDL-C lowering agents, attentions are mainly focused on statins, niacin, bile acid sequestrants, ezetimibe, fibrates and omega-3 fatty acids. On the other hand, emerging drugs that differ in mechanisms include: intervention of cholesterol biosynthesis downstream enzyme (squalene synthase inhibitors), inhibition of lipoprotein assembly (antisense mRNA inhibitors of apolipoprotein B and microsomal transfer protein inhibitors), enhanced lipoprotein clearance (proprotein convertase subtilisin kexin type 9, thyroid hormone analogues), inhibition of intestinal cholesterol absorption (Niemann-Pick C1-like 1 protein and acyl coenzyme A:cholesterol acyltransferase inhibitors) and interrupting enterohepatic circulation (apical sodium-dependent bile acid transporter inhibitors). Several ongoing agents are in their different stages of clinical trials, in expectation of promising antihyperlipidemic drugs. Therefore, alternative drugs monotherapy or in combination with statins will be sufficient to reduce LDL-C concentrations to optimal levels, and a new era for better LDL-C managements is plausible.

Title: Novel therapeutic agents for lowering low density lipoprotein cholesterol.
Authors: Joy TR.
Journal: Pharmacol Ther. 2012 Jul;135(1):31-43. [Epub 2012 Mar 23]
Comment: Elevated low-density lipoprotein cholesterol (LDL-C) levels have been associated with an increased risk for cardiovascular disease (CVD). Despite a 25-30% reduction in CVD risk with LDL-C reducing strategies, there is still a significant residual risk. Moreover, achieving target LDL-C values in individuals at high CVD risk is sometimes limited because of tolerability and/or efficacy. Thus, novel therapeutic agents are currently being developed to lower LDL-C levels further. This review highlights some of these therapeutic agents including anti-sense oligonucleotides focused on apolipoprotein B, proprotein convertase subtilisin/kexin

type 9 (PCSK9) inhibitors, microsomal triglyceride transfer protein inhibitors, and thyromimetics. For each therapeutic class, an overview of the mechanism of action, pharmacokinetic data, and efficacy/safety evidence is provided.

Title: Proprotein convertase subtilisin Kexin9 (PCSK9): A novel target for cholesterol regulation.
Authors: Basak A, Palmer-Smith H, Mishra P.
Journal: Protein Pept Lett. 2012 Jun 1;19(6):575-85. [Epub 2012 Apr 18]
Comment: Interest in lipid-lowering therapy has led to the discovery of "statin" compounds which inhibit the rate-limiting enzyme called hydroxymethylglutaryl coenzyme A reductase (HMG-CoA) responsible for cholesterol synthesis in the liver. Nowadays statins are also prescribed to treat diabetic dyslipidemia by effectively lowering low-density lipoprotein cholesterol (LDL-C). In addition, statins were found to be well tolerated by the humans. Though effective in most cases, statins can cause serious side effects for others. Therefore alternate approaches have been pursued. These include NPC1L1 (Niemann-Pick C1 like-1, an intestinal sterol transporter) blocker such as Ezetimibe© which reduces LDL-C level by additional 20%. Colesevelam, torcetrapib, avasimibe, implitapide, and niacin represent another group of anti-hyperlipidemic drugs. Development of potent Acyl-Coenzyme A cholesterol acyl-Transferase (ACAT) inhibitors, avasimibe and pactimibe had some initial promise but is now expected to stop after two clinical studies showed increasing atheromas. Implitapide and other Microsomal Triglyceride Transfer Protein (MTP) inhibitors have been associated with hepatic toxicity in humans. Squalene synthase inhibitors also showed early promise in clinical triasl but later met with some serious questions. Despite success, all the above drugs often exhibited serious side effects and are not suitable for all patients. Clearly, alternate innovative strategy and therapy are required for those who cannot take the above drugs for various reasons. Since the discovery of PCSK9 (Proprotein Convertase Subtilisin Kexin9), initially called NARC-1 (Neural Apoptosis Regulated Convertase1) in 2003 and demonstration of its unique functional activity to degrade the LDL receptor (LDL-R), it has become a subject of intense research for possible treatment of hypercholesterolemia as an alternative to statins and other drugs. PCSK9 is the ninth member of the mammalian proprotein convertase super family of serine endoproteases and is closely related to the bacterial Proteinase K. PCSK9 is expressed highly in the liver/biliary system, followed by kidney or renal system, intestine/digestive system including pancreas and the brain. It is synthesized as a pre-proprotein which first loses its signal peptide by the action of signal peptidase to produce ~72 kDa (for human sequence calculated 71.045 kDa) during its exit from the endoplasmic reticulum. The potential therapeutic consequence of PCSK9 was revealed only when it was identified as the third gene, along with LDL Receptor (LDL-R) and apolipoprotein B (apoB) to be responsible for Autosomal Dominant Hypercholesterolemia (ADH). More recently additional genes on HCHOLA4 locus have been identified that are also involved in ADH. Further studies have shown that over-expression of PCSK9 in both animals and humans leads to a greater risk of hypercholesterolemia. By suppressing PCSK9 function one can enhance LDL-R level and hence diminish circulatory LDL-C. Since this may provide an alternate opportunity for the intervention of hypercholesterolemia, PCSK9 became a target for the development of new alternate therapeutic agents for lowering LDL-C.

Title: Effect of a monoclonal antibody to PCSK9 on LDL cholesterol.
Authors: Stein EA, Mellis S, Yancopoulos GD, Stahl N, Logan D, Smith WB, Lisbon E, Gutierrez M, Webb C, Wu R, Du Y, Kranz T, Gasparino E, Swergold GD.
Journal: N Engl J Med. 2012 Mar 22;366(12):1108-18.
Comment: Proprotein convertase subtilisin/kexin 9 (PCSK9), one of the serine proteases, binds to low-density lipoprotein (LDL) receptors, leading to their accelerated degradation and to increased LDL cholesterol (LDL-C) levels. The authors report 3 phase 1 studies of a monoclonal antibody to PCSK9 designated as REGN727/SAR236553 (REGN727). In healthy volunteers, they performed 2 randomized, single ascending-dose studies of REGN727 administered either intravenously (40 subjects) or subcutaneously (32 subjects), as compared with placebo. These studies were followed by a randomized, placebo-controlled, multiple-dose trial in adults with heterozygous familial hypercholesterolemia who were receiving atorvastatin (21 subjects) and those with nonfamilial hypercholesterolemia who were receiving treatment with atorvastatin (30 subjects) (baseline LDL-C >100 mg/dL [2.6 mmol/L]) or a modified diet alone (10 subjects) (baseline LDL-C >130 mg/dL [3.4 mmol/L]). REGN727 doses of 50, 100, or 150 mg were administered subcutaneously on days 1, 29, and 43. The primary outcome for all studies was the occurrence of adverse events. The principal secondary outcome was the

effect of REGN727 on the lipid profile. Among subjects receiving REGN727, there were no discontinuations because of adverse events. REGN727 significantly lowered LDL-C levels in all the studies. In the multiple-dose study, REGN727 doses of 50, 100, and 150 mg reduced measured LDL-C in the combined atorvastatin-treated populations to 77.5 mg/dL (2.00 mmol/L), 61.3 mg/dL (1.59 mmol/L), and 53.8 mgdL (1.39 mmol/L), for a difference in the change from baseline of -39.2, -53.7, and -61.0 percentage points, respectively, as compared with placebo (P<0.001 for all comparisons). Thus, in 3 phase 1 trials, a monoclonal antibody to PCSK9 significantly reduced LDL-C levels in healthy volunteers and in subjects with familial or nonfamilial hypercholesterolemia. (See additional comment by Young SG, Fong LG: Lowering plasma cholesterol by raising LDL receptors--revisited. N Engl J Med. 2012 Mar 22;366(12):1154-5.)

Title: Safety and efficacy of a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 serine protease, SAR236553/REGN727, in patients with primary hypercholesterolemia receiving ongoing stable atorvastatin therapy.
Authors: McKenney JM, Koren MJ, Kereiakes DJ, Hanotin C, Ferrand AC, Stein EA.
Journal: J Am Coll Cardiol. 2012 Jun 19;59(25):2344-53. [Epub 2012 Mar 28]
Comment: Serum proprotein convertase subtilisin kexin 9 (PCSK9) binds to low-density lipoprotein (LDL) receptors, increasing serum LDL-C. SAR236553 is a fully human monoclonal antibody to PCSK9. This double-blind, parallel-group, placebo-controlled trial evaluated the LDL cholesterol (LDL-C)-lowering efficacy of 5 SAR236553/REGN727 (SAR236553) dosing regimens *vs.* placebo at week 12 in patients with LDL-C ≥100 mg/dL on stable atorvastatin therapy. Secondary objectives included evaluation of effects on other lipid parameters and the attainment of LDL-C treatment goals of <100 mg/dL (2.59 mmol/L) and <70 mg/dL (1.81 mmol/L). A total of 183 patients with LDL-C ≥100 mg/dL (2.59 mmol/L) on stable-dose atorvastatin 10, 20, or 40 mg for ≥6 weeks were randomized to: subcutaneous placebo every 2 weeks (Q2W); SAR236553 50, 100, or 150 mg Q2W; or SAR236553 200 or 300 mg every 4 weeks (Q4W), alternating with placebo for a total treatment period of 12 weeks. SAR236553 demonstrated a clear dose-response relationship with respect to percentage LDL-C lowering for both Q2W and Q4W administration: 40%, 64%, and 72% with 50, 100, and 150 mg Q2W, respectively, and 43% and 48% with 200 and 300 mg Q4W. LDL-C reduction with placebo at week 12 was 5%. SAR236553 also substantially reduced non-high-density lipoprotein cholesterol (non-HDL-C), apolipoprotein B (apoB), and lipoprotein(a) (Lp[a]). SAR236553 was generally well tolerated. One patient on SAR236553 experienced a serious adverse event of leukocytoclastic vasculitis. The results show that, when added to atorvastatin, PCSK9 inhibition with SAR236553 further reduces LDL-C by 40% to 72%. These additional reductions are both dose- and dosing frequency-dependent.
(Comments on this article appeared in:
Lipids: Monoclonal antibody therapy lowers LDL-cholesterol levels. [Nat Rev Cardiol. 2012]
The therapeutic potential of PCSK9 inhibition in primary dyslipidemia, the example from SAR236553/REGN727. [Expert Opin Investig Drugs. 2012]
PCSK9 inhibition: the next statin? [J Am Coll Cardiol. 2012])

Title: Mipomersen, an antisense apolipoprotein B synthesis inhibitor.
Authors: Bell DA, Hooper AJ, Burnett JR.
Journal: Expert Opin Investig Drugs. 2011 Feb;20(2):265-72. [Epub 2011 Jan 6]
Comment: Mipomersen, an antisense apoB synthesis inhibitor, is a second-generation antisense oligonucleotide (ASO) targeted to human apolipoprotein (apo) B-100, a large protein synthesized by the liver that plays a fundamental role in human lipoprotein metabolism. Mipomersen predominantly distributes to the liver and decreases the production of apoB-100, the primary structural protein of the atherogenic lipoproteins including low-density lipoprotein (LDL), thereby reducing plasma LDL-C and apoB-100 concentrations. This article provides an understanding of the pharmacokinetic and pharmacodynamic characteristics of mipomersen and insight into its clinical efficacy and safety. In clinical trials, mipomersen produced dose-dependent and prolonged reductions in LDL-C and other apoB-containing lipoproteins, including lipoprotein (a) [Lp(a)] in healthy volunteers and in patients with mild to moderate hypercholesterolemia. Mipomersen has been shown to decrease apoB, LDL-C and Lp(a) in patients with heterozygous and homozygous familial hypercholesterolemia on maximally tolerated lipid-lowering therapy. According to these observations, mipomersen shows promise as an adjunctive agent by reducing apoB-containing lipoproteins in patients at high risk of atherosclerotic cardiovascular disease who are not at target or are intolerant of statins. Although the short-

term efficacy and safety of mipomersen has been established, concern exists regarding the long-term potential for hepatic steatosis with this ASO.

Title: Mipomersen, an apolipoprotein B synthesis inhibitor, lowers low-density lipoprotein cholesterol in high-risk statin-intolerant patients: a randomized, double-blind, placebo-controlled trial.
Authors: Visser ME, Wagener G, Baker BF, Geary RS, Donovan JM, Beuers UH, Nederveen AJ, Verheij J, Trip MD, Basart DC, Kastelein JJ, Stroes ES.
Journal: Eur Heart J. 2012 May;33(9):1142-9. [Epub 2012 Apr 16]
Comment: This randomized, double-blind, placebo-controlled study investigated the safety and efficacy of mipomersen, an apolipoprotein B-100 (apoB) synthesis inhibitor, in patients who are statin intolerant and at high risk for cardiovascular disease (CVD). Thirty-three subjects, not receiving statin therapy because of statin intolerance, received a weekly subcutaneous dose of 200 mg mipomersen or placebo (2:1 randomization) for 26 weeks. The primary endpoint was per cent change in LDL cholesterol (LDL-C) from the baseline to Week 28. The other efficacy endpoints were per cent change in apoB and lipoprotein a [Lp(a)]. Safety was determined using the incidence of treatment-emergent adverse events (AEs) and clinical laboratory evaluations. After 26 weeks of mipomersen administration, LDL-C was reduced by 47 ± 18% ($P < 0.001$ *vs.* placebo). apoB and Lp(a) were also significantly reduced by 46 and 27%, respectively ($P < 0.001$ *vs.* placebo). Four mipomersen (19%) and two placebo subjects (17%) discontinued dosing prematurely due to AEs. Persistent liver transaminase increases ≥3× the upper limit of normal were observed in seven (33%) subjects assigned to mipomersen. In selected subjects, liver fat content was assessed, during and after treatment, using magnetic resonance spectroscopy. Liver fat content in these patients ranged from 0.8 to 47.3%. Liver needle biopsy was performed in two of these subjects, confirming hepatic steatosis with minimal inflammation or fibrosis. The present data suggest that mipomersen is a potential therapeutic option in statin-intolerant patients at high risk for CVD. The long-term follow-up of liver safety is required.

Title: Novel HDL-based therapeutic agents.
Authors: Joy TR.
Journal: Pharmacol Ther. 2012 Jul;135(1):18-30. [Epub 2012 Mar 23]
Comment: Reduction in low-density lipoprotein cholesterol (LDL-C) levels has been associated with a 25-30% reduction in cardiovascular disease risk. However, there still remains a significant and quantifiable risk. Since epidemiologic data have demonstrated that low levels of high-density lipoprotein cholesterol (HDL-C) are associated with an increased risk for cardiovascular disease, novel therapeutic agents are currently being developed to either raise HDL-C levels or enhance HDL functionality. This review highlights some of these therapeutic agents including cholesteryl ester transfer protein inhibitors, apolipoprotein A-I mimetics, RVX-208, and apolipoprotein A-I based infusion therapies. For each therapeutic class, an overview of the mechanism of action, pharmacokinetic data, and efficacy/safety evidence is provided.

Title: MicroRNAs: emerging roles in lipid and lipoprotein metabolism.
Authors: Sacco J, Adeli K.
Journal: Curr Opin Lipidol. 2012 Jun;23(3):220-5. [Epub 2012 Apr 7]
Comment: MicroRNAs (miRNAs) regulate gene expression by binding to target mRNAs and control a wide range of biological functions. Recent reports have identified specific miRNAs as major regulators of fatty acid and cholesterol homeostasis. This review examined the biological function of various miRNAs and the emerging evidence linking specific miRNAs to critical pathways in lipid metabolism. Disruption of lipid balance can lead to metabolic disturbances and thus tight regulation is required to maintain lipid homeostasis. Recent studies have shown key roles for miR-33 and miR-122 in regulation of lipid metabolism, and further evidence implicates miR-370 in regulation of miR-122. In addition, miRNAs involved in adipogenesis (miR-378/378* and miR-27) as well as newly discovered miRNAs such as miR-613, miR-302a, and miR-168 have now been implicated in regulation of lipid metabolism. Thus, growing evidence supports key roles for miRNAs in regulating both cholesterol and fatty acid metabolism, leading to considerable interest in miRNAs as potential drug targets to modulate lipid and lipoprotein metabolism. MiRNA-based therapeutics also hold considerable promise for curtailing the epidemic of obesity and type 2 diabetes and the associated risk of atherosclerosis.

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