

LIPIDS

Classifications and Understandings

by Jere Segrest, M.D., Ph.D.

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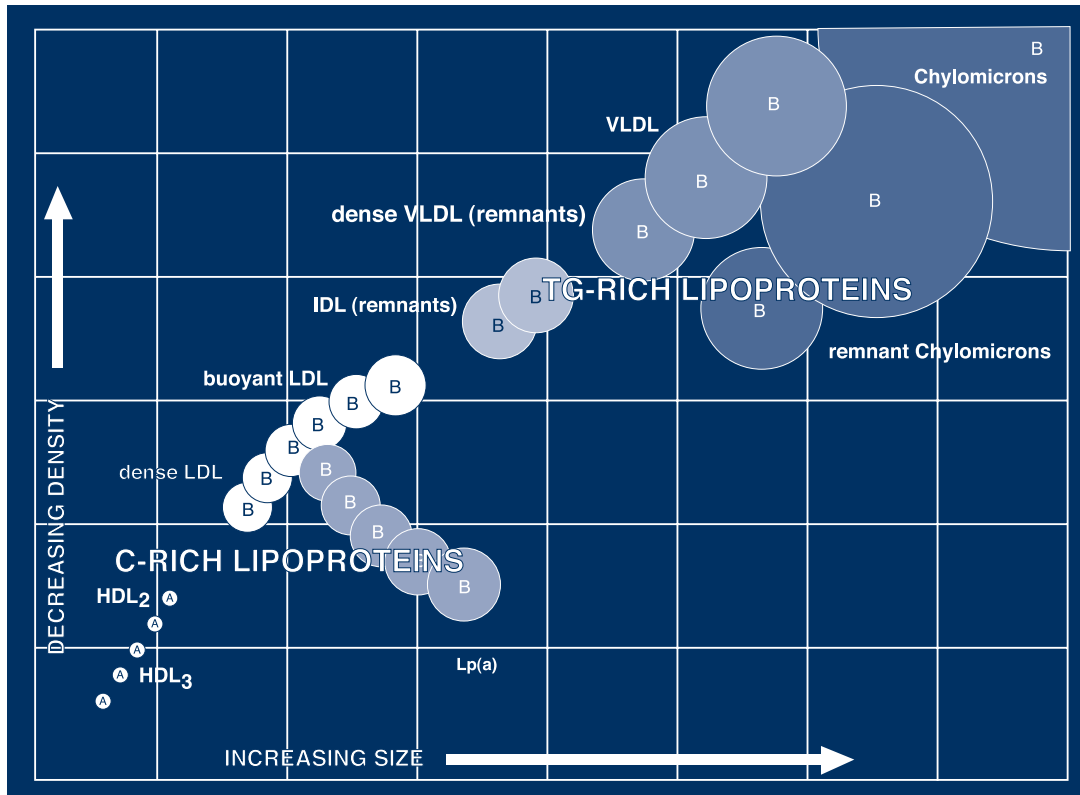
The author of over 140 peer-review publications, Dr. Segrest has been ranked as one of the 1,000 most referenced scientists in the world. He has been a guest lecturer at approximately 50 different universities around the country and at over 30 national and international symposia. Some of the committees he has served on include: the American Chemical Society Committee on Professional Training in Chemistry, the Molecular Cytology Study Section, the American Society for Biological Chemists Committee on Educational Affairs, and the Executive Committee for the Council on Arteriosclerosis of the American Heart Association. Currently, he belongs to the American Society of Biochemistry and Molecular Biology, the Biophysical Society, and is on the Board of Directors for the Birmingham Science Center. In addition to his extensive study of Atherosclerosis, he has conducted a vast amount of research in the areas of Metabolism and Digestive Diseases, Endocrinology, Enzymology, and Anatomic Pathology, in which he is board-certified.

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LIPOPROTEIN CLASSES



The general structure of plasma lipids (plasma lipoproteins) is that of a submicroscopic oil droplet containing an outer layer of phospholipids, unesterified cholesterol, and proteins, with a core of neutral lipids, predominately cholesterol ester and triglycerides. The lipoprotein classes differ in their lipid composition (cholesterol (C)-rich or triglyceride (TG)-rich), in their protein (apolipoprotein, apo) composition, and in their protein:lipid ratio (the higher the ratio, the greater the density). On the basis of protein composition, there are two types of lipoproteins, those that contain apo B and those that contain apo A. On the basis of lipid composition, there are also two types of lipoproteins, those that are C-rich and those that are TG-rich.

Lipoproteins are commonly classified by their density and/or size (**see above**) into six lipoprotein classes, each of which contains several subclasses. From lowest to highest density the six classes are:

- **Chylomicrons:** produced by the gut in response to dietary fat. Remnants are atherogenic.
- **VLDL (very low density lipoprotein):** produced by the liver. Remnants are atherogenic.
- **IDL (intermediate density lipoprotein):** an intermediate in VLDL catabolism. Atherogenic.
- **LDL (low density lipoprotein):** the end product of VLDL catabolism. "Bad" cholesterol.
- **HDL (high density lipoprotein):** produced by the liver and gut. "Good" cholesterol.
- **Lp(a):** consists of LDL plus a protein called apo(a). "Heart attack" cholesterol.

DYSLIPOPROTEINEMIAS

LIPOPROTEINS

Cholesterol, a lipid, is carried in lipoprotein particles which consist of proteins (called apolipoproteins from the Greek prefix apo, meaning out of or away from) and lipids such as cholesterol, cholesterol ester, triglycerides (fat), and phospholipids. These are present in the blood as discrete lipoprotein families, generally classified by their density as (from lowest to highest density) very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL), and high density lipoprotein (HDL). An additional clinically important lipoprotein is often present called lipoprotein (a) or Lp(a), which consists of LDL plus an additional apolipoprotein called apo(a).

RELATIONSHIP TO CAD RISK

Elevated levels of LDL have been shown to be associated with increased risk of development of atherosclerotic coronary artery disease (CAD). More recently it has been shown that elevations of VLDL, IDL, and Lp(a) also appear to be related to greater risk of CAD. In addition, Lp(a) may increase the thrombus (clot) formation that causes myocardial infarction (heart attack), and may be involved in restenosis (closing) of arteries following bypass operations or balloon angioplasty. Higher levels of HDL appear to be protective against development of CAD; low HDL is as (or more) potent a predictor of CAD as high LDL.

FAMILY HISTORY OF PREMATURE CAD

Of the CAD risk factors, the most important is inherited dyslipoproteinemia; this is especially true for premature coronary artery disease, defined as disease before the age of 55 in men and before 65 in women. In one study of large families with premature coronary artery disease by Roger Williams and his colleagues in the Mormon population of Utah (1), only 15% of family members with premature coronary artery disease did not have some form of dyslipoproteinemia. Further, from this study it is apparent that only 25% of premature coronary artery disease can be accounted for by elevated total cholesterol and LDL alone (1). Of importance to VAP, Inc. and the VAP-II procedure, 60% of premature coronary artery disease is accounted for by other lipoprotein abnormalities, including low HDL and elevated Lp(a), IDL, and VLDL (representing 20%, 15%, 5%, and 20% of premature coronary artery disease, respectively) (1).

VAP

The vast majority of individuals with dyslipidemia currently are undiagnosed and untreated. It is the goal of Atherotech to close the gap between diagnosis and treatment by allowing the effects of treatment on the different lipoprotein classes to be monitored on a routine basis with a single test.

IMPORTANCE OF COMPREHENSIVE LIPID TESTING

Certain examples of the importance of comprehensive testing for lipoproteins abound: 1) In the Framingham study, low HDL is more of a risk factor for coronary artery disease than is LDL; 2) the risk linked to elevated Lp(a) is affected by associated abnormalities in HDL, LDL, IDL and VLDL; 3) familial combined hyperlipidemia, a highly prevalent disorder associated with abnormalities in LDL, IDL, VLDL and HDL, has a strong link to premature coronary artery disease; and 4) Type III dyslipoproteinemia, associated with premature coronary artery disease, involves an abnormality in IDL and can be diagnosed by VAP-II.

ATHEROGENIC LIPOPROTEINS

- **HDL₂**. "Best" cholesterol. Most protective of the HDL subclasses.
- **Lp(a)**. "Heart attack" cholesterol. Independent risk factor for CAD and heart attacks.
- **Dense LDL (pattern B)**. "Worst" cholesterol. The most atherogenic of the LDL subclasses.
- **IDL (remnants)**. "Bad" non-LDL cholesterol. Independent risk factor for CAD.
- **Dense VLDL₃ (remnants)**. "Bad" non-LDL cholesterol. Independent risk factor for CAD.
- **VLDL**. Main carrier of triglycerides, an independent risk factor for CAD.
- **Type III dyslipidemia**. Carries high risk for CAD. VAP is diagnostic for type III.

EFFECTS OF LIPID MODIFICATION ON CAD

Having established the pre-eminence of dyslipoproteinemia as a risk factor for coronary artery disease, it is necessary to know if interventional modifications of lipoproteins can influence the progression of atherosclerosis. This question has been addressed in a number of double blinded prospective prevention trials.

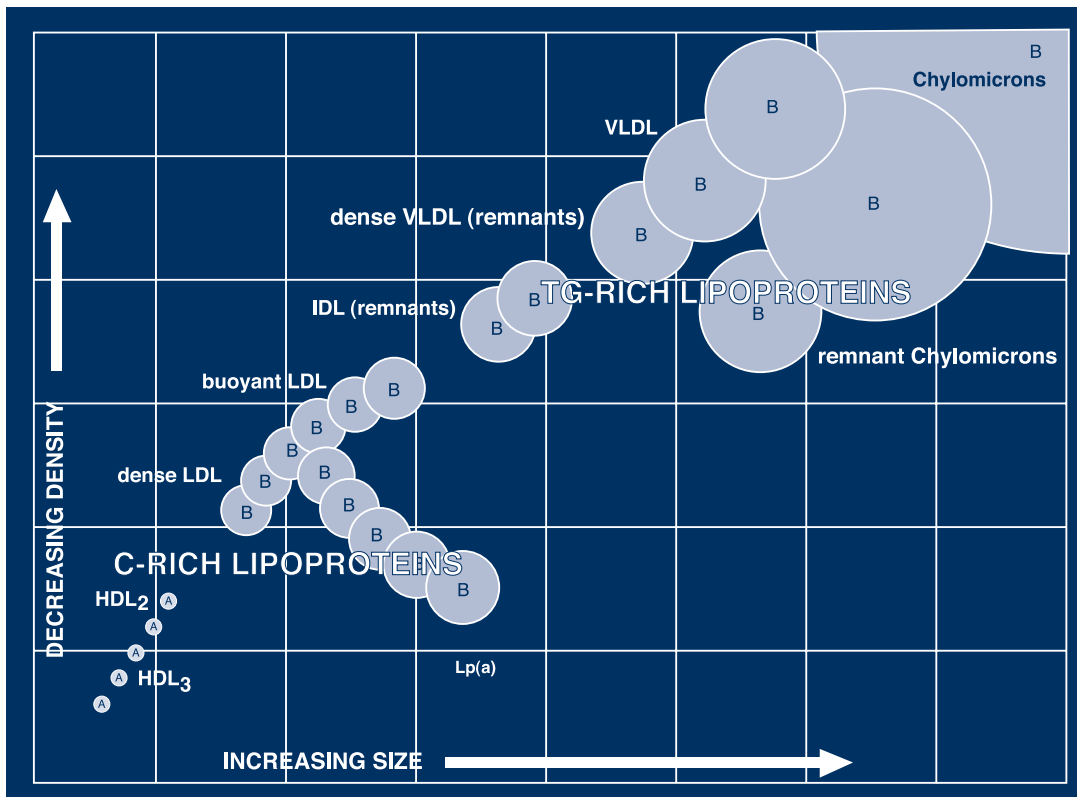
Primary prevention trials are studies in which, over a number of years, subjects without pre-existing coronary artery disease, are subject to lifestyle changes, such as a low fat, low cholesterol diet, and/or to lipoprotein-modifying drugs. These studies have clearly established that the incidence of coronary artery disease can be substantially reduced by these forms of intervention.

Secondary prevention trials are similar to primary prevention trials studies except that the subjects are patients with pre-existing coronary artery disease. Secondary prevention trials, especially the so-called regression studies, have shown that aggressive lipoprotein therapy with drugs (lowering LDL, Lp(a) and VLDL, and raising HDL), can open up pre-existing coronary artery blockages; perhaps more importantly, within 6 months most new clinical events, such as: myocardial infarction and angina have ceased in the treated, but not in the untreated group. The regression studies in particular have created a new enthusiasm in health professionals and among lay persons for the diagnosis and subsequent treatment of the various forms of dyslipoproteinemia.

GOOD CHOLESTEROL: HDL
BEST CHOLESTEROL: HDL₂

GOOD CHOLESTEROL: HDL

BEST CHOLESTEROL: HDL₂



HDL

HDL is an apo A-containing, C-rich lipoprotein, that, as the name implies, is dense, and also small. The major protein component of HDL is apo A-1. Since **HDL** levels are inversely related (high is good, low is bad) with risk for atherosclerosis and CAD, **HDL** is considered the protective, or so-called "good" cholesterol.

HDL SUBCLASSES

Two major subclasses have been identified by ultracentrifugation:

- **HDL₂**. This is the largest and lightest (most buoyant) of the two subclasses and the most protective (i.e., the "**best**" cholesterol, see below).
- **HDL₃**. This is the smallest and most dense of the two subclasses and the least protective (i.e., the "**worst**" "good" cholesterol, see below).
- **Further subdivision of subclasses**. Five major HDL subclasses have been identified by nondenaturing gradient gel electrophoresis. In order of increasing density and decreasing size these are: **HDL_{2b}**, HDL_{2a}, **HDL_{3a}**, and HDL_{3c}. **HDL_{2b}** is the "**best**" and **HDL_{3b}** is the "**worst**" (perhaps is even atherogenic) of these cholesterol (see below).

HDL₂ IS THE PROTECTIVE HDL SUBCLASS

- **Coronary artery disease**. Many clinical studies have demonstrated that HDL₂ (HDL_{2b} in particular) is more protective against risk for coronary disease than either total HDL or HDL₃.
 - **A recent prospective analysis of 1169 men, showed that those men with the highest (top quarter) levels of HDL₂ had a 79% reduction in risk compared to men with the lowest levels of HDL₂, while men with the highest levels of HDL₃ had a 63% reduction in risk compared to men with the lowest levels of HDL₃:**

Larnarache B, Moorjani S, Cantin B, Dagenais GR, Lupien PJ, Despres JP. Associations of HDL₂ and HDL₃ subfractions with ischemic heart disease in men. Prospective results from the Quebec Cardiovascular Study. Arterioscler Thromb Vasc Biol. 1997 Jun; 17(6):1098-105

– **Other prospective studies support the "best" cholesterol role for HDL₂:**

Salonen JT, Salonen R, Seppanen K, Rauramaa R, Tuomilehto J. HDL, HDL₂, and HDL₃ sub-fractions, and the risk of acute myocardial infarction. A prospective population study in eastern Finnish men. *Circulation* 1991 Jul;84(1):129-39

Gaziano JM, Buring JE, Breslow JL, Goldhaber SZ, Rosner B, VanDenburgh M, Willett W, Hennekens CH. Moderate alcohol intake, increased levels of high-density lipoprotein and its subfractions, and decreased risk of myocardial infarction. *N Engl J Med.* 1993 Dec

– **Children of parents with premature CAD have low HDL₂ but normal HDL₃:**

Bodurtha JN, Schieken R, Segrest JP, Nance WE. High-density lipoprotein-cholesterol subfractions in adolescent twins. *Pediatrics* 1987 Feb;79(2):181-9

– **Low HDL₂ is a risk for CAD in patients with normal cholesterol:**

Campos H, Roederer GO, Lussier-Cacan S, Davignon I, Krauss RM. Predominance of large LDL and reduced HDL₂ cholesterol in normolipidemic men with coronary artery disease. *Arterioscler Thromb Vasc Biol.* 1995 Aug;15(8):1043-8

Franceschini G, Bondioli A, Granata D, Mercuri V, Negri M, Tosi C, Sirtori CR. Reduced HDL₂ levels in myocardial infarction patients without risk factors for atherosclerosis. *Atherosclerosis* 1987 Dec;68(3):213-9

– **Low HDL₂ is a component of the atherogenic lipoprotein profile:**

Austin MA, King MC, Vranizan KM, Krauss RM. Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk. *Circulation* 1990 Aug;82(2):495-506

– **Additional references concerning HDL₂ and CAD:**

Sich D, Saidi Y, Giral P, Lagrost L, Egloff M, Auer C, Gautier V, Turpin G, Beucler I. Hyperalphalipoproteinemia: characterization of a cardioprotective profile associating increased high-density lipoprotein levels and decreased hepatic lipase activity. *Metabolism* 1998 Aug;47(8):965-73

Roheim PS, Asztalos BF. Clinical significance of lipoprotein size and risk for coronary atherosclerosis. *Clin Chem.* 1995 Jan;41(1):147-52

Drexel H, Amann FW, Rentsch K, Neuenschwander C, Luethy A, Khan SI, Follath F. Relation of the level of high-density lipoprotein subfractions to the presence and extent of coronary artery disease. *Am J Cardiol.* 1992 Aug 15;70(4):436-40

Krauss RM. The tangled web of coronary risk factors. *Am J Med.* 1991 Feb 21;90(2A):36S- 41S

Manttari M, Huttunen JK, Koskinen P, Manninen V, Tenkanen L, Heinonen OP, Frick MH. Lipoproteins and coronary heart disease in the Helsinki Heart Study. *Eur Heart J.* 1990 Dec: Suppl H:26-31

Steenkamp HJ, Jooste PL, Benade AJ, Langenhoven ML, Rossouw JE. Relationship between high density lipoprotein subfractions and coronary risk factors in a rural white population. *Arteriosclerosis* 1990 Nov-Dec; 10(6):1026-31

Schieken RM. The management of the family at high risk for coronary heart disease. *Cardiol Clin.* 1989 May;7(2):467-77

Kaappinen-Makelin R, Nikkila EA. Serum lipoproteins in patients with myocardial infarction, *Atherosclerosis* 1988 Nov;74 (1-2):65-74

Hamsten A, Walldius G, Szamosi A, Dahlen G, de Faire U. Relationship of angiographically defined coronary artery disease to serum lipoproteins and apolipoproteins in young survivors of myocardial infarction. *Circulation* 1986 Jun;73(6):1097-110

Ballantyne FC, Clark RS, Simpson HS, Ballantyne D. High density and low density lipoprotein subfractions in survivors of myocardial infarction and in control subjects. *Metabolism* 1982 May;31(5):433-7

Miller NE, Hammett F, Saltissi S, Rao S, van Zeller H, Coltart J, Lewis B. Relation of angiographically defined coronary artery disease to plasma lipoprotein subfractions and apolipoproteins. *Br Med J. (Clin Res Ed)* 1981 May 30;282(6278):1741-4

● **Peripheral vascular disease. A low level of HDL₂ is an independent risk factor for peripheral vascular disease and cerebrovascular disease.**

Laakso M, Pyorala K. Lipid and lipoprotein abnormalities in diabetic patients with peripheral vascular disease. *Atherosclerosis* 1988 Nov;74(1-2):55-63

Fellin R, Baroni L, Baiocchi MR, Baldo E, Q Grego F, Valerio G. Selective determination of cholesterol in high density lipoprotein subfractions (HDL₂ and HDL₃) in patients with cerebral and peripheral arteriosclerosis. *Clin Chim Acta*. 1985 Apr 30;147(3):233-40

● **Visceral adiposity. A low level of HDL₂ is associated independently with truncal obesity or visceral adiposity, a component of the insulin resistance syndrome and type 2 diabetes.**

Walton C, Lees B, Crook D, Worthington M, Godsland IIF, Stevenson JC. Body fat distribution, rather than overall adiposity, influences serum lipids and lipoproteins in healthy men independently of age. *Am J Med*. 1995 Nov;99(5):459-64

Katzel LI, Coon PJ, Busby MJ, Gottlieb SO, Krauss RM, Goldberg AP. Reduced HDL₂ cholesterol subspecies and elevated postheparin hepatic lipase activity in older men with abdominal obesity and asymptomatic myocardial ischemia. *Arterioscler Thromb* 1992 Jul;12(7):814-23

● **Insulin resistance. A low level of HDL₂ is associated independently with hyperinsulinemia and insulin resistance.**

Conway GS, Jacobs HS. Clinical implications of hyperinsulinemia in women. *Clin Endocrinol. (Oxf)* 1993 Dec;39(6):623-32

Ferns GA, Lanham J, Stocks I, Ritchie C, Katz J, Galton DJ. The measurement of high density lipoprotein subfractions in patients with primary gout using a simple precipitation method. *Ann Clin Biochem*. 1985 Sep;22 (Pt 5):526-32

Ostlund RE Jr, Staten M, Kohrt WM, Schultz J, Malley M. The ratio of waist-to-hip circumference, plasma insulin level, and glucose intolerance as independent predictors of the HDL₂ cholesterol level in older adults. *N Engl J Med*. 1990 Jan 25;322(4):229-34

● **Type 2 diabetes. A low level of HDL₂ is associated independently with adult onset diabetes.**

Syvanne M, Ahola M, Lahdenpera S, Kahri J, Kuusi T, Virtanen KS, Taskinen MR. High density lipoprotein subfractions in non-insulin-dependent diabetes mellitus and coronary artery disease. *J Lipid Res*. 1995 Mar;36(3):573-82

Laakso M, Lehto S, Penttila I, Pyorala K. Lipids and lipoproteins predicting coronary heart disease mortality and morbidity in patients with non-insulin-dependent diabetes. *Circulation* 1993 Oct;88(4 Pt 1):1421-30

Billingham MS, Milles JJ, Bailey CJ, Hall RA. Lipoprotein subfraction composition in non-insulin-dependent diabetes treated by diet, sulphonylurea, and insulin. *Metabolism* 1989 Sep;38(9):850-7

FACTORS THAT MODIFY HDL₂

Lifestyle

● **Exercise. HDL₂, but not HDL₃, is increased by aerobic exercise.**

Raitakari OT, Taimela S, Porkka KV, Telama R, Valimaki I, Akerblom HK, Viikari JS. Associations between physical activity and risk factors for coronary heart disease: the Cardiovascular Risk in Young Finns Study. *Med Sci Sports Exerc*. 1997 Aug;29(8):1055-61

Pronk NP. Short term effects of exercise on plasma lipids and lipoproteins in humans. *Sports Med*. 1993 Dec;16(6):431-48

Lakka TA, Salonen JT. Physical activity and serum lipids: a cross-sectional population study in eastern Finnish men. *Am J Epidemiol*. 1992 Oct 1;136(7):806-18

Cook TC, Laporte RE, Washburn RA, Traven ND, Slemenda CW, Metz KF. Chronic low level physical activity as a determinant of high density lipoprotein cholesterol and subfractions. *Med Sci Sports Exerc* 1986 Dec;18(6):653-7

● **Alcohol. Alcohol consumption increases all fractions of HDL, HDL₂ more than HDL₃.**

Valimaki M, Laitinen K, Ylikahri R, Ehnholm C, Jauhiainen M, Bard JM, Fruchart JC, Taskinen MR. The effect of moderate alcohol intake on serum apolipoprotein A-I-containing lipoproteins and lipoprotein(a). *Metabolism* 1991 Nov;40(11):1168-72

Veenstra J, Ockhuizen T, van de Pol H, Wedel M, Schaafsma G. Effects of a moderate dose of alcohol on blood lipids and lipoproteins postprandially and in the fasting state. *Alcohol Alcohol* 1990;25(4):371-7

Taskinen MR, Nikkila EA, Valimaki M, Sane T, Kuusi T, Kesaniemi A, Ylikahri R. Alcohol-induced changes in serum lipoproteins and in their metabolism. *Am Heart J.* 1987 Feb; 113(2 Pt 2):458-64

Burr ML, Fehily AM, Butland BK, Bolton CH, Eastham RD. Alcohol and high-density-lipoprotein cholesterol: a randomized controlled trial. *Br J Nutr.* 1986 Jul;56(1):81-6

Dai WS, LaPorte RE, Hom DL, Kuller LH, D'Antonio JA, Gutai JP, Wozniczak M, Wohlfahrt B. Alcohol consumption and high density lipoprotein cholesterol concentration among alcoholics. *Am J Epidemiol.* 1985 Oct;122(4):620-7

● **Smoking. Both active and passive cigarette smoking decrease HDL₂.**

Richard F, Marecaux N, Dallongeville J, Devienne M, Tiem N, Fruchart JC, Fantino M, Zylberberg Q, Amouyel P. Effect of smoking cessation on lipoprotein A-I and lipoprotein A-I:A-II levels. *Metabolism* 1997 Jun;46(6):711-5

Moskowitz WB, Mosteller M, Schieken RM, Bossano R, Hewitt JK, Bodurtha IN, Segrest JP. Lipoprotein and oxygen transport alterations in passive smoking preadolescent children. The MCV Twin Study. *Circulation* 1990 Feb;81(2):586-92

● **Diet. A diet high in carbohydrate and polyunsaturated fat lowers HDL₂.**

Kuusi T, Ehnholm C, Huttunen JK, Kostiaainen E, Pietinen P, Leino U, Uusitalo U, Nikkari T, Iacono JM, Puska P. Concentration and composition of serum lipoproteins during a low-fat diet at two levels of polyunsaturated fat. *J Lipid Res.* 1985 Mar;26(3):360-7

Genetics

● **Inheritance. HDL₂ is highly inherited but HDL₃ is not.**

Moskowitz WB, Mosteller M, Schieken RM, Bossano R, Hewitt JK, Bodurtha JN, Segrest JP. Lipoprotein and oxygen transport alterations in passive smoking preadolescent children. The MCV Twin Study. *Circulation* 1990 Feb;81(2):586-92

● **Gender. HDL₂ is higher in women than in men and accounts for perhaps 50% of women's increased longevity.**

Williams PT, Krauss RM, Vranizan KM, Stefanick NIL, Wood PD, Lindgren FT. Associations of lipoproteins and apolipoproteins with gradient gel electrophoresis estimates of high density lipoprotein subfractions in men and women. *Arterioscler Thromb.* 1992 Mar;12(3):332-40

Hazzard WR, Applebaum-Bowden D. Why women live longer than men: the biologic mechanism of the sex differential in longevity. *Trans Am Clin Climatol Assoc.* 1989;101:168-88; discussion 188-9

Porkka KV, Viikari JS, Taimela S, Dahl M, Akerblom HK. Tracking and predictiveness of serum lipid and lipoprotein measurements in childhood: a 12-year follow-up. The Cardiovascular Risk in Young Finns study. *Am J Epidemiol* 1994 Dec 15;140(12):1096-110

● **Postprandial lipemia. Fasting HDL₂ is the best lipoprotein predictor of the severity of postprandial lipemia; postprandial hyperlipidemia, resulting in high levels of cholesterol-rich IDL and chylomicron remnants, is considered by many to represent the major atherogenic insult.**

Patsch JR. Triglyceride-rich lipoproteins and atherosclerosis. *Atherosclerosis* 1994 Oct;110 Suppl:S23-6

Drugs

● **Nicotinic acid. Niacin is the most effective drug for raising HDL₂.**

Wailidius C, Wahlberg G. Effects of nicotinic acid and its derivatives on lipid metabolism and other metabolic factors related to atherosclerosis. *Adv Exp Med Biol* 1985;183:281-93

● **Gemfibrozil. Lopid increases HDL₃, not HDL₂.**

Manttari M, Huttunen JK, Koskinen P, Manninen V, Tenkanen L, Heinonen OP, Frick MH. Lipoproteins and coronary heart disease in the Helsinki Heart Study. *Eur Heart J.* 1990 Dec;11 Suppl H:26-31

● **Hormone replacement therapy. HRT raises HDL₂.**

Vadlamudi S, MacLean P, Israel RG, Marks RH, Hickey M, Otvos J, Barakat H. Effects of oral combined hormone replacement therapy on plasma lipids and lipoproteins. *Metabolism* 1998 Oct;47(10):1222-6

Hart DM, Farish E, Fletcher CD, Howie C, Kitchener H. Ten years post-menopausal hormone replacement therapy--effect on lipoproteins. *Maturitas* 1984 Apr;5(4):271-6

● **β blockers. These drugs decrease HDL₂.**

Superko HR, Haskell WL, Krauss RM. Association of lipoprotein subclass distribution with use of selective and non-selective beta-blocker medications in patients with coronary heart disease. *Atherosclerosis* 1993 Jun;101(1): 1-8

● **Androgens. Androgenic steroids drastically lower HDL₂.**

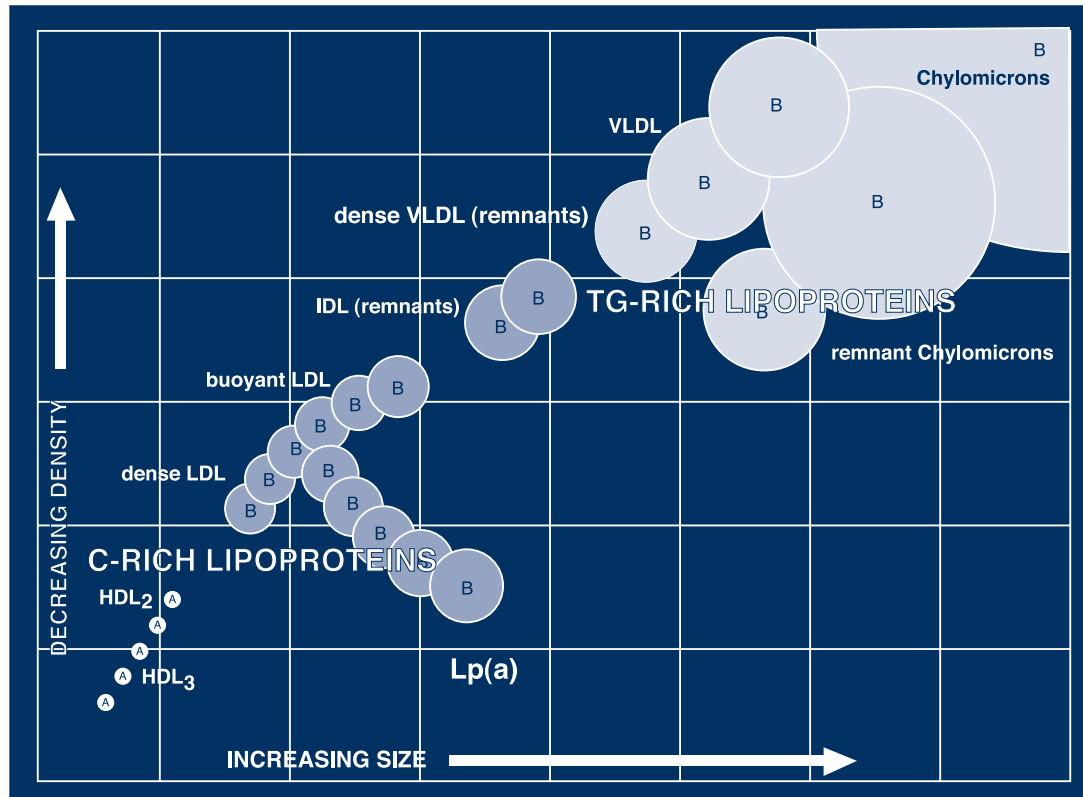
Hurley BF, Seals DR, Hagberg JM, Goldberg AC, Ostrove SM, Holloszy JO, Wiest WG, Goldberg AP. High-density lipoprotein cholesterol in bodybuilders vs. powerlifters. Negative effects of androgen use. *JAMA* 1984 Jul 27;252(4):507-13

CAVEAT

Not all studies have supported HDL₂ as more protective than total HDL or even HDL₃. A major problem has been the procedure used to measure the two HDL subclasses. In general, the studies using ultracentrifugation (mostly case-control because of the smaller number of subjects and the difficulty of standard ultracentrifugation analysis) support HDL₂ as most protective, while studies using questionable precipitation techniques (almost all prospective studies have used this technique because of the large number of subjects) often do not support HDL₂ as most protective, although some do.

HEART ATTACK CHOLESTEROL: Lp(a)

HEART ATTACK CHOLESTEROL: Lp(a)



Lp(a)

Lp(a) (lipoprotein (a)), consists of LDL attached to an additional apolipoprotein called apo(a). It is an apo B-containing, C-rich lipoprotein, that is generally denser yet larger than LDL and larger and generally less dense than HDL. Thus **Lp(a)** has the reverse characteristic of other lipoproteins that decrease in density the larger they are; **Lp(a)** increases in density the larger it becomes.

The relationship of **Lp(a)** to atherosclerosis and CAD is direct (high is bad, low is good) but complex; while related to **Lp(a)** levels, atherogenicity is also related to the number of kringles in apo(a) (see below). Since **Lp(a)** is thought to alter the thrombotic system and seems to be associated with increased myocardial infarction, it is often called the "heart attack" cholesterol.

Lp(a) SUBCLASSES

As many as 40 or more subclasses of **Lp(a)** occur in the human population as a whole. However, a given individual can have no more than two subclasses, since a single subclass is inherited from each parent. Apo(a) contains multiple repeated structural regions, called kringles, that are similar to those contained in the fibrinolytic enzyme, plasminogen. The more kringles, the more dense the **Lp(a)** particle; the fewer the kringles, the less dense and the more atherogenic the **Lp(a)**. Diet, exercise and most drugs (except nicotinic acid) have little effect on levels of **Lp(a)**.

Lp(a) CARRIES A HIGH RISK

- **Coronary artery disease.** The lipoprotein **Lp(a)** is a direct risk factor for CAD (high is bad, low is good) but the relationship is complex, since risk is also related to the number of kringles in apo(a).
 - **Because Lp(a) seems, in particular, to be associated with increased myocardial infarction, it has been called the "heart attack" cholesterol:**

Sandkamp M, Funke H, Schulte H, Kohler E, Assmann G. Lipoprotein(a) is an independent risk factor for myocardial infarction at a young age. Clin Chem. 1990 Jan;36(1):20-3

- **In a recent meta-analysis of prospective studies, it was found that in 12 of 14 studies, Lp(a) concentrations are higher in subjects who later develop CAD than in those who do not. The extent of the effect was similar in men and women.**

Craig WY, Neveux LM, Palomaki GE, Cleveland MM, Haddow JE. Lipoprotein(a) as a risk factor for ischemic heart disease: meta-analysis of prospective studies. *Clin Chem.* 1998 Nov;44(11):2301-6

- **The Framingham study found that elevated Lp(a) carries an increased risk for coronary artery disease of 1.9:**

Bostom AG, Cupples LA, Jenner JL, Ordovas JM, Seman LJ, Wilson PW, Schaefer EJ, Castelli WP. Elevated plasma lipoprotein(a) and coronary heart disease in men age 55 years and younger. A prospective study. *JAMA* 1996 Aug 21;276(7):544-8

- **In the FATS regression trial, in multivariate analyses, the best correlate of baseline CAD severity was Lp(a):**

Maher VM, Brown BG, Marcovina SM, Hillger LA, Zhao XQ, Albers JJ. Effects of lowering elevated LDL cholesterol on the cardiovascular risk of lipoprotein(a). *JAMA* 1995 Dec 13;274(22):1771-4

- **In men studied in the Stanford Five-City Project, the most efficient threshold value of Lp(a) concentration for separating cases and control subjects was 35 nmol/L (VAP cholesterol units = 14mg/dl):**

Wild SH, Fortmann SP, Marcovina SM. A prospective case-control study of lipoprotein(a) levels and apo(a) size and risk of coronary heart disease in Stanford Five-City Project participants. *Arterioscler Thromb Vasc Biol.* 1997 Feb;17(2):239-45

- **In most studies, Lp(a) was found to be an independent risk factor. However, in the Quebec Cardiovascular Study, Lp(a) was not an independent risk factor for coronary artery disease but appeared, rather, to increase the risk associated with other lipids:**

Cantin B, Gagnon F, Moojani S, Despres JP, Lainarche B, Lupien PJ, Dagenais GR. Is lipoprotein(a) an independent risk factor for ischemic heart disease in men? The Quebec Cardiovascular Study. *J Am Coll Cardiol.* 1998 Mar 1;31(3):519-25

- **When elevated Lp(a) is associated with the atherogenic lipoprotein profile (low HDL2, elevated dense LDL, IDL, dense VLDL and VLDL, the increased risk is 25.**

If two or more non-lipid risk factors are also present (hypertension, diabetes, cigarette smoking, or high total homocysteine) the increased risk is 122:

Hopkins PN, Wu LL, Hunt SC, James BC, Vincent GM, Williams RR. Lipoprotein(a) interactions with lipid and nonlipid risk factors in early familial coronary artery disease. *Arterioscler Thromb Vasc Biol.* 1997 Nov;17(11):2783-92

- **Multivariate analyses has shown that apo(a) phenotypes were the best predictors of premature CHD (age < 60).**

Gazzaruso C, Garzaniti A, Buscaglia P, Bonetti G, Falcone C, Fratino P, Finardi G, Geroldi D. Association between apolipoprotein(a) phenotypes and coronary heart disease at a young age. *J Am Coll Cardiol.* 1999 Jan;33(1):157-63

- **In another study, it was found that the risk for coronary artery disease increased continuously with a decreasing number of kringle repeats, from a risk of 0.3 to 4.6:**

Kraft HG, Lingenhel A, Kochl S, Hoppichler F, Kronenberg F, Abe A, Muhlberger V, Schonitzer D, Utennann G. Apolipoprotein(a) kringle IV repeat number predicts risk for coronary heart disease. *Arterioscler Thromb Vasc Biol.* 1996 Jun; 16(6):713-9

- **Review of Lp(a) as a risk factor for coronary artery disease, carotid disease and stroke:**

Kronenberg F, Steinmetz A, Kostner GM, Dieplinger H. Lipoprotein(a) in health and disease. *Crit Rev Clin Lab Sci.* 1996 Dec;33(6):495-543

● **Stroke. Lp(a) levels are a predictor for stroke in many studies.**

Peynet J, Beaudoux JL, Woimant F, Flourie F, Giraudeau V, Vicaut E, Launay JM. Apolipoprotein(a) size polymorphism in young adults with ischemic stroke. *Atherosclerosis* 1999 Jan;142(1):233-9

Jurgens Q, Taddei-Peters WC, Koltringer P, Petek W, Chen Q, Greilberger J, Macomber PF, Butman BT, Stead AG, Ransom JH. Lipoprotein(a) serum concentration and apolipoprotein(a) phenotype correlate with severity and presence of ischemic cerebrovascular disease. *Stroke* 1995 Oct;26(10):1841-8

Watts GF, Mazurkiewicz JC, Tonge K, Nelson V, Warburton FG, Slavin BM. Lipoprotein(a) as a determinant of the severity of angiographically defined carotid atherosclerosis. *QJM* 1995 May;88(5):321-6

● **Carotid disease. Lp(a) levels predict the presence and severity of carotid artery disease.**

Yamamoto M, Egusa G, Yamakido M. Carotid atherosclerosis and serum lipoprotein(a) concentrations in patients with NIDDM. *Diabetes Care* 1997 May;20(5):829-31

Tato F, Keller C, Schuster H, Spengel F, Wolfram G, Zollner N. Relation of lipoprotein(a) to coronary heart disease and duplexsonographic findings of the carotid arteries in heterozygous familial hypercholesterolemia. *Atherosclerosis* 1993 Jun;101(1):69-77

● **Post angioplasty restenosis. Reduction in Lp(a) levels significantly reduced restenosis rates.**

Daida H, Lee YJ, Yokoi H, Kanoh T, Ishiwata S, Kato K, Nishikawa H, Takatsu F, Kato H, Kutsunii Y, et al. Prevention of restenosis after percutaneous transluminal coronary angioplasty by reducing lipoprotein(a) levels with low-density lipoprotein apheresis. *Low Density Lipoprotein Apheresis Angioplasty Restenosis Trial (L-ART) Group. Am J Cardiol.* 1994 Jun 1;73(15):1037-40

● **Hypertension. The quantification of Lp(a) levels and the characterization of apo(a) phenotypes was found to be useful for assessment of familial predisposition to coronary artery disease in hypertensives.**

Gazzaruso C, Buscaglia P, Garzaniti A, Bonetti G, Savino S, Mariotti S, Jucci A, Finardi G, Geroldi D. Lipoprotein(a) plasma concentrations, apolipoprotein(a) polymorphism and family history of coronary heart disease in patients with essential hypertension. *J Cardiovasc Risk* 1996 Apr;3(2):191-7

● **Type 2 diabetes. In type 2 diabetics elevated levels of Lp(a) were independently associated with coronary heart disease with an increased risk of 3.48.**

Ruiz J, Thillet J, Huby T, James RW, Erlich D, Flandre P, Froguel P, Chapman J, Passa P. Association of elevated lipoprotein(a) levels and coronary heart disease in NIDDM patients. Relationship with apolipoprotein(a) phenotypes. *Diabetologia* 1994 Jun;37(6):585-91

● **Acute phase response. Lp(a) is an acute phase reactant and increases with the acute phase response.**

Min WK, Lee JO, Huh JW. Relation between lipoprotein(a) concentrations in patients with acute-phase response and risk analysis for coronary heart disease. *Clin Chem.* 1997 Oct;43(10):1891-5

FACTORS THAT MODIFY Lp(a)

Lifestyle

● **Lp(a) levels are not affected by diet or modest exercise.**

Mackinnon LT, Hubinger L, Lepre F. Effects of physical activity and diet on lipoprotein(a). *Med Sci Sports Exerc* 1997 Nov;29(11):1429-36

Genetics

● **Inheritance. Lp(a) is highly inherited; Lp(a) levels in children predict coronary artery disease in parents.**

Routi T, Ronnema T, Jokinen E, Viikari J, Niinikoski H, Leino A, Simell O. Correlation of toddlers' serum lipoprotein(a) concentration with parental values and grandparents' coronary heart disease: the STRIP baby study. *Acta Paediatr.* 1996 Apr;85(4):407-12

Vella JC, Jover E. Relation of lipoprotein(a) in 11 to 19-year-old adolescents to parental cardiovascular heart disease. *Clin Chem.* 1993 Mar;39(3):477-80

● **Ethnicity. Lp(a) levels vary greatly in different ethnic groups.**

Sandholzer C, Saha N, Kark JD, Rees A, Jaross W, Dieplinger H, Hoppichler F, Boerwinkle E, Uterinann G. Apo(a) isoforms predict risk for coronary heart disease. A study in six populations. *Arterioscler Thromb.* 1992 Oct;12(10):1214-26

● **Menopause. Lp(a) levels predict coronary artery disease in both premenopausal and postmenopausal women, with an increase in risk of 5.1 and 2.4 respectively.**

Orth-Gomer K, Mittleman MA, Schenck-Gustafsson K, Wamala SP, Eriksson M, Belkic K, Kirkeeide R, Svane B, Ryden L. Lipoprotein(a) as a determinant of coronary heart disease in young women. *Circulation* 1997 Jan 21;95(2):329-34

- **Familial hypercholesterolemia. Lp(a) levels are much higher in patients with familial hypercholesterolemia and its presence is a strong risk factor for coronary artery disease.**

Drugs

- **Nicotinic acid. Niacin is effective in lowering Lp(a) levels.**

Rodriguez CR, Seman LJ, Ordovas JM, Jenner J, Genest MS Jr, Wilson PW, Schaefer EJ. Lipoprotein(a) and coronary heart disease. *Chem Phys Lipids* 1994 Jan;67-68:389-98

Crouse JR 3rd. New developments in the use of niacin for treatment of hyperlipidemia: new considerations in the use of an old drug. *Coron Artery Dis.* 1996 Apr;7(4):321-6

Illingworth DR, Stein EA, Mitchel YB, Dujovne CA, Frost PH, Knopp RH, Tun P, Zupkis RV, Greguski RA. Comparative effects of lovastatin and niacin in primary hypercholesterolemia. A prospective trial. *Arch Intern Med.* 1994 Jul 25;154(14):1586-95

Seed M, O'Connor B, Perombelon N, O'Donnell M, Reaveley D, Knight BL. The effect of nicotinic acid and acipimox on lipoprotein(a) concentration and turnover. *Atherosclerosis* 1993 Jun;101(1):61-8

- **Statins. HMG CoA reductase inhibitors fail to lower Lp(a) levels.**

Raal FJ, Pilcher G, Rubinsztein DC, Lingenhel A, Utermann G. Statin therapy in a kindred with both apolipoprotein B and low density lipoprotein receptor gene defects. *Atherosclerosis* 1997 Feb 28;129(1):97-102

Illingworth DR, Stein EA, Mitchel YB, Dujovne CA, Frost PH, Knopp RH, Tun P, Zupkis RV, Greguski RA. Comparative effects of lovastatin and niacin in primary hypercholesterolemia. A prospective trial. *Arch Intern Med.* 1994 Jul 25;154(14):1586-95

QUOTES

- **"There is no standard assay for Lp(a)."**
- **"Measuring Lp(a) could be reserved for subjects in whom there is equivocation over how aggressively to treat the traditional CHD risk factors, such as elevated plasma LDL cholesterol. If Lp(a) were found to be high in such a subject, the modifiable CHD risk factors should be addressed more aggressively."**

Hegele RA. Is it time to measure Lp(a) as part of coronary heart disease risk assessment? *Clin Biochem.* 1997 Jul;30(5):443-5

- **"...specific therapy to lower lipoprotein Lp(a) may be indicated for patients with premature coronary atherosclerosis, a strong family history of premature atherosclerosis or refractory hypercholesterolemia."**

Stein JH, Rosenson RS. Lipoprotein Lp(a) excess and coronary heart disease. *Arch Intern Med.* 1997 Jun 9;157(11):1170-6

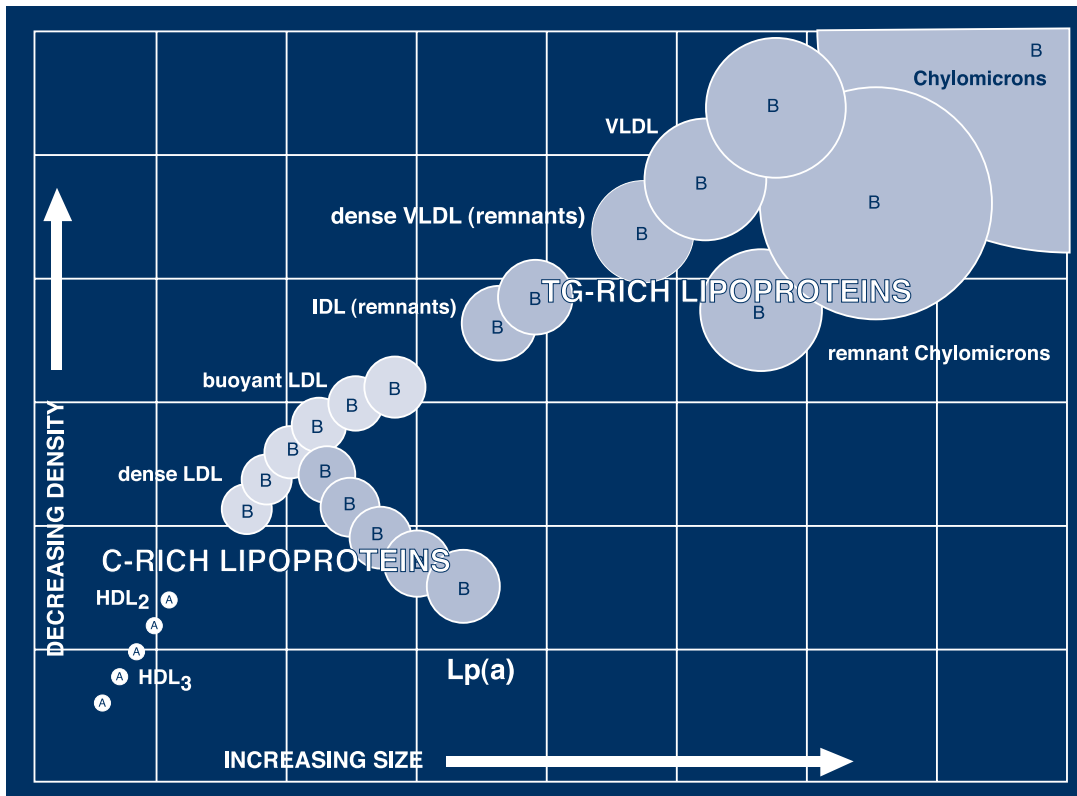
- **"Measurement of Lp(a) may be useful to guide management of individuals with a family history of IHD or with existing disease."**

Craig WY, Neveux LM, Palomaki GE, Cleveland MM, Haddow JE. Lipoprotein(a) as a risk factor for ischemic heart disease: meta-analysis of prospective studies. *Clin Chem.* 1998 Nov;44(11):2301-6

BAD CHOLESTEROL: LDL
WORST CHOLESTEROL: DENSE LDL

BAD CHOLESTEROL: LDL

WORST CHOLESTEROL: DENSE LDL



LDL

LDL (is an apo B-containing, C-rich lipoprotein, that is intermediate in size and density between HDL and IDL. The sole protein component of **LDL** is apo B. Since **LDL** levels are directly related (high is bad, low is good) to risk for atherosclerosis, **LDL** is considered an atherogenic, or so-called "bad" cholesterol.

LDL SUBCLASSES

Six major subclasses have been identified by ultracentrifugation and non-denaturing gradient gel electrophoresis:

- **Pattern B.** The two smallest and most dense of the six subclasses comprise **pattern B (small, dense LDL)**. On a per mg. cholesterol basis these are the most atherogenic of the LDL subclasses (i.e., the "**worst**" cholesterol, see below).
- **Pattern A.** The two largest and least dense of the six subclasses are called **pattern A (large, buoyant LDL)**. On a per mg. cholesterol basis these are the least atherogenic of the LDL subclasses (see below). The treatment goal for **pattern B** is to convert it to **pattern A**.
- **Pattern AB.** The two intermediate particles in size and dense of the six subclasses are called **pattern AB**. On a per mg. cholesterol basis these are intermediate in atherogenic of the LDL subclasses (see below).

DENSE LDL IS THE "WORST" LDL SUBCLASS

- **Coronary artery disease.** A number of clinical studies have demonstrated that on a per mg. cholesterol basis dense LDL is more atherogenic than "bad" cholesterol or total LDL. Thus **dense LDL** can be considered the "**worst**" cholesterol.

– **Reviews of dense LDL and CAD:**

Superko HR. Did grandma give you heart disease? The new battle against coronary artery disease. *Am J Cardiol.* 1998 Nov 5;82(9A):34Q-46Q. **Review.**

Zambon A, et al. Lipoprotein classes and coronary disease regression. *Curr Opin Lipidol.* 1998 Aug;9(4):329-36. **Review.**

Ballantyne CM. Current thinking in lipid lowering. *Am J Med.* 1998 Jun 22; 104(6A):33S- 41S. **Review.**

Lamarche B, et al. Atherosclerosis prevention for the next decade: risk assessment beyond low density lipoprotein cholesterol. *Can J Cardiol.* 1998 Jun;14(6):841-51. **Review.**

Superko HR. What can we learn about dense low density lipoprotein and lipoprotein particles from clinical trials? *Curr Opin Lipidol.* 1996 Dec;7(6):363-8. **Review.**

Austin MA. Genetic epidemiology of dyslipidaemia and atherosclerosis. *Ann Med.* 1996 Oct;28(5):459-63. **Review.**

Austin MA, et al. Small, dense low density lipoproteins, the insulin resistance syndrome and non-insulin dependent diabetes. *Curr Opin Lipidol.* 1996 Jun;7(3):167-71. **Review.**

Superko HR. New aspects of risk factors for the development of atherosclerosis, including small low-density lipoprotein, homocysteine, and lipoprotein(a). *Curr Opin Cardiol.* 1995 Jul;10(4):347-54. **Review.**

Krauss RM. Dense low density lipoproteins and coronary artery disease. *Am J Cardiol.* 1995 Feb 23;75(6):53B-57B. **Review.**

Austin MA, et al. Characterization of low-density lipoprotein subclasses: methodologic approaches and clinical relevance. *Curr Opin Lipidol.* 1994 Dec;5(6):395-403. **Review.**

– **40-50% of patients with CAD have dense LDL:**

Krauss RM. Dense low density lipoproteins and coronary artery disease. *Am J Cardiol.* 1995 Feb 23;75(6):53B-57B

- **Dense LDL is associated with a 3 - 4.5 fold increased risk for coronary artery disease and a 6.9 fold increased risk for myocardial infarction (heart attack); in contrast, total cholesterol and total LDL are associated with only a 2-fold increase in risk for coronary artery disease:**

Griffin BA, Freeman DJ, Tait GW, Thomson J, Caslake MJ, Packard CJ. Role of plasma triglyceride in the regulation of plasma low density lipoprotein (LDL) subfractions: relative contribution of small, dense LDL to coronary heart disease risk. *Atherosclerosis* 1994 Apr;106(2):241-53

Krauss RM. Dense low density lipoproteins and coronary artery disease. *Am J Cardiol.* 1995 Feb 23;75(6):53B-57B.

Kannel WB, Wilson PW. Efficacy of lipid profiles in prediction of coronary disease. *Am Heart J.* 1992 Sep;124(3):768-74

- **Dense LDL was a predictor of future CAD in the prospective Physicians' Health Study:**

Stampfer MJ, et al. A prospective study of triglyceride level, low-density lipoprotein particle diameter, and risk of myocardial infarction. *JAMA* 1996 Sep 18;276(11):882-8

- **LDL-cholesterol lowering by diet and drug treatment resulted in reduced coronary angiographic progression in CAD subjects with predominantly dense LDL, but an equivalent lowering of LDL cholesterol in subjects with more buoyant LDL was not associated with angiographic benefit.**

Gardner CD, Fortmann SP, Krauss RM. Association of small low-density lipoprotein particles with the incidence of coronary artery disease in men and women. *JAMA* 1996 Sep 18;276(11):875-81

- **Conversion from dense to less dense LDL accounts for up to 50% of the regression seen in the FATS trial:**

Brown BG, et al. Use of niacin, statins, and resins in patients with combined hyperlipidemia. *Am J Cardiol.* 1998 Feb 26;81(4A):52B-59B. **Review.**

- **Dense LDL is a better predictor of outcome in two additional regression studies than is LDL:**

Miller BD, et al. Predominance of dense low-density lipoprotein particles predicts angiographic benefit of therapy in the Stanford Coronary Risk Intervention Project. *Circulation* 1996 Nov 1;94(9):2146-53

Watts GF, Mandalia S, Brunt JN, Slavin BM, Coltart DJ, Lewis B. Independent associations between plasma lipoprotein subtraction levels and the course of coronary artery disease in the St. Thomas' Atherosclerosis Regression Study (STARS). *Metabolism* 1993 Nov;42(11):1461-7

- **Dense LDL is a risk for CAD in patients with normal cholesterol:**

Kazurni T, et al. Low density lipoprotein particle diameter in young, non-obese, normolipidemic Japanese men. *Atherosclerosis* 1999 Jan;142(1):113-9

- **Dense LDL is a component of the atherogenic lipoprotein profile:**

Austin MA, King MC, Vranizan KM, Krauss RM. Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk. *Circulation* 1990 Aug;82(2):495-506

- **Peripheral vascular disease. Dense LDL is an independent risk factor for cerebrovascular diseases.**

Landray MJ, et al. Association of atherogenic low-density lipoprotein subfractions with carotid atherosclerosis. *QJM* 1998 May;91(5):345-51

- **Visceral adiposity. Dense LDL is associated independently with truncal obesity or visceral adiposity, a component of the insulin resistance syndrome and type 2 diabetes.**

Despres JP. The insulin resistance-dyslipidemic syndrome of visceral obesity: effect on patients' risk. *Obes Res.* 1998 Apr;6 Suppl 1:8S-17S. **Review.**

- **Insulin resistance. Small, dense LDL is an integral feature of the insulin resistance syndrome. Behavioral or environmental factors are important for the expression of the phenotype and for its association with other heart disease risk factors.**

Hsueh WA, et al. Cardiovascular risk continuum: implications of insulin resistance and diabetes. *Am J Med.* 1998 Jul 6; 105(1 A):4S-14S

Haffner SM. The prediabetic problem: development of non-insulin-dependent diabetes mellitus and related abnormalities. *J Diabetes Complications* 1997 Mar-Apr;11(2):69-76

Austin MA, et al. Small, dense low density lipoproteins, the insulin resistance syndrome and non-insulin-dependent diabetes. *Curr Opin Lipidol.* 1996 Jun;7(3):167-71

Haffner SM, et al. A preponderance of small dense LDL is associated with specific insulin, proinsulin and the components of the insulin resistance syndrome in non-diabetic subjects. *Diabetologia* 1995 Nov;38(11):1328-36

Selby JV, Austin MA, Newman B, Zhang D, Quesenberry CP Jr, Mayer EJ, Krauss RM. LDL subclass phenotypes and the insulin resistance syndrome in women. *Circulation* 1993 Aug;88(2):381-7

- **Type 2 diabetes. Dense LDL predicts future type 2 diabetes.**

Siegel RD, et al. Lipoproteins, apolipoproteins, and low-density lipoprotein size among diabetics in the Framingham offspring study. *Metabolism* 1996 Oct;45(10):1267-72

FACTORS THAT MODIFY DENSE LDL

Lifestyle

- **Diet**

- **Men with dense LDL subclass pattern B had significantly greater reductions in LDL cholesterol (LDL-C) and apolipoprotein B than men with pattern A.**

Dreon DM, Krauss RM. Diet-gene interactions in human lipoprotein metabolism. *J Am Coll Nutr.* 1997 Aug;16(4):313-24

- **The reduction in LDL in women on a low-fat, high-carbohydrate diet is directly related to their number of parents with dense LDL.**

Dreon DM, Fernstrom HA, Williams PT, Krauss RM. LDL subclass patterns and lipoprotein response to a low-fat, high-carbohydrate diet in women. *Arterioscler Thromb Vasc Biol.* 1997 Apr;17(4):707-14

Genetics

- **Inheritance. Dense LDL has a complex inheritance.**

Juo SH, et al. Etiologic heterogeneity of hyperapobetalipoproteinemia (hyperapoB). Results from segregation analysis in families with premature coronary artery disease. *Arterioscler Thromb Vasc Biol.* 1997 Nov;17(11):2729-36

Austin MA. Genetic epidemiology of dyslipidemia and atherosclerosis. *Ann Med.* 1996 Oct;28(5):459-63

- **Gender. Dense LDL is higher in women than in men.**

Gardner CD, et al. Association of small low-density lipoprotein particles with the incidence of coronary artery disease in men and women. *JAMA* 1996 Sep 18;276(11):875-81

Drugs

- **Nicotinic acid. Niacin is the most effective drug for converting dense (class B) to less dense (class A) LDL.**

Brown BG, et al. Use of niacin, statins, and resins in patients with combined hyperlipidemia. *Am J Cardiol.* 1998 Feb 26;81(4A):52B-59B

- **Statins. HMG CoA reductase inhibitors lower all LDL subclasses equally.**

Brown BG, et al. Use of niacin, statins, and resins in patients with combined hyperlipidemia. *Am J Cardiol.* 1998 Feb 26;81(4A):52B-59B

- **β blockers. These drugs increase dense LDL.**

Superko HR, Haskell WL, Krauss RM. Association of lipoprotein subclass distribution with use of selective and non-selective beta-blocker medications in patients with coronary heart disease. *Atherosclerosis* 1993 Jun;101(1):1-8

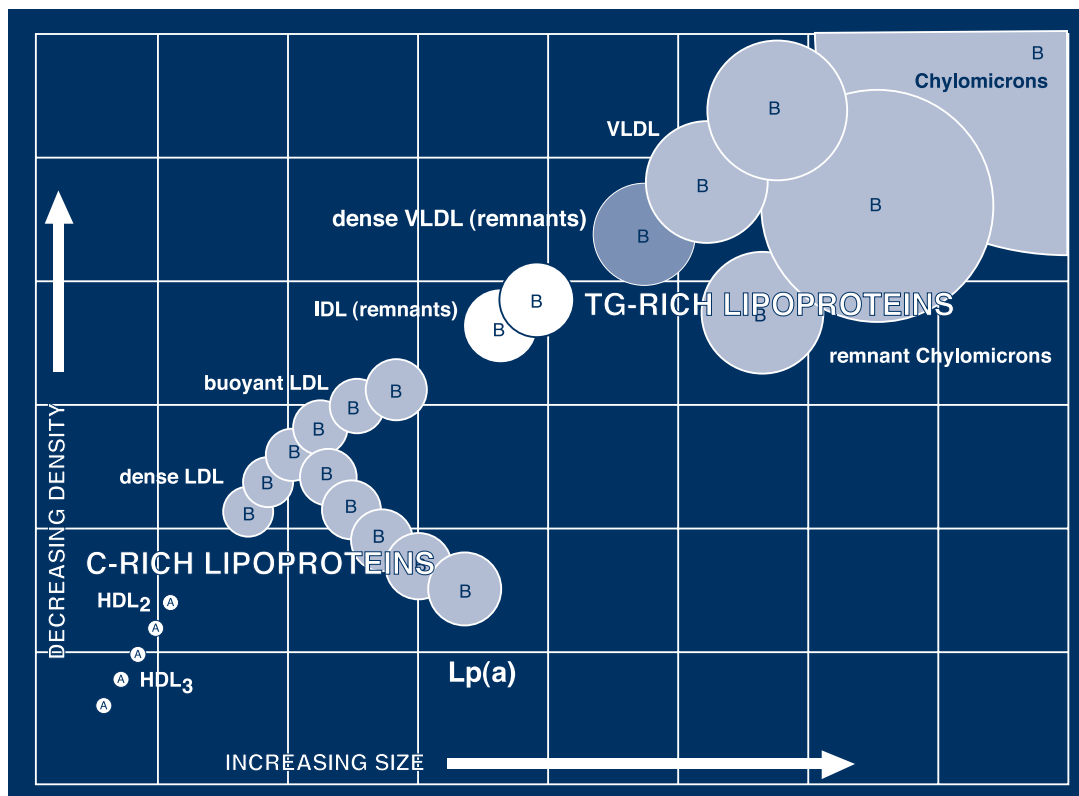
CAVEAT

A number of studies have suggested that dense LDL is not significant in multi-regression analysis, but since there is an inverse association of dense LDL to triglycerides, the univariate significance of dense LDL is the result of elevated triglycerides. However, many other studies do support dense LDL as an independent risk factor from elevated triglycerides. In particular, in the triglyceride range of 70 to 250 mg/dl, up to 50% of patients cannot be classified as to pattern B or A based upon triglyceride alone. Unfortunately, this is the triglycerides range most commonly found in patients with coronary artery disease.

Superko, HR. Small dense LDL: The new coronary artery disease risk factor and how it is changing the treatment of CAD. *Preventive Cardio.* 1998;1:16-24

BAD TRIGLYCERIDES
IDL, DENSE VLDL AND REMNANTS

BAD TRIGLYCERIDES IDL, DENSE VLDL AND REMNANTS



IDL AND VLDL REMNANTS

IDL (intermediate density lipoprotein, is a TG-rich, apo B-containing, lipoprotein intermediate in density and size between LDL and VLDL. It is an intermediate or remnant product of the breakdown of VLDL. Much of the triglyceride content of the original VLDL has been removed to produce a much more cholesterol-rich particle. **IDL** consists of at least two subclasses. This subclass also may contain **chylomicron remnants**. Several studies indicate that **IDL** is an independent risk factor for CAD and in several studies was more atherogenic than LDL (see below).

DENSE VLDL AND CHYLOMICRON REMNANTS

VLDL (very low density lipoprotein) consists of at least three subclasses. The smallest and most dense of these, **dense VLDL** is more cholesterol-rich than are the other larger and less dense VLDL particles. This subclass also may contain **chylomicron remnants**. Studies suggest that both **dense VLDL** and **chylomicron remnants** are independent risk factors for CAD and may be as or more atherogenic than LDL (see below).

IDL IS A "BAD" TRIGLYCERIDE

● **Coronary artery disease.** Multiple clinical studies have demonstrated that IDL, a cholesterol enriched TG-rich lipoprotein, is an independent risk factor for coronary artery disease.

– **Reviews of IDL and coronary artery disease:**

Zambon A, et al. Lipoprotein classes and coronary disease regression. *Curr Opin Lipidol.* 1998 Aug;9(4):329-36.

Thompson GR. Angiographic evidence for the role of triglyceride-rich lipoproteins in progression of coronary artery disease. *Eur Heart J.* 1998 Jul;19 Suppl H:H31-6

Ballantyne CM. Current thinking in lipid lowering. *Am J Med.* 1998 Jun 22;104(6A):33S-41S

Krauss RM. Relationship of intermediate and low-density lipoprotein subspecies to risk of coronary artery disease. *Am Heart J.* 1987 Feb;113(2 Pt 2):578-82

– **At least three prospective studies support the atherogenicity of IDL:**

Mack WJ, Krauss RM, Hodis HN. Lipoprotein subclasses in the Monitored Atherosclerosis Regression Study (MARS). Treatment effects and relation to coronary angiographic progression. *Arterioscler Thromb Vasc Biol.* 1996 May;16(5):697-704

Krauss RM, Lindgren FT, Williams FT, Kelsey SF, Brensike J, Vranizan K, Detre KM, Levy RI. Intermediate-density lipoproteins and progression of coronary artery disease in hypercholesterolaemic men. *Lancet* 1987 Jul 11;2(8550):62-6

Phillips NR, Waters D, Havel RJ. Plasma lipoproteins and progression of coronary artery disease evaluated by angiography and clinical events. *Circulation* 1993 Dec;88(6):2762-70

Quote from Phillips NR, et al. "In patients with established coronary heart disease, increased levels of remnants of triglyceride-rich lipoproteins and decreased levels of high-density lipoproteins appear to promote progression of coronary artery atherosclerosis, which in turn may lead to an untoward clinical event. No such relation could be shown for the level of components of low-density lipoproteins."

– **A number of case-control studies support the atherogenicity of IDL:**

Steiner G, Schwartz L, Shumak S, Poapst M. The association of increased levels of intermediate-density lipoproteins with smoking and with coronary artery disease. *Circulation* 1987 Jan;75(1):124-30

Lipinska I, Gurewich V, Meriam CM, Kosowsky BD, Ramaswamy K, Philbin E, Losordo D. Lipids, lipoproteins, fibrinogen and fibrinolytic activity in angiographically assessed coronary heart disease. *Artery* 1987;15(1):44-60

Meyer E, Westerveld HT, de Ruyter-Meijstek FC, Van Greevenbroek MM, Rienks R, van Rijn HJ, Erkelens DW, de Bruin TW. Abnormal postprandial apolipoprotein B-48 and triglyceride responses in normolipidemic women with greater than 70% stenotic coronary artery disease: a case-control study. *Atherosclerosis* 1996 Aug 2;124(2):221-35

Hamsten A, Wallius G, Szamosi A, Dahlen G, de Faire U. Relationship of angiographically defined coronary artery disease to serum lipoproteins and apolipoproteins in young survivors of myocardial infarction. *Circulation* 1986 Jun;73(6):1097-110

Tatami R, Mabuchi H, Ueda K, Ueda R, Haba T, Kametani T, Ito S, Koizumi J, Ohta M, Miyamoto S, Nakayama A, Kanaya H, Oiwake H, Genda A, Takeda R. Intermediate-density lipoprotein and cholesterol-rich very low density lipoprotein in angiographically determined coronary artery disease. *Circulation* 1981 Dec;64(6):1174-84

– **Elevated IDL is a component of the atherogenic lipoprotein profile:**

Zambon A, et al. Lipoprotein classes and coronary disease regression. *Curr Opin Lipidol.* 1998 Aug;9(4):329-36.

Austin MA, King MC, Vranizan KM, Krauss RM. Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk. *Circulation* 1990 Aug;82(2):495-506

– **Elevated IDL is a component of familial hypercholesterolemia and may be the more atherogenic than the elevated LDL usually thought of as the major atherogenic lipoprotein in this disorder:**

Yanagi K, Yamashita S, Kihara S, Nakamura T, Nozaki S, Nagai Y, Funahashi T, Kameda-Takemura K, Ueyama Y, Jiao S, Kubo M, Tokunaga K, Matsuzawa Y. Characteristics of coronary artery disease and lipoprotein abnormalities in patients with heterozygous familial hypercholesterolemia associated with diabetes mellitus or impaired glucose tolerance. *Atherosclerosis* 1997 Jul 11;132(1):43-51

Brugger D, Schuster H, Zollner N. Familial hypercholesterolemia and familial defective apolipoprotein B - 100: comparison of the phenotypic expression in 116 cases. *Eur J Med Res.* 1996 May 24;1(8):383-6

● **Insulin resistance. Hyperinsulinemia and insulin resistance are associated with elevated levels of IDL.**

Bavenholm P, Karpe F, Proudler A, Tomvall P, Crook D, Hamsten A. Association of insulin and insulin propeptides with an atherogenic lipoprotein phenotype. *Metabolism* 1995 Nov;44(11):1481-8

- **Type 2 diabetes.** Non-insulin dependent diabetics is associated with elevated IDL.

Steiner G. Intermediate-density lipoproteins, diabetes and coronary artery disease. *Diabetes Res Clin Pract.* 1998 Jul;40 Suppl:S29-33

Betteridge DJ. Lipids and atherogenesis in diabetes mellitus. *Atherosclerosis* 1996 Jul;124 Suppl:S43-7

Yoshino G, Hirano T, Kazumi T. Dyslipidemia in diabetes mellitus. *Diabetes Res Clin Pract.* 1996 Jun;33(1):1-14

Quote from Yoshino G, et al. "plasma lipid levels of diabetic subjects must be more strictly controlled than for the non-diabetic population in order to avoid an increased risk for coronary heart disease."

Lifestyle

- **Since IDL is under strong genetic control, diet and exercise lower IDL only modestly.**

Zambon A, et al. Lipoprotein classes and coronary disease regression. *Curr Opin Lipidol.* 1998 Aug(4):329-36

Segrest JP, unpublished observations.

Genetics

- **Inheritance. Elevated IDL is a component of both the atherogenic lipoprotein profile and familial combined hyperlipidemia and thus is under strong genetic control.**

Juo SH, et al. Etiologic heterogeneity of hyperapobetalipoproteinemia (hyperapoB). Results from segregation analysis in families with premature coronary artery disease. *Arterioscler Thromb Vasc Biol.* 1997 Nov;17(11):2729-36

Kontopoulos AG, Athyros VG, Papageorgiou AA, Hatzikonstandinou HA, Mayroudi MC, Boudoulas H. Effects of simvastatin and ciprofibrate alone and in combination on lipid profiles, plasma fibrinogen and low density lipoprotein particle structure and distribution in patients with familial combined hyperlipidemia and coronary artery disease. *Coron Artery Dis.* 1996 Nov;7(11):843-50

Austin MA. Genetic epidemiology of dyslipidemia and atherosclerosis. *Ann Med.* 1996 Oct;28(5):459-63

- **Gender. IDL is higher in men than in women.**

Gardner CD, et al. Association of small low-density lipoprotein particles with the incidence of coronary artery disease in men and women. *JAMA.* 1996 Sep 18;276(11):875-81

Austin MA, King MC, Vranizan KM, Krauss RM. Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk. *Circulation.* 1990 Aug;82(2):495-506

Drugs

- **Both nicotinic acid and statins lower IDL and do it most effectively in combination. Fibrates may also lower IDL but less effectively.**

Zambon A, et al. Lipoprotein classes and coronary disease regression. *Curr Opin Lipidol.* 1998 Aug;9(4):329-36.

Thompson GR. Angiographic evidence for the role of triglyceride-rich lipoproteins in progression of coronary artery disease. *Eur Heart J* 1998 Jul;19 Suppl H:H31-6

Ballantyne CM. Current thinking in lipid lowering. *Am J Med* 1998 Jun 22;104(6A):33S-41S

Kontopoulos AG, Athyros VG, Papageorgiou AA, Hatzikonstandinou HA, Mayroudi MC, Boudoulas H. Effects of simvastatin and ciprofibrate alone and in combination on lipid profiles, plasma fibrinogen and low density lipoprotein particle structure and distribution in patients with familial combined hyperlipidemia and coronary artery disease. *Coron Artery Dis.* 1996 Nov;7(11):843-50

Segrest JP, unpublished observations.

DENSE VLDL IS ALSO A "BAD" TRIGLYCERIDE

– Review of dense VLDL, another cholesterol enriched TG-rich lipoprotein:

Zambon A, et al. Lipoprotein classes and coronary disease regression. *Curr Opin Lipidol.* 1998 Aug;9(4):329-36. Review. IDL, SMALL VLDL

– Dense VLDL is the best predictor of progression of coronary artery disease in the MARS regression trial:

Mack WJ, et al. Lipoprotein subclasses in the Monitored Atherosclerosis Regression Study (MARS). Treatment effects and relation to coronary angiographic progression. *Arterioscler Thromb Vasc Biol.* 1996 May;16(5):697-704

– Dense VLDL is a predictor of severity of coronary artery disease:

Tornvall P, et al. Relation of plasma levels and composition of apolipoprotein B-containing lipoproteins to angiographically defined coronary artery disease in young patients with myocardial infarction. *Circulation* 1993 Nov;88(5 Pt 1):2180-9

● Insulin resistance. Hyperinsulinemia and insulin resistance are associated with elevated levels of dense VLDL.

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● Type 2 diabetes. Non-insulin dependent diabetics is associated with elevated dense VLDL.

Erkelens DW. Diabetic dyslipidaemia. *Eur Heart J.* 1998 Jul;19 Suppl H:H27-40. Review.

TG-RICH REMNANTS ARE YET ANOTHER FORM OF "BAD" TRIGLYCERIDE

● Coronary artery disease. Multiple clinical studies have demonstrated that TG-rich lipoprotein remnants, cholesterol enriched TG-rich lipoproteins, are an independent risk factor for coronary artery disease.

– Reviews of remnants and coronary artery disease:

Rubinfeld M, Coletti AT, Mosea L. Treatment strategies for management of serum lipids: lessons learned from lipid metabolism, recent clinical trials, and experience with the HMG CoA reductase inhibitors. *Prog Cardiovasc Dis.* 1998 Sep-Oct;41(2):95-116

Thompson GR. Angiographic evidence for the role of triglyceride-rich lipoproteins in progression of coronary artery disease. *Eur Heart J.* 1998 Jul;19 Suppl H:H31-6

Ballantyne CM. Current thinking in lipid lowering. *Am J Med.* 1998 Jun 22;104(6A):33S-41S

Cohn JS. Postprandial lipidemia: emerging evidence for atherogenicity of remnant lipoproteins. *Can J Cardiol.* 1998 May;14 Suppl B:18B-27B.

LaRosa JC. Triglycerides and coronary risk in women and the elderly. *Arch Intern Med.* 1997 May 12;157(9):961-8

Davignon J, Cohn JS. Triglycerides: a risk factor for coronary heart disease. *Atherosclerosis* 1996 Jul;124 Suppl:S57-64

Quote from Davignon J, et al. "...subgroup and meta-analyses have supported an independent association between TG and CHD. The strength of TG to predict the CHD lies in its ability to reflect the presence of atherogenic plasma TG-rich lipoprotein (TRL) remnants. Emerging data from angiographic intervention trials have implicated TRL in atherosclerotic disease progression independently of low-density lipoproteins (LDL). Thus, in hypertriglyceridemic patients, physicians should conduct a thorough clinical evaluation, a family survey, an assessment of associated risk factors and a complete analysis of the plasma lipoprotein profile, in order to assess the atherogenic potential of this hyperlipidemia."

Havel RJ. Postprandial hyperlipidemia and remnant lipoproteins. *Curr Opin Lipidol.* 1994 Apr;5(2):102-9

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Quote from Breslow JL. "Four types of abnormalities are frequently seen: increased LDL cholesterol levels; decreased high density lipoprotein cholesterol levels, usually accompanied by increased triglyceride or very low density lipoprotein levels; increased concentrations of chylomicron remnants and intermediate density lipoproteins; and increased concentrations of an abnormal lipoprotein, lipoprotein(a). One

or more of these abnormalities is present in 50 - 80% of myocardial infarction survivors."

– **A number of case-control studies support the atherogenicity of IDL:**

Masuoka H, Ishikura K, Kamei S, Obe T, Seko T, Okuda K, Koyabu S, Tsuneoka K, Tamai T, Sugawa M, Nakano T. Predictive value of remnant-like particles cholesterol/high-density lipoprotein cholesterol ratio as a new indicator of coronary artery disease. *Am Heart J.* 1998 Aug;136(2):226-30

Devaraj S, Vega G, Lange R, Grundy SM, Jialal I. Remnant-like particle cholesterol levels in patients with dysbetalipoproteinemia or coronary artery disease. *Am J Med.* 1998 May;104(5):445-50

Sakata K, Miho N, Shirohani M, Yoshida H, Takada Y, Takada A. Remnant-like particle cholesterol is a major risk factor for myocardial infarction in vasospastic angina with nearly normal coronary artery. *Atherosclerosis* 1998 Feb;136(2):225-31

Nakajima K, Saito T, Tamura A, Suzuki M, Nakano T, Adachi M, Tanaka A, Tada N, Nakamura H, Campos E, et al. Cholesterol in remnant-like lipoproteins in human serum using monoclonal anti apo B-100 and anti apo A-I immunoaffinity mixed gels. *Clin Chim Acta.* 1993 Dec 31;223(1-2):53-71

Phillips NR, Waters D, Havel RJ. Plasma lipoproteins and progression of coronary artery disease evaluated by angiography and clinical events. *Circulation* 1993 Dec;88(6):2762-70

Quote from Phillips NR, et al. "In patients with established coronary heart disease, increased levels of remnants of triglyceride-rich lipoproteins and decreased levels of high-density lipoproteins appear to promote progression of coronary artery atherosclerosis, which in turn may lead to an untoward clinical event. No such relation could be shown for the level of components of low-density lipoproteins."

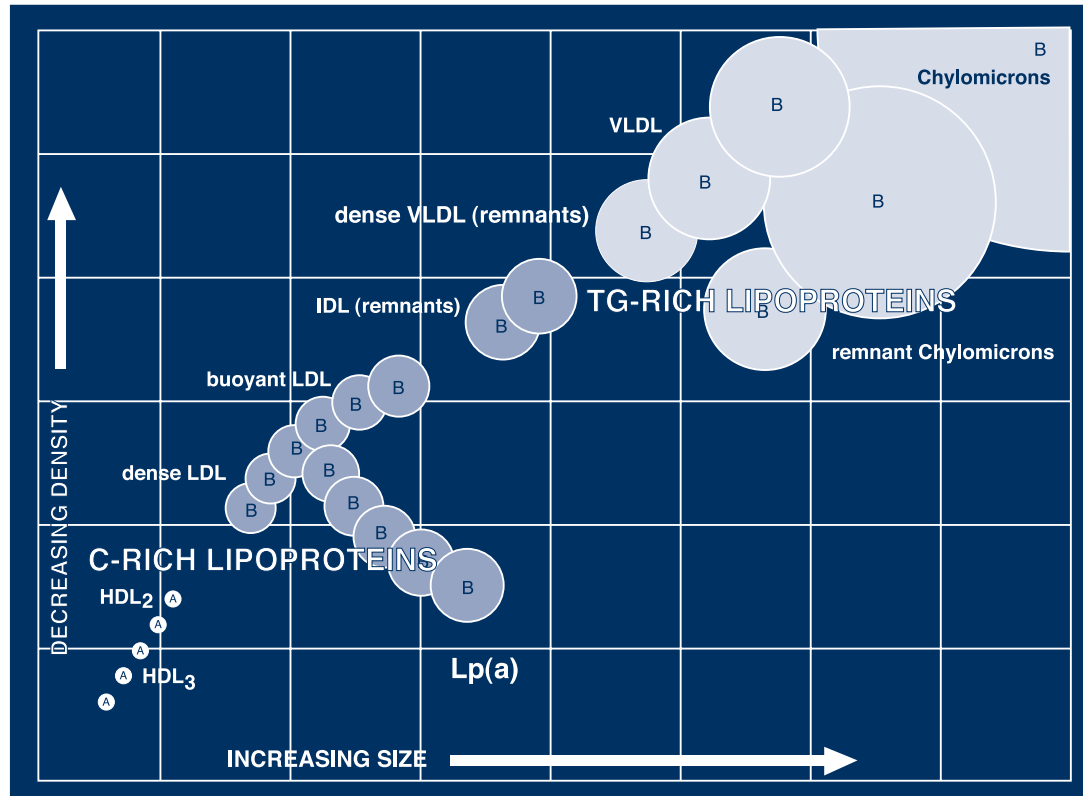
– **Remnants impair endothelium-dependent acetylcholine-induced coronary artery relaxation:**

Inoue T, Saniabadi AR, Matsunaga R, Hoshi K, Yaguchi I, Morook. Impaired endothelium-dependent acetylcholine-induced coronary artery relaxation in patients with high serum remnant lipoprotein particles. *Atherosclerosis* 1998 Aug;139(2):363-7

Kugiyama K, Doi H, Motoyama T, Soejima H, Misumi K, Kawano H, Nakagawa O, Yoshimura M, Ogawa H, Matsumura T, Sugiyama S, Nakano T, Nakajima K, Yasue H. Association of remnant lipoprotein levels with impairment of endothelium-dependent vasomotor function in human coronary arteries. *Circulation* 1998 Jun 30;97(25):2519-26

CALCULATED VS. MEASURED LDL

CALCULATED VS. MEASURED LDL



CALCULATED LDL

- **LDL is calculated by the Friedewald equation, $LDL = [Total\ C] - [HDL\text{-}C] - [TG/5]$.**

Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem.* 1972 Jun;18(6):499-502

- **Correlation coefficients for calculated LDL compared to measured LDL from normal subjects are good to excellent [$r=0.94 - 0.99$].**

Rifai N, Warnick GR, McNamara JR, Belcher JD, Grinstead GF, Frantz ID Jr. Measurement of low-density-lipoprotein cholesterol in serum: a status report. *Clin Chem.* 1992 Jan;38(1):150-60

- **Unfortunately, patients at high risk for coronary artery disease do not have normal lipoprotein profiles.**

- **40 - 50% of patients with coronary artery disease have the atherogenic lipoprotein profile with elevated IDL, triglycerides, dense LDL and dense VLDL, and low HDL₂:**

Austin MA, King MC, Vranizan KM, Krauss RM. Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk. *Circulation* 1990 Aug;82(2):495-506

- **Most subjects with insulin resistance and/or type 2 diabetes have an atherogenic lipoprotein profile:**

Siegel RD, et al. Lipoproteins, apolipoproteins, and low-density lipoprotein size among diabetics in the Framingham offspring study. *Metabolism* 1996 Oct;45(10):1267-72

- **Hypertension is often associated with both the insulin resistance syndrome and an atherogenic lipoprotein profile:**

Williams RR, Hopkins PN, Hunt SC, Schumacher MC, Elbein SC, Wilson DE, Stults BM, Wu LL, Hasstedt SJ, Lalouel JM. Familial dyslipidemic hypertension and other multiple metabolic syndromes. *Ann Med.* 1992 Dec;24(6):469-75

Williams RR, Hunt SC, Hopkins PN, Stults BM, Wu LL, Hasstedt SJ, Barlow GK, Stephenson SH, Lalouel JM, Kuida H. Familial dyslipidemic hypertension. Evidence from 58 Utah families for a syndrome present in approximately 12% of patients with essential hypertension. *JAMA* 1988 Jun 24;259(24):3579-86

- **20% or so with premature coronary artery disease or a family history of premature coronary artery disease have elevated Lp(a):**

Schaefer EJ, Genest JJ Jr, Ordovas JM, Salem DN, Wilson PW. Familial lipoprotein disorders and premature coronary artery disease. *Atherosclerosis* 1994 Aug;108 Suppl:S41-54

● **Calculated LDL is particularly inaccurate in the high risk patient, the patient who most needs an LDL measurement.**

- **The Friedewald formula "is not in fact a true estimate of LDL cholesterol but rather of LDL cholesterol along with variable, usually smaller, amounts of intermediate-density lipoprotein (IDL) cholesterol and lipoprotein(a)."**

Frost PH, Havel RJ. Rationale for use of non-high-density lipoprotein cholesterol rather than low-density lipoprotein cholesterol as a tool for lipoprotein cholesterol screening and assessment of risk and therapy. *Am J Cardiol.* 1998 Feb 26;81(4A):26B-31

- **The Friedewald formula becomes progressively less accurate as plasma triglyceride concentrations increase. An accuracy of 10% or better was achieved when TG levels were: > 200 mg/dl, 84% of the time; 200-300 mg/dl, 77% of the time; 300-400 mg/dl, 59% of the time; 400-600 mg/dl, 41% of the time.**

Rifai N, Warnick GR, McNamara JR, Belcher JD, Grinstead GF, Frantz ID Jr. Measurement of low-density lipoprotein cholesterol in serum: a status report. *Clin Chem.* 1992 Jan;38(1):150-60

McNamara JR, Cohn JS, Wilson PW, Schaefer EJ. Calculated values for low-density lipoprotein cholesterol in the assessment of lipid abnormalities and coronary disease risk. *Clin Chem.* 1990 Jan;36(1):36-42

- **The Friedewald formula should not be used for management of lipoprotein abnormalities in patients with type 2 diabetes:**

Whiting MJ, Shephard MD, Tallis GA. Measurement of plasma LDL cholesterol in patients with diabetes. *Diabetes Care* 1997 Jan;20(1):12-4

Rubies-Prat J, Reverter JL, Senti M, Pedro-Botet J, Salinas I, Lucas A, Nogues X, Sanmarti A. Calculated low-density lipoprotein cholesterol should not be used for management of lipoprotein abnormalities in patients with diabetes mellitus. *Diabetes Care* 1993 Aug;16(8):1081-6

- **The Friedewald formula is inaccurate in patients with IDL elevation:**

Senti M, Pedro-Botet J, Nogues X, Rubies-Prat J. Influence of intermediate-density lipoproteins on the accuracy of the Friedewald formula. *Clin Chem.* 1991 Aug;37(8):1394-7

“DIRECT LDL”

- **The LDL measurements using these kits marketed by Sigma are generally no more accurate, and some times less accurate, than LDL calculations using the Friedewald formula:**

Whitting MJ, Shepard MD, Tallis GA. Measurement of plasma LDL cholesterol in patients with diabetes. *Diabetes Care* 1997 Jan;20(1):12-4

Schectman G, Patsches M, Sasse EA. Variability in cholesterol measurements: comparison of calculated and direct LDL cholesterol determinations. *Clin Chem* 1996 May;42(5):732-7

Jialal I, Hirany SV, Devaraj S, Sherwood TA. Comparison of an immunoprecipitation method for direct measurement of LDL-cholesterol with beta-qualification (ultracentrifugation). *Am J Clin Pathol.* 1995 Jul;104(1):76-81

COMPARISON OF THE VAP PROCEDURE WITH ALTERNATE METHODS FOR MEASURING LDL AND OTHER LIPOPROTEIN CLASSES

Measurement of total cholesterol in the blood has been the most common method for assessment of risk for coronary artery disease. The National Cholesterol Education Program (NCEP) recommended in 1985 that all Americans should know their "cholesterol number." However, it is now well recognized that a total cholesterol value provides very incomplete information regarding relative risk, and that a cholesterol profile is far more valuable in assessing risk. The NCEP has in fact recently (1993) amended its guidelines to suggest that HDL cholesterol be included in all routine cholesterol screening. Many physicians believe that even the inclusion of HDL is insufficient for the determination of risk, and that a complete cholesterol profile, such as is provided by VAP, is necessary. Most clinical laboratories offer a number of standard lipid (including cholesterol and triglyceride) profiles, while a number of more specialized tests are becoming available to address this issue. These tests, starting with the VAP, will be discussed here.

VAP METHOD

VAP (Vertical Auto Profile) is a procedure for determining a cholesterol profile; that is, the cholesterol content of all lipoprotein particles in the blood. Alterations in lipoprotein levels are called dyslipidemias, and can be classified by the lipoprotein cholesterol profile or by the cause of the dyslipidemia. Different dyslipidemias require different dietary and drug treatments. Thus, measurement of all lipoproteins is important in assessing risk of CAD, as well as establishing and monitoring treatment.

- **Vertical density gradient ultracentrifugation.** VAP separates lipoproteins, according to density, by density gradient ultracentrifugation. Serum samples are placed in an ultracentrifuge tube and adjusted to a high density; a lower density solution is layered on top of serum samples, and the tubes are placed in a vertical ultracentrifuge rotor. During centrifugation, the two density layers blend to form a continuous density gradient, and the lipoproteins present in the serum sample float toward the top of the tube and stop at their characteristic density. Thus, VLDL will float to the top of the tube, LDL will form a band in the middle, and HDL will remain near the bottom of the tube. Use of the vertical rotor provides an optimal combination of resolution of individual lipoprotein families and speed of separation.
- **VAP profile.** Following separation, the tubes are placed in a fractionator assembly and punctured through the bottom with a needle. The material in the tube is drained from the bottom into a Technicon AutoAnalyzer (VAP-I) or a reaction coil (VAP-II), not yet used in the clinical laboratory, where it is mixed with an enzymatic reagent. The enzymatic reagent reacts with the cholesterol in the sample to form a colored product which can be detected and measured photometrically. Thus, a cholesterol profile is developed due to the amount of color formation from the bottom to the top of the tube.

The use of a vertical rotor to provide a spin time of less than one hour, semi-automated continuous flow cholesterol analysis and computer programs to decompose the individual lipoprotein curves allow up to 49 individual VAP profiles to be run in a given 8 hr. shift per ultracentrifuge per VAP analyzer. Each rotor contains a control sample of known cholesterol content, as determined by the UAB Hospital Clinical Laboratory and the Northwest Lipid Research Laboratory (NWLRL), part of the National Cholesterol Reference Laboratory Network coordinated by the Centers for Disease Control.

- **Quantification of lipoprotein subclasses.** Total cholesterol in each sample is determined by comparison of the total area under the profile with the area of the control. Subcurves corresponding to each lipoprotein are decomposed from the total profile using computer software developed in our laboratory, and the cholesterol content of each lipoprotein is again determined by measuring the area under the subcurve. Accuracy of VAP is ensured by extensive quality control procedures. Variability of results is minimized by constant monitoring of total and lipoprotein cholesterol values in the control; rotors whose control values are out of acceptable range are repeated. Accuracy is ensured by frequent comparison of random samples with results from NWLRL, and participation in the AAB Proficiency program.

There are two basic definitions of LDL: that which we term "real LDL" and that which we term "LDL-C(NCEP)"; the latter contains Lp(a), "real LDL" and IDL. Additionally, "real LDL" occurs within the population as a number of different density particles; small, dense "real LDL" is associated with a high risk for coronary artery disease. A wide variety of methodologies have been used for the determination of serum LDL-C concentrations: none, except VAP, measure "real LDL" and determine its density. Many of these techniques are cumbersome and time consuming.

OTHER METHODS

It is clear that methods are needed for measuring LDL and other lipoprotein classes. In this section, available methods for measuring lipoproteins will be compared and contrasted to the VAP procedure.

- **HDL precipitation.** The simplest lipid profile includes measurement of total and HDL cholesterol. Generally, total cholesterol is first measured, then VLDL, IDL, and LDL are removed from the blood by a chemical precipitation method.

The remaining cholesterol is presumed to be in HDL. Precipitation methods are simple and rapid, but generally over-estimate HDL, since the other lipoproteins are not completely removed from the blood. In addition, precipitation methods give inaccurate results when serum triglyceride values are high. The ability of VAP to measure lipoprotein cholesterol levels is generally unaffected by triglyceride levels.

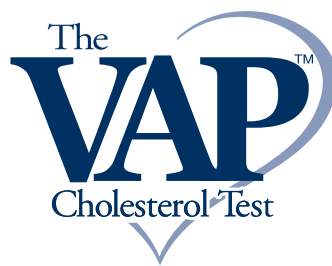
- **HDL precipitation plus triglyceride measurement.** Lipid profiles are also commonly available which include triglyceride levels as well as total and HDL cholesterol. A value for LDL cholesterol is calculated using the Friedewald formula, which assumes that VLDL cholesterol can be numerically related to the triglyceride level. LDL cholesterol is calculated to be total cholesterol (HDL + VLDL cholesterol). Thus, IDL and Lp(a) cholesterol are not separately measured, and their values are included in the LDL cholesterol level reported. Using this procedure, neither LDL or VLDL cholesterol are directly measured. In addition, multiple measurements increase accuracy.

Accuracy of this formula depends on the observation that serum triglyceride concentrations are highly correlated with VLDL-C concentrations. The Friedewald equation tends to become less reliable for estimating LDL-C when plasma triglyceride concentrations are high (>250 - 400 mg./dl) because the ratio of VLDL cholesterol to serum triglyceride changes as serum triglyceride levels increase because the ratio of VLDL-C to serum triglyceride gradually changes as serum triglyceride concentrations increase. The multiple analyses involved in this procedure, along with assumptions made may result in inaccurate lipoprotein cholesterol values. The ability of the VAP procedure to measure LDL is unaffected by triglyceride levels.

- **Density-Gradient Ultracentrifugation.** With the density-gradient ultracentrifugation technique, different density solutions are carefully layered into each tube along with the sample. After ultracentrifugation to equilibrium, each of the lipoproteins will have migrated into its respective isopycnic density region. These approaches have the advantages of separating all lipoprotein classes in a single centrifugation step; the drawbacks include: 1) the time involved in layering the gradients, 2) collecting the fractions after centrifugation, and 3) analyzing each of the fractions. The VAP procedure, which is a variation on the density-gradient ultracentrifugation procedure, overcomes all of these drawbacks by use of a vertical rotor for more rapid separation and by use of an autosampler for continuous fraction analysis.
- **Electrophoretic methods.** These separate lipoproteins according to their charge and size. Studies suggest that many of the electrophoretic methods do not achieve acceptable precision. In addition, proficiency surveys demonstrate substantial inaccuracy for the electrophoretic methods in common use in routine laboratories.
- **Sequential Gradient Ultracentrifugation.** Sequential gradient ultracentrifugation involves one or more successive ultracentrifugation steps, depending on the desired lipoprotein class separation (19). VLDL cholesterol is calculated as plasma cholesterol minus infranatant cholesterol; LDL-C is calculated as infranatant cholesterol minus HDL cholesterol. This procedure is called the quantification method and has been adopted for use by Lipid Research Clinics (20). Additional ultracentrifugation steps can be performed to individually separate additional density classes. The drawbacks of this method for a complete lipoprotein profile are: 1) the limited number of samples possible to analyze per day per centrifuge (less than 3), 2) the inability to separate Lp(a) from LDL and the full extra day required to measure IDL means that "real LDL" cannot be measured, and 3) the potential for the lipoprotein particles being disrupted during centrifugation. The VAP procedure overcomes all of these drawbacks.
- **Chromatographic methods.** Several chromatographic techniques based upon the great differences in size between the various lipoproteins for quantification of difference lipoprotein have been described. The major drawbacks of the commonly used agarose column chromatography are the length of the procedure (24 h) and the inability to obtain homogeneous lipoprotein fractions. The development of methodologies based on high pressure liquid chromatography has alleviated some of these problems. The major drawback with this procedure is the inability to separate "real LDL" from Lp(a) and IDL. The use of chromatographic techniques has been restricted to research laboratories because of the complexity of procedures and instruments and the length of analysis time.
- **Precipitation methods.** Three methods for selective chemical precipitation of LDL have been introduced: heparin precipitation, polyvinyl sulfate precipitation, and the bio-Merieux method. The precipitation methods give inaccurate results when serum triglyceride values are high (indeed, they seem to be worse than the Friedewald calculation in that regard) and they give falsely high results in the presence of VLDL remnants. The precipitation methods, like all routine methods other than VAP, do not distinguish between Lp(a) and "real LDL."
- **Direct LDL.** Kits from Genzyme and Sigma Chemical Company purport to measure LDL directly. Literature from these companies suggest that the tests use antibodies to precipitate HDL and perhaps VLDL. Cholesterol is then measured on the remaining material. It is extremely likely that Lp(a) would be included in the reported LDL value. In addition, it is not clear as to whether IDL or VLDL are removed before cholesterol measurement. Unlike VAP, which does directly measure real LDL separately from Lp(a), IDL, and VLDL, the Direct LDL test gives no information regarding density of the LDL particles.
- **Lp(a) immunoassays.** Measurement of Lp(a) by common immunological techniques is complicated by the presence of multiple classes of Lp(a), each of which may react differently in these techniques. These tests measure the apo(a) protein level; Lp(a) levels are commonly expressed as "mass units," which assume a constant protein:lipid ratio in all the Lp(a) classes. Although this assumption simplifies calculation of Lp(a) levels, it can lead to inaccurate reporting of these levels, depending on the major Lp(a) class present. VAP measures cholesterol in Lp(a), which is not dependent on the class of Lp(a) present.

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