

Smith SC Jr, Blair SN, Criqui MH, Fletcher GF, Fuster V, Gersh BJ, Gotto AM, Gould KL, Greenland P, Grundy SM, Hill MN, Hlatky MA, Houston-Miller N, **Krauss RM**, LaRosa J, Ockene IS, Oparil S, Pearson TA, Rapaport E, Starke RD

**Preventing heart attack and death in patients with coronary disease. Endorsed by the board of trustees of the American College of Cardiology. [Review] [13 refs]**

Cardiovascular Nursing 1996 Jul-Aug;32(4):26-8

**Krauss RM**

Donner Laboratory, Lawrence Berkeley Laboratory, University of California, Berkeley 94720.

**Dense low density lipoproteins and coronary artery disease. [Review] [35 refs]**

American Journal of Cardiology 1995 Feb 23;75(6):53B-57B

A common, genetically influenced lipoprotein subclass profile characterized by a predominance of small, dense low density lipoprotein (LDL) particles is associated with relative increases in plasma triglyceride and apolipoprotein (apo) B-100, and reduced levels of high density lipoprotein cholesterol and apoA1. Recently, this phenotype has also been associated with the insulin resistance syndrome and familial combined hyperlipidemia. Case-control studies of patients with myocardial infarction and angiographically documented coronary artery disease (CAD) have demonstrated that 40-50% of patients have the small, dense LDL phenotype and that this is associated with a 2- to 3-fold increase in disease risk. However, because of strong statistical correlations among the multiple features of the phenotype, it has been difficult to determine whether 1 of its metabolic alterations are primarily responsible for increased CAD susceptibility. More direct evidence for enhanced atherogenicity of lipoproteins in this trait derives from a recent report that LDL-cholesterol lowering by diet and drug treatment resulted in reduced coronary angiographic progression in CAD subjects with predominantly dense LDL, but that an equivalent lowering of LDL cholesterol in subjects with more buoyant LDL was not associated with angiographic benefit. Further, in vitro findings have indicated increased susceptibility of small, dense LDL to oxidative modification and relatively greater binding of these particles to arterial wall proteoglycans. Thus, the small, dense LDL trait may underlie familial predisposition to CAD in a large proportion of the population, and its presence may indicate the potential for benefit from specific therapeutic interventions. [References: 35]

**Krauss RM**

Life Sciences Division, Lawrence Berkeley Laboratory, University of California, Berkeley.

**Heterogeneity of plasma low-density lipoproteins and atherosclerosis risk. [Review] [98 refs]**

Current Opinion In Lipidology 1994 Oct;5(5):339-49

Increased levels of IDL and small, dense LDL are associated with the risk of coronary artery disease. Possible mechanisms include increased susceptibility of small, dense LDL to oxidation, and to other pathologic effects, such as increased retention in the arterial wall. Beneficial effects of a low-fat diet and certain lipid-lowering therapies on the levels and properties of small, dense LDL or their precursors may contribute substantially to the reductions in coronary atherosclerosis observed in several lipid-lowering trials. [References: 98]

**Superko HR, Krauss RM**

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**Coronary artery disease regression. Convincing evidence for the benefit of aggressive lipoprotein management. [Review] [75 refs]**

Circulation 1994 Aug;90(2):1056-69

BACKGROUND: Numerous reports suggest that coronary artery disease can regress with lipoprotein manipulation. Many of these reports lack control groups and contain relatively small numbers. METHODS AND RESULTS: Ten randomized controlled clinical trials using coronary arteriography to assess the effect of lipoprotein manipulation on the rate of progression and regression of atherosclerosis have been either published or

reported as an abstract at a national meeting. These studies were critically reviewed for individual differences and combined clinically applicable lessons. These trials involved a total of 2095 subjects and have consistently reported reduction in the percentage of patients arteriographically defined as progressing (mean, 23.6%) and an increase in the percentage regressing (mean, 20.0%) compared with control groups. Compared with large clinical trials using clinical end points, lipoprotein change was greater, achieving on average a 28% reduction in low-density lipoprotein cholesterol, 11% reduction in triglycerides, and 11% increase in high-density lipoprotein cholesterol compared with control groups. Four investigations used a nonpharmacological approach, and seven used single and multiple drug therapy combined with diet. Despite the relatively brief treatment time of often 2 to 4 years, clinical events were fewer in the treatment groups; within some studies, this reached statistical significance. Side effects from the different therapies were tolerated by most patients, and severe adverse clinical events were few. CONCLUSIONS: These trials present convincing evidence that aggressive lipoprotein manipulation can result in improved arteriographic measurements and fewer cardiovascular events in a relatively short period of time of 2 to 4 years. Extrapolation of this information to the larger population with known coronary artery disease suggests that directed lipoprotein manipulation can reduce clinical events in a cost-effective manner. [References: 75]

**Tribble DL, Krauss RM**

Department of Molecular and Nuclear Medicine, Lawrence Berkeley Laboratory, University of California, Berkeley.

**HDL and coronary artery disease. [Review] [193 refs]**

Advances in Internal Medicine 1993;38:1-29

**Hodis HN, Mack WJ, Dunn M, Liu C, Liu C, Selzer RH, Krauss RM**

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**Intermediate-density lipoproteins and progression of carotid arterial wall intima-media thickness.**

Circulation 1997 Apr 15;95(8):2022-6

BACKGROUND: Although LDL cholesterol (LDL-C) is generally accepted to be a major risk factor for progression of atherosclerosis, the traditional measurement of LDL-C includes measurement of IDL. Little is known about the relationship between IDL and progression of atherosclerosis. Therefore, we investigated the association of plasma lipoprotein subclasses with progression of preinvasive carotid artery atherosclerosis in the Monitored Atherosclerosis Regression Study (MARS). METHODS AND RESULTS: MARS was a randomized, double-blind, placebo-controlled serial arterial imaging trial conducted in subjects 37 to 67 years old with angiographically defined coronary artery disease. Analytical ultracentrifugation was used to determine lipoprotein subclasses, including LDL (Sf 0 to 12), IDL (Sf 12 to 20), VLDL (Sf 20 to 400), and HDL (F1.20 0 to 9) in 188 subjects. Subjects were randomized to a cholesterol-lowering diet plus placebo or lovastatin 80 mg/d. The outcome measure, the annual progression rate of the distal common carotid artery far wall intima-media thickness determined by high resolution B-mode ultrasonography, was determined at baseline and every 6 months on trial. When the major apolipoprotein B-containing lipoproteins were measured independently, IDL ( $r=.21$ ,  $P<.005$ ) but not VLDL ( $r=-.09$ ,  $P=.24$ ) or LDL ( $r=.09$ ,  $P=.26$ ) was associated with the progression of carotid artery intima-media thickness. CONCLUSIONS: These data provide further evidence for the role of triglyceride-rich lipoproteins in the progression of atherosclerosis and support the evidence that indicates that the risk of atherosclerosis attributable to LDL-C may in part be the result of lipoproteins in the IDL fraction (Sf 12 to 20) that is included within the traditional measurements of LDL-C.

**Dreon DM, Fernstrom HA, Williams PT, Krauss RM**

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**LDL subclass patterns and lipoprotein response to a low-fat, high-carbohydrate diet in women.**

Arteriosclerosis, Thrombosis & Vascular Biology 1997 Apr;17(4):707-14

A predominance of small, dense LDL particles (subclass pattern B) characterizes a metabolic trait that is associated with higher levels of triglyceride-rich lipoproteins and lower levels of HDL compared with those of individuals with predominantly larger LDL (pattern A). This trait appears to be under the influence of one or more genes, with maximal expression in adult males and reduced expression in premenopausal females. In a previous study, men with LDL subclass pattern B had significantly greater reductions in LDL cholesterol (LDL-C) and apolipoprotein B than men with pattern A. We hypothesized that despite the low prevalence of pattern B in premenopausal women, genetic predisposition to this trait could affect dietary responsiveness. Specifically, we predicted that LDL-C reduction on a low-fat, high-carbohydrate diet would be greatest in daughters of two pattern B parents, intermediate in daughters with one pattern B parent, and least in daughters with no pattern B parents. When 72 premenopausal women were placed on a 20% fat diet for 8 weeks, the changes in LDL-C (mmol/L) compared with levels on basal diets were significantly related to the number of pattern B parents (two B parents:  $-0.92 \pm 0.61$ , one B parent:  $-0.23 \pm 0.10$ , no B parents:  $-0.05 \pm 0.06$ ) and could not be explained by diet adherence or baseline characteristics including initial lipoprotein profile or body mass index. The number of pattern B parents was also related to reductions in plasma mass concentrations of IDL, total LDL, and large LDL and to increases in plasma triglycerides. There was a significant inverse correlation between changes in triglyceride and LDL-C induced by the low-fat, high-carbohydrate diet. Thus, genetic and metabolic factors underlying LDL subclass pattern B may result in enhanced LDL and triglyceride responsiveness to substitution of dietary carbohydrate for fat in premenopausal women.

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### Variability of plasma HDL subclass concentrations in men and women over time.

Arteriosclerosis, Thrombosis & Vascular Biology 1997 Apr;17(4):702-6

Plasma HDL subclasses were examined by gradient gel electrophoresis in repeated samples to assess variability over time. Absorbance of the protein stain was used as an index of mass concentrations at 0.01-nm intervals within five HDL subclasses: HDL3c (7.2 to 7.8 nm), HDL3b (7.8 to 8.2 nm), HDL3a (8.2 to 8.8 nm), HDL2a (8.8 to 9.7 nm), and HDL2b (9.7 to 12 nm). Three separate longitudinal studies of men showed that repeated samples of HDL over time were correlated most strongly within HDL2b, somewhat less within HDL2a, and more weakly within HDL3a, HDL3b, and HDL3c. As in men, repeated samples in women from two studies were significantly correlated within the HDL2b, HDL2a, and HDL3b intervals. Plasma HDL2b levels were significantly more stable in men than in women. Although the variability of HDL subclass measurements includes both methodological and physiological sources, differences in laboratory measurement error do not appear to explain the differences in correlations among subclasses. Specifically, analysis of 288 replications from frozen aliquots suggested that laboratory error had the least effect on correlations involving HDL3 subclasses and only slightly greater effect on correlations involving HDL2 subclasses. Our results suggest that for plasma sampled over time, the stability of HDL subclass levels increases with particle size. Prior reports of subclass-specific correlations between HDL and other variables (eg, diet, exercise, and other lipids) are unlikely to be artifacts of laboratory precision but could arise from subclass differences in variability that are physiological.

Krauss RM

### Understanding the basis for variation in response to cholesterol-lowering diets [editorial; comment].

Comment on: Am J Clin Nutr 1997 Mar;65(3 ):823-30  
American Journal of Clinical Nutrition 1997 Mar;65(3):885-6

Kotchen TA, Krauss RM

### Dietary sodium and blood pressure [letter; comment].

Comment on: JAMA 1996 May 22-29;275(20):1590-7

JAMA 1996 Nov 13;276(18):1468; discussion 1469-70

Stone NJ, Nicolosi RJ, Kris-Etherton P, Ernst ND, Krauss RM, Winston M

### AHA conference proceedings. Summary of the scientific conference on the efficacy of hypocholesterolemic dietary interventions. American Heart Association.

Circulation 1996 Dec 15;94(12):3388-91

Tribble DL, Chu BM, Levine GA, Krauss RM, Gong EL

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### Selective resistance of LDL core lipids to iron-mediated oxidation. Implications for the biological properties of iron-oxidized LDL.

Arteriosclerosis, Thrombosis & Vascular Biology 1996 Dec;16(12):1580-7

Although the nature and consequences of oxidative changes in the chemical constituents of low density lipoproteins (LDLs) have been extensively examined, the physical dynamics of LDL oxidation and the influence of physical organization on the biological effects of oxidized LDLs have remained relatively unexplored. To address these issues, in the present studies we monitored surface- and core-specific peroxidative stress relative to temporal changes in conjugated dienes (CDs), particle charge (an index of oxidative protein modification), and LDL-macrophage interactions. Peroxidative stress in LDL surface and core compartments was evaluated with the site-specific, oxidation-labile fluorescent probes parinaric acid (PnA) and PnA cholesteryl ester (PnCE), respectively. When oxidation was initiated by  $\text{Cu}^{2+}$ , oxidative loss of the core probe (PnCE) closely followed that of the surface probe (PnA), as indicated by the time to 50% probe depletion ( $t_{1/2}$ ;  $15.5 \pm 7.8$  and  $30.4 \pm 12$  minutes for PnA and PnCE, respectively). Both probes were more resistant in LDL exposed to  $\text{Fe}^{3+}$  ( $t_{1/2}$ ,  $53.2 \pm 8.1$  and  $346.7 \pm 155.4$  minutes), although core probe resistance was much greater with this oxidant (PnCE  $t_{1/2}$ /PnA  $t_{1/2}$  5.8 vs 2.0 for  $\text{Cu}^{2+}$ ). Despite differences in the rate and extent of oxidative changes in  $\text{Cu}^{2+}$ - versus  $\text{Fe}^{3+}$ -exposed LDLs, PnCE loss occurred in close correspondence with CD formation and appeared to precede changes in particle charge under both conditions. Exposure of LDLs to hemin, a lipophilic  $\text{Fe}^{3+}$ -containing porphyrin that becomes incorporated into the LDL particle, resulted in rapid loss of PnCE and simultaneous changes in particle, charge, even at concentrations that yielded increases in CDs and thiobarbituric acid-reactive substances similar to those obtained with free  $\text{Fe}^{3+}$ . These results suggest that oxidation of the LDL hydrophobic core occurs in conjunction with accelerated formation of CDs and may be essential for LDL protein modification. In accordance with the known effects of oxidative protein modifications on LDL receptor recognition, exposure of LDLs to  $\text{Cu}^{2+}$  and hemin but not  $\text{Fe}^{3+}$  produced particles that were readily processed by macrophages. Thus, the physical site of oxidative injury appears to be a critical determinant of the chemical and biological properties of LDLs, particularly when oxidized by  $\text{Fe}^{3+}$ .

Miller BD, Alderman EL, Haskell WL, Fair JM, Krauss RM

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### 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

BACKGROUND: LDL particles differ in size and density. Individuals with LDL profiles that peak in relatively small, dense particles have been reported to be at increased risk of coronary artery disease. We hypothesized that response to coronary disease therapy in such individuals might differ from response in individuals whose profiles peak in larger, more buoyant LDL. We examined this hypothesis in the Stanford Coronary Risk Intervention Project, an angiographic trial that compared multifactorial risk-reduction intervention with the usual care of physicians. METHODS AND RESULTS: For 213 men, a bimodal frequency distribution of peak LDL density (g/mL) determined by analytical ultracentrifugation was used to classify baseline LDL profiles as "buoyant mode" (density  $< 1.0378$ ) or "dense mode" (density  $> 1.0378$ ). Coronary

disease progression after 4 years was assessed by rates of change (mm/y, negative when arteries narrow) of minimum artery diameter. Rates for buoyant-mode subjects were  $-0.038 \pm 0.007$  (mean  $\pm$  SEM) in usual care (n = 65) and  $-0.039 \pm 0.010$  in intervention (n = 56; P = .6). Rates for dense-mode subjects were  $-0.054 \pm 0.012$  in usual care (n = 51) and  $-0.008 \pm 0.009$  in intervention (n = 41, P = .007). Lipid changes did not account for this difference in angiographic response. CONCLUSIONS: Different types of LDL profile may predict different-responses to specific therapies, perhaps because metabolic processes determine both LDL profiles and responses to therapies.

**Krauss RM**, Deckelbaum RJ, Ernst N, Fisher E, Howard BV, Knopp RH, Kotchen T, Lichtenstein AH, McGill HC, Pearson TA, Prewitt TE, Stone NJ, Horn LV, Weinberg R  
Office of Scientific Affairs, American Heart Association, Dallas, TX 75231-4596, USA.

**Dietary guidelines for healthy American adults. A statement for health professionals from the Nutrition Committee, American Heart Association.**

Circulation 1996 Oct 1;94(7):1795-800

Mack WJ, **Krauss RM**, Hodis HN

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**Lipoprotein subclasses in the Monitored Atherosclerosis Regression Study (MARS). Treatment effects and relation to coronary angiographic progression.**

Arteriosclerosis, Thrombosis & Vascular Biology 1996 May;16(5):697-704

Accumulating evidence suggests that triglyceride-rich lipoproteins contribute to coronary artery disease. Using data from the Monitored Atherosclerosis Regression Study, an angiographic trial of middle-aged men and women randomized to lovastatin or placebo, we investigated relationships between lipoprotein subclasses and progression of coronary artery atherosclerosis. Coronary artery lesion progression was determined by quantitative coronary angiography in low-grade (< 50% diameter stenosis), high-grade (50% diameter stenosis), and all coronary artery lesions in 220 baseline/2-year angiogram pairs. Analytical ultracentrifugation was used to measure lipoprotein masses that were statistically evaluated for treatment group differences and relationships to progression of coronary artery atherosclerosis. All low density lipoprotein (LDL), intermediate density lipoprotein (IDL), and very low density lipoprotein (VLDL) masses were significantly lowered and all high density lipoprotein (HDL) masses were significantly raised with lovastatin therapy. The mass of smallest LDL (Svedberg flotation rate [Sf] 0 to 3), IDL (Sf 12 to 20), all VLDL subclasses (Sf 20 to 60, Sf 60 to 100, and Sf 100 to 400), and peak LDL flotation rate were significantly related to the progression of coronary artery lesions, specifically low-grade lesions. Greater baseline levels of HDL3, were related to a lower likelihood of coronary artery lesion progression. In multivariate analyses, small VLDL (Sf 20 to 60) and HDL3 mass were the most important correlates of coronary artery lesion progression. These results provide further evidence for the importance of triglyceride-rich lipoproteins in the progression of coronary artery disease. In addition, these results present new evidence for the possible protective role of HDL3 in the progression of coronary artery lesions. More specific information on coronary artery lesion progression may be obtained through the study of specific apolipoprotein B-containing lipoproteins.

Stampfer MJ, **Krauss RM**, Ma J, Blanche PJ, Holl LG, Sacks FM, Hennekens CH

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**A prospective study of triglyceride level, low-density lipoprotein particle diameter, and risk of myocardial infarction [see comments].**

Comment in: JAMA 1996 Sep 18;276(11):914-5  
JAMA 1996 Sep 18;276(11):882-8

OBJECTIVE: To test whether a predominance of small, dense low-density lipoprotein (LDL) particles and elevated triglyceride levels are independent risk factors for myocardial infarction (MI). DESIGN: Nested case-control study with prospectively collected samples. SETTING:

Prospective cohort study. PARTICIPANTS: Blood samples were collected at baseline (85% nonfasting samples) from 14916 men aged 40 to 84 years in the Physicians' Health Study. MAIN OUTCOME MEASUREMENTS: Myocardial infarction diagnosed during 7 years of follow-up. RESULTS: Cases (n=266) had a significantly smaller LDL diameter (mean [SD], 25.6 [0.9] nm) than did controls (n=308) matched on age and smoking (mean [SD], 25.9 [8] nm; P<.001). Cases also had higher median triglyceride levels (1.90 vs 1.49 mmol/L [168 vs 132 mg/dL]; P<.001). The LDL diameter had a high inverse correlation with triglyceride level (r=-0.71), and a high direct correlation with high-density lipoprotein cholesterol (HDL-C) level (r=0.60). We observed a significant multiplicative interaction between triglyceride and total cholesterol (TC) levels (P=.01). After simultaneous adjustment for lipids and a variety of coronary risk factors, LDL particle diameter was no longer a statistically significant risk indicator, with a relative risk (RR) of 1.09 (95% confidence interval [CI], 0.85-1.40) per 0.8-nm decrease. However, triglyceride level remained significant with an RR of 1.40 (95% CI, 1.10-1.77) per 1.13 mmol/L (100-mg/dL) increase. The association between triglyceride level and MI risk appeared linear across the distribution; men in the highest quintile had a risk about 2.5 times that of those in the lowest quintile. The TC level, but not HDL-C level, also remained significant, with an RR of 1.80 (95% CI, 1.44-2.26) per 1.03-mmol/L (40-mg/dL) increase. CONCLUSIONS: These findings indicate that nonfasting triglyceride levels appear to be a strong and independent predictor of future risk of MI, particularly when the total cholesterol level is also elevated. In contrast, LDL particle diameter is associated with risk of MI, but not after adjustment for triglyceride level. Increased triglyceride level, small LDL particle diameter, and decreased HDL-C levels appear to reflect underlying metabolic perturbations with adverse consequences for risk of MI; elevated triglyceride levels may help identify high-risk individuals.

Gardner CD, Fortmann SP, **Krauss RM**

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**Association of small low-density lipoprotein particles with the incidence of coronary artery disease in men and women [see comments].**

Comment in: JAMA 1996 Sep 18;276(11):914-5  
JAMA 1996 Sep 18;276(11):875-81

OBJECTIVE: To investigate the prospective association of low-density lipoprotein (LDL) particle diameter with the incidence of fatal and nonfatal coronary artery disease (CAD). DESIGN: A nested case-control study. SETTING: Cases and controls were identified from a population-based sample of men and women combining all of the 5 cross-sectional surveys conducted from 1979 to 1990 of the Stanford Five-City Project (FCP). PARTICIPANTS: Incident CAD cases were identified through FCP surveillance between 1979 and 1992. Controls were matched by sex, 5-year age groups, survey time point, ethnicity, and FCP treatment condition. The sample included 124 matched pairs: 90 pairs of men and 34 pairs of women. MAIN OUTCOME MEASURES: LDL peak particle diameter (LDL size) was determined by gradient gel electrophoresis on plasma samples collected during the cross-sectional surveys (stored at 70 degrees C for 5-15 years). Established CAD risk-factor data were available from FCP baseline measurements. RESULTS: LDL size was smaller among CAD cases than controls (mean  $\pm$  SD) ( $26.17 \pm 1.00$  nm vs  $26.68 \pm 0.90$  nm; P<.001). The association was graded across control quintiles of LDL size. The significant case-control difference in LDL size was independent of levels of high-density lipoprotein cholesterol (HDL-C), non-HDL cholesterol (non-HDL-C), triglyceride, smoking, systolic blood pressure, and body mass index, but was not significant after adjusting for the ratio of total cholesterol (TC) to HDL-C (TC:HDL-C). Among all the physiological risk factors, LDL size was the best differentiator of CAD status in conditional logistic regression. However, when added to the physiological parameters above, the TC:HDL-C ratio was found to be a stronger independent predictor of CAD status. CONCLUSION: LDL size was significantly smaller in CAD cases than in controls in a prospective, population-based study. These findings support other evidence of a role for small, dense LDL particles in the etiology of atherosclerosis.

Rotter JI, Bu X, Cantor RM, Warden CH, Brown J, Gray RJ, Blanche PJ, **Krauss RM**, Lusis AJ

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### **Multilocus genetic determinants of LDL particle size in coronary artery disease families.**

American Journal of Human Genetics 1996 Mar;58(3):585-94

Recent interest in atherosclerosis has focused on the genetic determinants of low-density lipoprotein (LDL) particle size, because of (i) the association of small dense LDL particles with a three-fold increased risk for coronary artery disease (CAD) and (ii) the recent report of linkage of the trait to the LDL receptor (chromosome 19). By utilizing nonparametric quantitative sib-pair and relative-pair analysis methods in CAD families, we tested for linkage of a gene or genes controlling LDL particle sizes with the genetic loci for the major apolipoproteins and enzymes participating in lipoprotein metabolism. We confirmed evidence for linkage to the LDL receptor locus ( $P=0.008$ ). For six candidate gene loci, including apolipoprotein(apo)B, apoAII, apo(a), apoE-CI-CII, lipoprotein lipase, and high-density lipoprotein-binding protein, no evidence for linkage was observed by sib-pair linkage analyses ( $P$  values ranged from .24 to .81). However, in addition, we did find tentative evidence for linkage with the apoAI-CIII-AIV locus (chromosome 11) ( $P=.06$ ) and significant evidence for linkage of the cholesteryl ester transfer protein locus (chromosome 16) ( $P=.01$ ) and the manganese superoxide dismutase locus (chromosome 6) ( $P=.001$ ), thus indicating multilocus determination of this atherogenic trait.

Campos H, Arnold KS, Balestra ME, Innerarity TL, **Krauss RM**  
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### **Differences in receptor binding of LDL subfractions.**

Arteriosclerosis, Thrombosis & Vascular Biology 1996 Jun;16(6):794-801

Differences in low density lipoprotein (LDL) receptor-binding affinity among LDL particles of different size were examined in competitive binding assays in human skin fibroblasts and LDL ( $d = 1.020$  to  $1.050$  g/mL) from subjects with a predominance of large ( $> 272$  Å), medium (259 to 271 Å), and small ( $< 257$  Å) LDL. Among 57 normolipidemic subjects with LDL cholesterol (-C) levels  $< 160$  mg/dL, binding affinity was reduced by 16% in those with predominantly large LDL and by 14% in those with small LDL compared with most subjects who had a predominance of medium-size LDL and in all LDL size subgroups in 66 subjects with LDL-C  $> 160$  mg/dL. Differences in LDL receptor-binding affinity were further investigated by using LDL density subfractions (I,  $d = 1.026$  to  $1.032$  g/mL; II,  $d = 1.032$  to  $1.038$  g/mL; and III,  $d = 1.038$  to  $1.050$  g/mL) from three subjects with predominantly large (pattern A) and small (pattern B) LDL particles. The binding affinity (Kd) of LDL-II was similar for patterns A and B ( $9.2 \pm 1.4$  and  $9.4 \pm 0.7$ , respectively) and 30% lower in LDL-III from both groups ( $P < .05$ ). The binding affinity of LDL-I in pattern A ( $12.6 \pm 1.5$   $\mu$ g/mg) was lower ( $P < .05$ ) than that in LDL-II and LDL-I from pattern B ( $8.0 \pm 2.4$   $\mu$ g/mg). After incubation with a monoclonal antibody that specifically blocked the LDL receptor-binding domain of apoE, LDL-I from two pattern B subjects showed substantially lower binding affinity (Kd = 20.0 and 19.2  $\mu$ g/mg) than in pattern A (Kd = 13.2 and 14.2  $\mu$ g/mg), a result consistent with our finding of a higher apoE content in pattern B LDL-I ( $P < .001$ ). Thus, factors associated with variations in particle size and apoE content in LDL subclasses in normolipidemic subjects contribute to the differences in LDL receptor binding that may result in differing metabolic behavior in vivo.

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### **Hormone replacement therapy, hormone levels, and lipoprotein cholesterol concentrations in elderly women.**

American Journal of Obstetrics & Gynecology 1996 Mar;174(3):897-902

OBJECTIVE: Our purpose was to assess the relationships of lipid and lipoprotein cholesterol levels to hormone replacement therapy and

hormone levels in elderly women. STUDY DESIGN: A sample of 292 postmenopausal women 55 to 99 years old (mean 76 years) was drawn from Leisure World Laguna Hills, California, an upper-middle-class, white independent-living population. We compared 84 women receiving unopposed estrogen replacement therapy and 38 women taking combination hormone replacement therapy with 170 women who had never used hormone replacement therapy. Nonparametric tests for differences in lipid and lipoprotein cholesterol levels among groups and multiple stepwise regression models were used. RESULTS: Estrogen users (with and without progestin) had lower total and low-density lipoprotein cholesterol and higher high-density lipoprotein and high-density lipoprotein subfraction types 2, 2a, and 2b cholesterol levels. High density lipoprotein type 3 subfractions were lower in combination hormone replacement therapy users but higher in unopposed estrogen users relative to nonusers. The conjugated equine estrogen dose was negatively correlated with total ( $p = 0.0009$ ) and low-density lipoprotein cholesterol ( $p < 0.0001$ ) levels and positively correlated to high-density lipoprotein cholesterol ( $p = 0.002$ ) and its subfractions. The medroxyprogesterone acetate dose showed no consistent effect on cholesterol levels. CONCLUSION: The associations found here reaffirm the significant role of estrogen replacement therapy on lipid and lipoprotein cholesterol levels and provide no evidence of a reduction in the beneficial effect of estrogen with the addition of a progestational agent to the replacement regimen.

**Krauss RM**, Chait A, Stone NJ

### **Soy protein and serum lipids [letter; comment].**

Comment on: N Engl J Med 1995 Aug 3;333(5):276-82

New England Journal of Medicine 1995 Dec 21;333(25):1715-6

Tribble DL, Thiel PM, van den Berg JJ, **Krauss RM**

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### **Differing alpha-tocopherol oxidative lability and ascorbic acid sparing effects in buoyant and dense LDL.**

Arteriosclerosis, Thrombosis & Vascular Biology 1995 Nov;15(11):2025-31

The enhanced oxidizability of smaller, more dense LDL is explained in part by a lower content of antioxidants, including ubiquinol-10 and alpha-tocopherol. In the present studies, we also observed greater rates of depletion of alpha-tocopherol (mole per mole LDL per minute) in dense ( $d = 1.040$  to  $1.054$  g/mL) compared with buoyant ( $d = 1.026$  to  $1.032$  g/mL) LDL in the presence of either  $\text{Cu}^{2+}$  or the radical-generating agent 2-2'-azobis (2-amidinopropane)dihydrochloride. Differences were particularly pronounced at the lowest  $\text{Cu}^{2+}$  concentration tested (0.25  $\mu$ mol/L), with a fivefold greater rate in dense LDL. At higher concentrations (1.0 and 2.5  $\mu$ mol/L  $\text{Cu}^{2+}$ ), there was a greater dependence of depletion rate on initial amount of alpha-tocopherol, which was reduced in dense LDL, thus resulting in smaller subfraction-dependent differences in depletion rates. Inclusion of ascorbic acid (15  $\mu$ mol/L), an aqueous antioxidant capable of recycling alpha-tocopherol by hydrogen donation, was found to extend the course of  $\text{Cu}^{2+}$ -induced alpha-tocopherol depletion in both buoyant and dense LDL, but this effect was more pronounced in dense LDL (time to half-maximal alpha-tocopherol depletion was extended 15.6-fold and 21.2-fold in buoyant and dense LDL, respectively, at 2.5  $\mu$ mol/L  $\text{Cu}^{2+}$ ;  $P < .05$ ). Thus, dense LDL exhibits more rapid alpha-tocopherol depletion and conjugated diene formation than buoyant LDL when oxidation is performed in the absence of ascorbic acid, but these differences are reversed in the presence of ascorbic acid.(ABSTRACT TRUNCATED AT 250 WORDS)

Fan J, McCormick SP, **Krauss RM**, Taylor S, Quan R, Taylor JM, Young SG

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### **Overexpression of human apolipoprotein B-100 in transgenic rabbits results in increased levels of LDL and decreased levels of HDL.**

Arteriosclerosis, Thrombosis & Vascular Biology 1995 Nov;15(11):1889-99

In this study, and 80-kb human genomic DNA fragment spanning the human apoB gene was used to generate transgenic New Zealand White rabbits that expressed human apoB-100. The concentration of human apoB in the plasma of the transgenic rabbits ranged between 5 and 100 mg/dL. The transgenic rabbits had nearly threefold elevations in the plasma levels of triglycerides and cholesterol compared with nontransgenic controls. Nearly all the cholesterol and human apoB in the plasma was in the LDL fraction. Pronounced triglyceride enrichment of the LDL fraction was a striking feature of human apoB overexpression in the transgenic rabbits, in which the LDL fraction contained more than 75% of the plasma triglycerides. The triglyceride-enriched LDL particles were smaller and more dense than the native rabbit LDL and contained markedly increased amounts of apoE and apoC-III. In the nontransgenic control animals most of the triglycerides were in the VLDL, and most of the apoE and apoC-III were in the VLDL and HDL fractions. In addition to increased LDL levels, overexpression of human apoB in rabbits resulted in lower plasma levels of HDL cholesterol and apoA-I. In our prior studies on transgenic mice expressing human apoB, we documented triglyceride-rich LDL and reduced levels of HDL cholesterol. These prior findings in mice, together with the present findings in transgenic rabbits, suggest that triglyceride-rich LDL and lowered levels of HDL cholesterol may be hallmark features of apoB overexpression.

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### **Predominance of large LDL and reduced HDL2 cholesterol in normolipidemic men with coronary artery disease.**

*Arteriosclerosis, Thrombosis & Vascular Biology* 1995 Aug;15(8):1043-8

Previous studies have indicated that a predominance of small, dense LDL particles is associated with coronary artery disease (CAD) risk. In the present study we examined the LDL peak particle diameter (determined by lipid-stained 2% to 16% gradient gel electrophoresis) in 92 normolipidemic men with CAD (total cholesterol < 200 mg/dL and triglyceride < 250 mg/dL) and 92 matched healthy controls. Plasma triglyceride, LDL cholesterol, and apo B levels were similar in subjects with CAD and in control subjects, whereas subjects with CAD had decreased HDL2 cholesterol levels (mean  $\pm$  SEM,  $10 \pm 0.7$  compared with  $15 \pm 0.7$  mg/dL in control subjects;  $P < .0002$ ). Mean LDL particle diameter ( $\pm$  SEM) was increased in the subjects with CAD compared with control subjects ( $26.8 \pm 0.08$  and  $26.4 \pm 0.08$  nm, respectively;  $P < .001$ ). The association between large LDL size and CAD was significant ( $P < .0001$ ) after adjustments were made for age, body mass index, HDL cholesterol levels, and VLDL cholesterol levels. An LDL particle size distribution characterized by a predominance of the largest of three classes of LDL particles ( $> 26.8$  nm) was more prevalent among subjects with CAD (43%) than among control subjects (25%) ( $P < .002$ ). Among subjects with this LDL size profile, subjects with CAD had significantly higher ( $P < .05$ ) VLDL triglyceride, VLDL cholesterol, and VLDL apo B levels and significantly lower ( $P < .0001$ ) HDL2 cholesterol levels than controls. (ABSTRACT TRUNCATED AT 250 WORDS)

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### **Low-density-lipoprotein subclasses and response to a low-fat diet in healthy men.**

*American Journal of Clinical Nutrition* 1995 Aug;62(2):478S-487S

Lipid and lipoprotein responses to reduced dietary fat intake were investigated in relation to differences in distribution of low-density-lipoprotein (LDL) subclasses among 105 healthy men consuming high-fat (46% fat) and low-fat (24% fat) diets in random order for 6 wk each. With high-fat diets, 87 subjects had predominantly large, buoyant LDL (pattern A), whereas the remainder had primarily smaller, denser LDL (pattern B). With low-fat diets, 36 men changed from pattern A to B. Compared with the 51 men with pattern A with both diets (stable A group), men in the stable B group ( $n = 18$ ) had significantly greater reductions in plasma LDL cholesterol, apolipoprotein B, and mass of mid-sized (LDL II) and small (LDL III) LDL subfractions. In both the stable A and change groups, there

was a shift in LDL particle mass from larger, lipid-enriched (LDL I and II) to smaller, lipid-depleted (LDL III and IV) subfractions, suggestive of change in LDL composition with minimal change in particle number, and consistent with the observation of reduced plasma LDL cholesterol without reduced apolipoprotein B. Stable B subjects had significantly greater increases in the largest very-low-density-lipoprotein subfraction with the low-fat diet than the stable A group, and also had greater decreases in the high-density-lipoprotein (HDL) subclass HDL3 but smaller reductions in HDL2. Genetic and environmental factors influencing LDL subclass distributions thus may also contribute substantially to interindividual variation in plasma lipoprotein response to a low-fat diet.

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### **Greater oxidative susceptibility of the surface monolayer in small dense LDL may contribute to differences in copper-induced oxidation among LDL density subfractions.**

*Journal of Lipid Research* 1995 Apr;36(4):662-71

We monitored peroxidative stress in the surface monolayer as compared with the outer core of large, buoyant ( $d$  1.025-1.032 g/ml) and small, dense ( $d$  1.040-1.054 g/ml) low density lipoprotein (LDL) subfractions using the oxidation-labile fluorescent probes parinaric acid (PnA) and parinaric acid methyl ester (PnME), which partition preferentially into these respective regions of LDL. Oxidation was initiated either with  $\text{CuSO}_4$  ( $5 \mu\text{M}$ ) or the iron ( $\text{Fe}^{3+}$ )-containing lipophilic complex hemin ( $1.0 \mu\text{M}$ ) plus cumene hydroperoxide to facilitate heme degradation. In the presence of  $\text{Cu}^{2+}$ , PnA was depleted significantly more rapidly than PnME in dense ( $P = 0.039$ ) but not in buoyant LDL, suggesting that surface vulnerability is enhanced in dense LDL particles. With hemin, PnA and PnME were similarly susceptible within both subfractions, although there was a trend toward slower loss of PnA in buoyant LDL ( $P = 0.10$ ), consistent with the internal site of initiation and a greater surface resistance in buoyant particles. As indicated by conjugated diene lag times, dense LDL was more susceptible than buoyant LDL to oxidation by  $\text{Cu}^{2+}$  ( $P = 0.03$ ) but not hemin ( $P = 0.68$ ). These results suggest that the increased susceptibility of dense LDL to oxidation by external agents such as  $\text{Cu}^{2+}$  is at least partially mediated by an enhanced vulnerability of the surface compartment.

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### **Associations of hepatic and lipoprotein lipase activities with changes in dietary composition and low density lipoprotein subclasses.**

*Journal of Lipid Research* 1995 Mar;36(3):462-72

To test whether lipoprotein lipase or hepatic lipase activities are associated with lipoprotein subclasses, and to assess the effects of dietary manipulations on these associations, enzyme activities were measured in postheparin plasma ( $75 \text{ U heparin/kg}$ ) from 43 healthy men who were randomly allocated to a low-fat (24% fat, 60% carbohydrate) and a high-fat (46% fat, 38% carbohydrate) diet for 6 weeks each in a cross-over design. The high-fat diet significantly increased both lipoprotein lipase (+20%,  $P = 0.02$ ) and hepatic lipase (+8%,  $P = 0.007$ ) activities. On both diets, hepatic lipase activity was significantly positively correlated ( $P < 0.01$ ) with plasma apolipoprotein (apo)B concentrations, and with levels of small dense low density lipoprotein (LDL) III, measured by analytic ultracentrifugation as mass of lipoproteins of flotation rate (Sof) 3-5, while lipoprotein lipase activity was inversely associated with levels of LDL III ( $P < 0.05$ ). Despite the cross-sectional correlations, increased hepatic lipase activity was not significantly correlated with the reduction in LDL III mass observed on the high-fat diet. Rather, changes in hepatic lipase were correlated inversely with changes in small very low density lipoproteins (VLDL) of Sof 20-40, and small intermediate density lipoproteins (VLDL) of Sof 10-16. Moreover, changes in lipoprotein lipase activity were not significantly correlated with changes in small LDL, but were positively associated with changes in small IDL of Sof 10-14, and large LDL I of Sof 7-10. Thus, while increased levels of small dense LDL are associated with a metabolic state characterized by relatively increased hepatic lipase and decreased lipoprotein lipase activity, changes in these

enzymes do not appear to be primary determinants of diet-induced changes in levels of this LDL subfraction. On the other hand, increased lipoprotein lipase activity induced by high-fat feeding may contribute to the accumulation in plasma of both large LDL I and small IDL, whereas increased hepatic lipase may promote catabolism or clearance of triglyceride-rich lipoprotein remnants.

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**Solvent drag of LDL across mammalian endothelial barriers with increased permeability.**  
American Journal of Physiology 1995 May;268(5 Pt 2):H1982-91

We investigated the mechanisms of hamster low-density lipoprotein (LDL) transport across the endothelial barrier in individually perfused venular microvessels in hamster mesentery. These experiments are the first to use microperfusion techniques and quantitative fluorescence microscopy to investigate LDL transport across mammalian microvessel endothelium. The apparent permeability coefficient for hamster LDL, PsLDL, rose from  $2.7 \times 10^{-7}$  cm/s at control to  $23.2 \times 10^{-7}$  cm/s at the peak of the biphasic increase in microvessel permeability after exposure of the vessels to 100  $\mu$ M histamine. Close to the peak, PsLDL rose  $1.85 \times 10^{-7}$  cm/s for every centimeter of H<sub>2</sub>O increase in hydrostatic pressure. Thus, at a mean pressure of 11.3 cmH<sub>2</sub>O, 90% of the LDL flux was coupled to transendothelial water flow by a solvent drag mechanism. The corresponding solvent drag reflection coefficient for hamster LDL was estimated to be approximately 0.8. These results are consistent with sieving hamster LDL (effective radius 14.9 nm) through equivalent pores of approximately 22 nm radius. Similar results were found with human LDL (effective radius 13.2 nm) in hamster microvessels. The results provide a bridge between studies of LDL transport across cultured endothelial barriers, where high diffusive permeability coefficients to LDL may obscure the contributions of solvent drag, and studies in whole animals, where the consequences of sieving of LDL at the vessel wall, even in the high permeability state, have not received much attention.

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**Effects of dietary fat on high-density-lipoprotein subclasses are influenced by both apolipoprotein E isoforms and low-density-lipoprotein subclass patterns.**

American Journal of Clinical Nutrition 1995 Jun;61(6):1234-40

We examined the effects of replacing dietary fat with carbohydrates on high-density-lipoprotein (HDL) subclasses as measured by nondenaturing polyacrylamide-gradient-gel electrophoresis. One hundred five men received a 6-wk low-fat diet (24% of total energy) and a 6-wk high-fat diet (46% of energy) in a crossover design. Absorbency of protein stain was measured within five HDL subclasses: HDL3c (7.2-7.8 nm), HDL3b (7.8-8.2 nm), HDL3a (8.2-8.8 nm), HDL2a (8.8-9.7 nm), and HDL2b (9.7-12 nm). The low-density-lipoprotein-(LDL) subclass pattern was determined by gradient-gel electrophoresis, with pattern B men defined as having an LDL-predominant peak diameter  $\geq 25.5$  nm and an LDL distribution skewed toward larger size particles. On the high-fat diet, 18 men exhibited LDL-subclass pattern B and 87 men exhibited the alternative LDL pattern A. Twelve men had the apolipoprotein (apo) epsilon 2 allele. Replacing dietary fat with carbohydrates 1) significantly decreased HDL3a, HDL2a, and HDL2b; 2) reduced HDL2b significantly more in pattern A than in pattern B men; and 3) increased plasma HDL3b concentrations significantly more in those men with the epsilon 2 allele. Our results suggest that unfavorable HDL changes were significantly more likely to occur in men who had LDL-subclass pattern A or the apo epsilon allele than in men who had pattern B or lacked the epsilon 2 allele.

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**LDL physical and chemical properties in familial combined hyperlipidemia.**

Arteriosclerosis, Thrombosis & Vascular Biology 1995 Apr;15(4):452-9

Familial combined hyperlipidemia (FCHL) is characterized by elevations of triglyceride and/or cholesterol within families and an elevation in apoB. Although small dense LDL has been consistently associated with hypertriglyceridemia, small dense LDL persists despite reductions in triglyceride after treatment with gemfibrozil in FCHL. The current study evaluated potential differences in the distribution and chemical composition of LDL species in patients with FCHL and normolipidemic control subjects. LDL from FCHL patients was characterized by a relative abundance of a discrete LDL species with a mean peak analytic ultracentrifuge flotation rate (S<sub>0</sub>f) of  $4.7 \pm 0.5$  (SEM), a density of  $1.041 \pm 0.001$  g/mL, and a particle diameter of  $250 \pm 1$  Å as assessed by gradient gel electrophoresis. The major LDL species in the control subjects had a higher mean S<sub>0</sub>f rate ( $6.3 \pm 0.4$ ), was more buoyant (density,  $1.037 \pm 0.001$  g/mL), and was larger (diameter,  $262 \pm 2$  Å). In addition, in a series of six LDL fractions separated by equilibrium density gradient ultracentrifugation, particle diameters were significantly smaller in all fractions from FCHL patients compared with those from control subjects. LDL particles from patients contained less free cholesterol, cholesteryl ester, and phospholipid than LDL from control subjects. The amount of triglyceride per LDL particle, however, did not differ between FCHL patients and control subjects. Differences in flotation rate and mass of the major LDL species between patients and control subjects could not be fully accounted for by differences in plasma triglyceride levels. Thus, LDL particles from FCHL patients are smaller and more dense with less cholesterol and phospholipid. Many of these differences appear to be independent of plasma triglyceride. (ABSTRACT TRUNCATED AT 250 WORDS)

Katzel LI, Coon PJ, Rogus E, **Krauss RM**, Goldberg AP

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**Persistence of low HDL-C levels after weight reduction in older men with small LDL particles.**

Arteriosclerosis, Thrombosis & Vascular Biology 1995 Mar;15(3):299-305

LDL subclass pattern B is characterized by a predominance of small LDL particles (LDL peak particle size  $\geq 255$  Å) and is associated with increased plasma triglyceride (TG) and reduced HDL cholesterol (HDL-C) concentrations. This study compared the effect of weight loss on lipoprotein and glucose metabolism in 15 healthy, obese (body mass index [BMI],  $30.9 \pm 2.4$  kg/m<sup>2</sup>), older ( $60 \pm 9$  years) men with LDL pattern B and in 25 men of comparable age and BMI with LDL pattern A (LDL peak particle size  $\geq 260$  Å). At baseline, men with LDL pattern B had higher TG and lower apolipoprotein (apo) A-I, HDL-C, and HDL2-C levels ( $P < .001$ ) than men with LDL pattern A, while the total cholesterol and LDL cholesterol levels and fasting and 2-hour postprandial glucose and insulin levels did not differ between groups. With weight loss ( $10.1 \pm 3.6$  kg) there were significant decreases in 2-hour postprandial glucose and insulin levels in men with LDL patterns B and A ( $P < .05$ ). However, the change in plasma TG, HDL-C, HDL2-C, and apoA-I levels with weight loss differed between groups. In men with LDL pattern A, plasma TG levels decreased by 15% ( $P < .001$ ) compared with a 34% ( $P < .001$ ) decrease in LDL pattern B (two-factor ANOVA,  $P < .01$ ). Plasma HDL-C concentrations increased by 0.16 mmol/L ( $P < .001$ ) in the men with LDL pattern A but by only 0.07 mmol/L in the men with LDL pattern B (two-factor ANOVA,  $P < .05$ ). (ABSTRACT TRUNCATED AT 250 WORDS)

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**Apolipoprotein E isoform phenotype and LDL subclass response to a reduced-fat diet.**

Arteriosclerosis, Thrombosis & Vascular Biology 1995 Jan;15(1):105-11

We investigated the association of apolipoprotein (apo) E isoform phenotype with lipoprotein response to reduced dietary fat intake in 103

healthy men (apoE3/2, n = 10; apoE3/3, n = 65; and apoE4/3, 4/4, n = 28). In a randomized, crossover design, subjects consumed high-fat (46%) and low-fat (24%) diets for 6 weeks each. High-fat LDL cholesterol differed among phenotypes, with apoE4/3, 4/4 > apoE3/3 > apoE3/2. Reduction of LDL cholesterol on the low-fat diet was greater for apoE4/3, 4/4 than apoE3/3 (P < .05). There was no significant change in plasma apoB level within any of the apoE phenotype groups on the low-fat diet. This result, together with measurements of LDL subfraction mass by analytical ultracentrifugation, indicated that the primary basis for the diet-induced reduction in LDL cholesterol was not reduced LDL particle number but rather a shift from large, buoyant, cholesterol-rich LDL particles (floatation rate, 7 to 12) to smaller, denser LDL particles (floatation rate, 0 to 7). The magnitude of this effect was related to apoE phenotype, with progressively greater reductions in levels of large LDL (P < .01) from apoE3/2 to apoE3/3 to apoE4/3, 4/4. These results indicate that reduced dietary fat lowers levels of large, buoyant LDL particles by an apoE-dependent mechanism.

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**The associations of high-density lipoprotein subclasses with insulin and glucose levels, physical activity, resting heart rate, and regional adiposity in men with coronary artery disease: the Stanford Coronary Risk Intervention Project baseline survey.**

Metabolism: Clinical & Experimental 1995  
Jan;44(1):106-14

We used nondenaturing polyacrylamide gradient gel electrophoresis to examine the associations of high-density lipoprotein (HDL) subclasses with adiposity, physical activity, resting heart rate (an indicator of sympathetic drive), and plasma insulin and glucose levels in 97 men with angiographically documented coronary artery disease. These men neither smoked nor used medications known to affect lipoproteins. The absorbency of protein stain was used as an index of mass concentrations at intervals of 0.01 nm within five HDL subclasses: HDL3c (7.2 to 7.8 nm), HDL3b (7.8 to 8.2 nm), HDL3a (8.2 to 8.8 nm), HDL2a (8.8 to 9.7 nm), and HDL2b (9.7 to 12 nm). HDL peak diameter was determined from the predominant peak of the HDL particle distribution when plotted against particle diameter. Four men who were non-insulin-dependent diabetics as defined by a fasting glucose exceeding 140 mg/dL had significantly higher plasma HDL3b levels and significantly smaller HDL peak diameters than nondiabetic men, and were therefore excluded from further analyses. In the remaining 93 nondiabetic men, plasma HDL3b levels correlated positively with indices of truncal obesity (waist to hip ratio and subscapular skinfold), whereas plasma HDL2b levels correlated negatively with indices of total adiposity (body mass index [BMI]) and truncal obesity (subscapular and abdominal skinfold). Fasting plasma insulin levels correlated negatively with HDL3a, HDL2a, and HDL2b. Obesity significantly affected the relationships of resting heart rate with insulin and HDL subclasses. (ABSTRACT TRUNCATED AT 250 WORDS)

Austin MA, **Krauss RM**

**LDL density and atherosclerosis [letter; comment].**

Comment on: JAMA 1994 Jul 27;272(4):305-8  
JAMA 1995 Jan 11;273(2):115

Williams PT, **Krauss RM**, Stefanick ML, Vranizan KM, Wood PD

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**Effects of low-fat diet, calorie restriction, and running on lipoprotein subfraction concentrations in moderately overweight men.**

Metabolism: Clinical & Experimental 1994  
May;43(5):655-63

We studied the effects of exercise (primarily running), calorie restriction (dieting), and a low-fat, high-carbohydrate diet on changes in lipoprotein subfractions in moderately overweight men in a randomized controlled

clinical trial. After 1 year, complete data were obtained for 39 men assigned to lose weight through dieting without exercise, 37 men assigned to lose weight through dieting with exercise (primarily running), and 40 nondieting sedentary controls. We instructed both diet groups to consume no more than 30% total fat, 10% saturated fat, and 300 mg/d of cholesterol, and at least 55% carbohydrates, and the controls were instructed to maintain their usual food choices. Analytic ultracentrifugation was used to measure changes in plasma lipoprotein mass concentrations. In addition, the absorbance of protein-stained polyacrylamide gradient gels was used as an index of concentrations for five high-density lipoprotein (HDL) subclasses that have been identified by their particle sizes, ie, HDL3c (7.2 to 7.8 nm), HDL3b (7.8 to 8.2 nm), HDL3a (8.2 to 8.8 nm), HDL2a (8.8 to 9.7 nm), and HDL2b (9.7 to 12 nm). Relative to controls, weight decreased significantly in men who dieted with exercise (net difference  $\pm$  SE,  $-3.3 \pm 0.4$  kg/m<sup>2</sup>) and in men who dieted without exercise ( $-2.0 \pm 0.4$  kg/m<sup>2</sup>). Dieting with exercise significantly decreased very-low-density lipoprotein (VLDL)-mass concentrations and significantly increased plasma HDL2-mass, HDL3a, HDL2a, and HDL2b relative to both control and dieting without exercise. There were no significant changes in lipoprotein mass and HDL protein for dieters who did not run. (ABSTRACT TRUNCATED AT 250 WORDS)

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**Isolation of allele-specific, receptor-binding-defective low density lipoproteins from familial defective apolipoprotein B-100 subjects.**

Journal of Lipid Research 1994 Aug;35(8):1469-76

Familial defective apolipoprotein B-100 (FDB) is a genetic disorder apparently caused by a single amino acid substitution (Arg3500→Gln) that disrupts the binding of low density lipoproteins (LDL) to the LDL receptor. The plasma of FDB heterozygotes contains a mixture of normal LDL and LDL that is defective in binding to the LDL receptor. In this study, the monoclonal antibody MB19 (which recognizes an immunogenetic polymorphism in apolipoprotein B-100) was used to determine the percentage of defective LDL in the plasma of FDB heterozygotes and to isolate allele-specific receptor-binding-defective LDL. Several FDB heterozygotes were identified who were heterozygous for the MB19 polymorphism: one apolipoprotein B allotype in each of these individuals bound with low affinity to MB19 and possessed the Arg3500→Gln mutation, whereas the other apolipoprotein B allotype bound with high affinity to MB19 and normally to the LDL receptor. Using MB19 radio-immunoassay, we determined that an average of 73% (range 65-87) of the total LDL from FDB heterozygotes contained the Arg3500→Gln mutation. Antibody MB19-Sepharose immuno-affinity chromatography was used to separate the receptor-binding-defective LDL from the normal LDL. The isolated LDL contained primarily the Arg3500→Gln mutation and had only about 9% of normal LDL receptor-binding ability. Finally, the MB19-Sepharose chromatography procedure may be useful for isolating other allele-specific LDL that have functionally significant mutations.

**Krauss RM**, Kesaniemi YA

**Cardiovascular disease and hyperlipidaemia [editorial].**

Current Opinion In Lipidology 1994 Aug;5(4):249-51

Edwards KL, Austin MA, Newman B, Mayer E, **Krauss RM**, Selby JV

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**Multivariate analysis of the insulin resistance syndrome in women.**

Arteriosclerosis & Thrombosis 1994 Dec;14(12):1940-5

The insulin resistance syndrome (IRS) is characterized by a constellation of interrelated coronary heart disease (CHD) risk factors, including dyslipidemia, obesity, central obesity, elevated systolic blood pressure, and hyperinsulinemia. Factor analysis was used to investigate the clustering of

these risk factors in individuals by examining the correlational structure among these variables. Data from 281 genetically unrelated nondiabetic women who participated in exam 2 (1979 to 1980) of the Kaiser Permanente Women Twins Study were used. Factor analysis reduced 10 correlated risk factors to 3 uncorrelated factors, each reflecting a different aspect of the IRS: factor 1 (increased body weight, waist circumference, fasting insulin, and glucose), factor 2 (increased postload and fasting glucose and insulin and systolic blood pressure), and factor 3 (larger low-density lipoprotein particles, decreased plasma triglycerides, and increased high-density lipoprotein). Together, the factors explained nearly 66% of the total variance in the data. Thus, factor analysis defined three distinct aspects of the IRS in this sample of nondiabetic women. These factors may reflect separate underlying mechanisms of the syndrome, each of which may also be involved in CHD risk.

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### **Variations in high-density lipoprotein subclasses during the menstrual cycle.**

Metabolism: Clinical & Experimental 1994

Nov;43(11):1438-41

In a study of 41 healthy premenopausal women, plasma high-density lipoprotein-2a (HDL2a) levels (ie, HDL of diameter 8.8 to 9.7 nm) were significantly higher during the luteal phase than during the follicular phase of the cycle. There was no significant variation in HDL2b or any of the HDL3 subclasses.

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### **Genetic predictors of FCHL in four large pedigrees. Influence of ApoB level major locus predicted genotype and LDL subclass phenotype.**

Arteriosclerosis & Thrombosis 1994 Nov;14(11):1687-94

The genetic basis of familial combined hyperlipidemia (FCHL) has eluded investigators for 20 years, despite the apparent segregation of FCHL as an autosomal dominant disorder affecting 1% to 2% of individuals. Etiologic heterogeneity and additive effects of traits controlled by other genetic loci have been suggested. Two traits have been implicated in FCHL. The first is the predominance of a small, dense low-density lipoprotein (LDL), LDL subclass phenotype B, which segregates as a mendelian trait. The second is a mendelian locus with large effects on apolipoprotein (apo) B levels that is defined by complex segregation analysis (predicted apoB level genotype). This study shows that these factors appear to be separate genetic effects, both of which aid in the prediction of FCHL in four large pedigrees. The results suggest that FCHL may be best predicted by a threshold model in which apoB level genotype and LDL subclass phenotype each act to increase the risk of FCHL. Heterogeneity in the transmission of apoB levels among families is suggested, supporting the etiologic heterogeneity of FCHL. These results emphasize the advantages inherent in the study of large pedigrees when disease heterogeneity is suspected.

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### **Relations of plasma TG and HDL-C concentrations to body composition and plasma insulin levels are altered in men with small LDL particles.**

Arteriosclerosis & Thrombosis 1994 Jul;14(7):1121-8

Low-density lipoprotein (LDL) subclass pattern B is characterized by a predominance of small, dense LDL particles (LDL peak particle size > 255 Å), increased plasma triglyceride (TG) levels, reduced high-density lipoprotein (HDL) cholesterol levels, and glucose intolerance. This study tested the hypothesis that there are differences in the regulation of TG and HDL metabolism by insulin in patients with LDL pattern B. The study group comprised 160 healthy older (60 ± 8 years, mean ± SD) men. Forty-nine of the men (31%) had LDL pattern B. These men had a higher waist-to-hip ratio (WHR) (0.98 ± 0.06 versus 0.95 ± 0.06, P < .005) and lower maximal aerobic capacity (VO<sub>2</sub>max) (P < .005) than the 111 men of

comparable age with a predominance of larger LDL particles (LDL peak particle size > 255 Å, LDL pattern A). Men with LDL pattern B also had higher TG (1.76 ± 0.60 versus 1.03 ± 0.41 mmol/L, P < .0001) and lower HDL cholesterol (0.83 ± 0.13 versus 1.06 ± 0.29 mmol/L, P < .0001) and percent HDL2 subspecies (by gradient gel electrophoresis) (31 ± 4 versus 43 ± 6, P < .0001) levels than men with LDL pattern A, but the total cholesterol and LDL cholesterol levels did not differ between groups. Fasting glucose and insulin levels also did not differ between groups, but plasma glucose and insulin levels measured at 90 and 120 minutes during an oral glucose tolerance test were significantly higher in men with LDL pattern B. (ABSTRACT TRUNCATED AT 250 WORDS)

Haskell WL, Alderman EL, Fair JM, Maron DJ, Mackey SF, Superko HR, Williams PT, Johnstone IM, Champagne MA, **Krauss RM**, et al

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### **Effects of intensive multiple risk factor reduction on coronary atherosclerosis and clinical cardiac events in men and women with coronary artery disease. The Stanford Coronary Risk Intervention Project (SCRIP).**

Circulation 1994 Mar;89(3):975-90

BACKGROUND: Recent clinical trials have shown that modification of plasma lipoprotein concentrations can favorably alter progression of coronary atherosclerosis, but no data exist on the effects of a comprehensive program of risk reduction involving both changes in lifestyle and medications. This study tested the hypothesis that intensive multiple risk factor reduction over 4 years would significantly reduce the rate of progression of atherosclerosis in the coronary arteries of men and women compared with subjects randomly assigned to the usual care of their physician. METHODS AND RESULTS: Three hundred men (n = 259) and women (n = 41) (mean age, 56 ± 7.4 years) with angiographically defined coronary atherosclerosis were randomly assigned to usual care (n = 155) or multifactor risk reduction (n = 145). Patients assigned to risk reduction were provided individualized programs involving a low-fat and -cholesterol diet, exercise, weight loss, smoking cessation, and medications to favorably alter lipoprotein profiles. Computer-assisted quantitative coronary arteriography was performed at baseline and after 4 years. The main angiographic outcome was the rate of change in the minimal diameter of diseased segments. All subjects underwent medical and risk factor evaluations at baseline and yearly for 4 years, and reasons for all hospitalizations and deaths were documented. Of the 300 subjects randomized, 274 (91.3%) completed a follow-up arteriogram, and 246 (82%) had comparative measurements of segments with visible disease at baseline and follow-up. Intensive risk reduction resulted in highly significant improvements in various risk factors, including low-density lipoprotein cholesterol and apolipoprotein B (both, 22%), high-density lipoprotein cholesterol (+12%), plasma triglycerides (-20%), body weight (-4%), exercise capacity (+20%), and intake of dietary fat (-24%) and cholesterol (-40%) compared with relatively small changes in the usual-care group. No change was observed in lipoprotein(a) in either group. The risk-reduction group showed a rate of narrowing of diseased coronary artery segments that was 47% less than that for subjects in the usual-care group (change in minimal diameter, -0.024 ± 0.066 mm/y versus -0.045 ± 0.073 mm/y; P < .02, two-tailed). Three deaths occurred in each group. There were 25 hospitalizations in the risk-reduction group initiated by clinical cardiac events compared with 44 in the usual-care group (rate ratio, 0.61; P = .05; 95% confidence interval, 0.4 to 0.9). CONCLUSIONS: Intensive multifactor risk reduction conducted over 4 years favorably altered the rate of luminal narrowing in coronary arteries of men and women with coronary artery disease and decreased hospitalizations for clinical cardiac events.

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### **Reduced adipose tissue lipoprotein lipase responses, postprandial lipemia, and low high-density lipoprotein-2 subspecies levels in older athletes with silent myocardial ischemia.**

Metabolism: Clinical & Experimental 1994

Feb;43(2):190-8

Healthy older ( $64 \pm 1$  years, mean  $\pm$  SEM) athletic (maximal oxygen consumption [VO<sub>2</sub>max]  $> 40$  mL/kg/min) normocholesterolemic men with no prior history of coronary artery disease (CAD) were recruited for cardiovascular and metabolic studies. Thirty-three percent had asymptomatic exercise-induced ST segment depression on their exercise electrocardiogram (ECG), consistent with silent myocardial ischemia (SI). We hypothesized that abnormalities in high-density lipoprotein (HDL) and postprandial triglyceride (TG) metabolism may increase their risk for CAD. Compared with 12 nonischemic controls of comparable age, percent body fat, and VO<sub>2</sub>max, the 13 men with SI had decreased fasting HDL cholesterol ([HDL-C]  $41 \pm 2$  v  $50 \pm 2$  mg/dL,  $P < .001$ ) and %HDL<sub>2b</sub> subspecies levels as measured by gradient gel electrophoresis ( $22 \pm 2$  v  $34 \pm 3$ ,  $P < .001$ ). Fasting plasma TG and low-density lipoprotein cholesterol (LDL-C) levels were the same in both groups. Although plasma glucose levels during an oral glucose tolerance test (OGTT) were similar in both groups, the total insulin area was higher in men with SI ( $P < .05$ ). After consumption of a standard high-fat meal (680 kcal/m<sup>2</sup> body surface area of a formula in which 86% of the calories were derived from fat), postprandial plasma TG, chylomicron-TG, and very-low-density lipoprotein (VLDL)-TG levels and postprandial areas were higher in men with SI ( $P < .001$ ). (ABSTRACT TRUNCATED AT 250 WORDS)

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### Modification of LCAT activity and HDL structure. New links between cigarette smoke and coronary heart disease risk.

Arteriosclerosis & Thrombosis 1994 Feb;14(2):248-53

The mechanism(s) through which smoking influences the progression of atherosclerosis is poorly understood. Recent evidence suggests that oxidants present in the gas phase of cigarette smoke are involved. We exposed human plasma to the filtered gas phase of cigarette smoke to assess its effects on plasma components involved in the antiatherogenic reverse cholesterol transport pathway. In our model, freshly isolated plasma (24 mL) was exposed to filtered air or gas-phase cigarette smoke for up to 6 hours at 37 degrees C. Lecithin-cholesterol acyltransferase (LCAT) activity was dramatically inhibited by cigarette smoke. A single 15-minute exposure to the smoke from an eighth of a cigarette was sufficient to reduce LCAT activity by 7%; additional exposures resulted in further decreases in activity. At 6 hours, only 22% of control LCAT activity remained in plasma exposed to smoke. Compared with control, gas-phase cigarette smoke-exposed plasma possessed high-density lipoprotein (HDL) with increased (16%) negative charge and with cross-linked apolipoproteins AI and AII. These data demonstrate that gas-phase cigarette smoke can inhibit a key enzyme (LCAT) and modify an integral lipid transport particle (HDL) that are essential components for the normal function of the reverse cholesterol transport pathway. Gas-phase cigarette smoke-induced modification of the reverse cholesterol transport pathway may provide a new mechanistic link between cigarette smoke and coronary heart disease risk.

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### Oxidative susceptibility of low density lipoprotein subfractions is related to their ubiquinol-10 and alpha-tocopherol content.

Proceedings of the National Academy of Sciences of the United States of America 1994 Feb 1;91(3):1183-7

The conjugated polyene fatty acid parinaric acid (PnA) undergoes a stoichiometric loss in fluorescence upon oxidation and can be used to directly monitor peroxidative stress within lipid environments. We evaluated the course of potentially atherogenic oxidative changes in low density lipoproteins (LDL) by monitoring the oxidation of PnA following its incorporation into buoyant ( $p = 1.026$ - $1.032$  g/ml) and dense ( $p = 1.040$ - $1.054$  g/ml) LDL subfractions. Copper-induced oxidation of LDL-associated PnA exhibited an initial lag phase followed by an increased rate of loss until depletion. Increased PnA oxidation occurred immediately after the antioxidants ubiquinol-10 and alpha-tocopherol were consumed but before there were marked elevations in conjugated dienes. Despite

differences in sensitivity to early oxidation events, PnA oxidation and conjugated diene lag times were correlated ( $r = 0.582$ ;  $P = 0.03$ ), and both indicated a greater susceptibility of dense than buoyant LDL in accordance with previous reports. The greater susceptibility of PnA in dense LDL was attributed to reduced levels of ubiquinol-10 and alpha-tocopherol, which were approximately 50% lower than in buoyant LDL (mol of antioxidant/mol of LDL) and together accounted for 80% of the variation in PnA oxidation lag times. These results suggest that PnA is a useful probe of LDL oxidative susceptibility and may be superior to conjugated dienes for monitoring the initial stages of LDL lipid peroxidation. Differences in oxidative susceptibility among LDL density subfractions are detected by the PnA assay and are due in large part to differences in their antioxidant content.

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### Low-density lipoprotein subclass patterns and lipoprotein response to a reduced-fat diet in men. FASEB Journal 1994 Jan;8(1):121-6

Low-density lipoprotein (LDL) subclass pattern B is a common genetically influenced lipoprotein profile characterized by a predominance of small, dense LDL particles, and associated with increased levels of triglyceride-rich lipoproteins, reductions in high-density lipoprotein cholesterol (HDL-C), and increased risk of coronary artery disease compared to individuals with a predominance of larger LDL (pattern A). We sought to determine whether LDL subclass patterns are associated with response of plasma lipoprotein levels to changes in dietary fat and carbohydrate content. In a randomized cross-over study, 105 men consumed, for six weeks each, high-fat (46%) and low-fat (24%) solid food diets, with replacement of fat by carbohydrate. Diet-induced changes in subjects who exhibited pattern B ( $n = 18$ ) following the high-fat diet differed significantly from those in subjects with pattern A ( $n = 87$ ): in pattern B subjects LDL cholesterol (LDL-C) reductions were two-fold greater and plasma apolipoprotein (apo) B levels decreased significantly. These differences remained significant after adjustment for levels of plasma LDL-C, apo B, HDL-C, and body mass index. Thus, LDL subclass pattern is a factor that contributes significantly to interindividual variation of plasma lipoprotein response to a low-fat, high-carbohydrate diet.

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### Susceptibility of small, dense, low-density lipoproteins to oxidative modification in subjects with the atherogenic lipoprotein phenotype, pattern B [see comments].

Comment in: Am J Med 1993 Apr;94(4):347-9

American Journal of Medicine 1993 Apr;94(4):350-6

PURPOSE: To test whether low-density lipoprotein (LDL) from subjects with an atherogenic lipoprotein phenotype characterized by small, dense LDL (pattern B) demonstrates greater susceptibility to oxidative modification than LDL from subjects exhibiting primarily larger, more buoyant LDL particles (pattern A). PATIENTS AND METHODS: Measures of susceptibility to oxidative modification were compared in six density subfractions of LDL isolated from pattern A and pattern B subjects. Seven male and three female pattern A subjects and five male and two female pattern B subjects, classified on the basis of peak LDL particle size, were studied. Plasma lipid and lipoprotein levels, apolipoprotein B, mean LDL particle diameter, lag phase, and rate of oxidation after initiation of oxidation by copper sulfate were measured. RESULTS: The lag time, a measure of resistance to oxidative modification, was inversely related ( $p < 0.001$ ) to LDL density in both groups of subjects, without an independent effect of phenotype. The fraction that had the major LDL peak had a shorter lag time ( $p < 0.05$ ) in pattern B than in pattern A. Pattern B subjects also demonstrated an increased rate of oxidation ( $p < 0.005$ ) in fraction 1, which includes remnants of triglyceride-rich lipoproteins. CONCLUSIONS: The increased atherogenic risk associated with the pattern B phenotype may result in part from increased concentrations of lipoprotein subpopulations that are relatively susceptible to oxidative modification.

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**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

The relationship of beta-blocker drug use to plasma low density lipoprotein-cholesterol (LDL-C), lipoprotein mass distribution, (LDL, Sf0-12), intermediate density lipoproteins (IDL, Sf12-20), very low density lipoproteins (VLDL, Sf20-400), and high density lipoproteins (HDL, F(1.2)0-9) were examined in 206 men with coronary heart disease. Thirty-three used non-selective (NSEL), 49 used selective (SEL), and were compared to 124 who used no beta-blockade (NoBB). No significant between group differences were seen for potentially confounding variables. LDL and IDL mass, total cholesterol and LDL-cholesterol were not significantly different between groups. HDL-C was significantly lower in both NSEL ( $P < 0.005$ ) and SEL ( $P < 0.01$ ). NSEL and SEL had significantly lower HDL mass ( $P < 0.005$  and  $P < 0.005$ ) and SEL ( $P < 0.01$  and  $P = 0.06$ ), and HDL3 mass ( $P < 0.01$  and  $P < 0.05$ ). VLDL mass was significantly higher ( $P < 0.02$ ) only in NSEL. Small LDL (Sf0-7) was not significantly different between groups and large LDL (Sf7-12) was significantly lower in NSEL ( $P < 0.05$ ) and SEL ( $P < 0.05$ ). LDL peak Sf was significantly lower in both NSEL ( $P < 0.005$ ) and SEL ( $P < 0.02$ ) compared to NoBB. Despite the lack of differences in levels of LDL-cholesterol, beta-blocker use is associated with a significant difference in the distribution of larger, more buoyant to smaller, more dense LDL particles. Reduced HDL levels in subjects on beta-blockade therapy are associated with reductions in both HDL2 and HDL3 subclasses.(ABSTRACT TRUNCATED AT 250 WORDS)

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**Lipoprotein subclasses in genetic studies: the Berkeley data set.**

*Genetic Epidemiology* 1993;10(6):523-8

In conjunction with a study examining the inheritance of LDL subclass patterns in a healthy population, measurements of lipids, lipoproteins, and lipoprotein subclasses were performed in 301 individuals in 27 kindreds. Questionnaires were used to obtain information on use of medications, hormones, cigarettes, and alcohol. Laboratory data from this study (the Berkeley data set) include measurements of LDL and HDL size subclasses by nondenaturing gradient gel electrophoresis, and measurement of apolipoprotein A-I by radial immunodiffusion.

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**Associations of age, adiposity, alcohol intake, menstrual status, and estrogen therapy with high-density lipoprotein subclasses.**

*Arteriosclerosis & Thrombosis* 1993 Nov;13(11):1654-61

We used nondenaturing polyacrylamide gradient gel electrophoresis to examine the associations of age, adiposity, alcohol intake, and exogenous estrogen with high-density lipoprotein (HDL) subclasses in 427 members of 51 principally Mormon kindreds. The absorbency of protein stain was used as an index of mass concentrations at intervals of 0.01 nm within five HDL subclasses: HDL3c (7.2 to 7.8 nm), HDL3b (7.8 to 8.2 nm), HDL3a (8.2 to 8.8 nm), HDL2a (8.8 to 9.7 nm), and HDL2b (9.7 to 12 nm). Age and alcohol intake were obtained from questionnaires, and body mass index was computed from clinic measurements as weight (kg)/height (m)<sup>2</sup>. The results suggest that HDL3b concentrations were higher after menopause than before. Adult men (18 years old) had significantly higher HDL3c and HDL3b and significantly lower HDL2b and HDL2a levels than younger boys. Compared with the women, adult men had higher levels of HDL3c and HDL3b and lower levels of HDL2b, HDL2a, and larger-diameter HDL3a particles. There were no significant differences between the HDL profiles of women and younger boys, suggesting that

divergence in HDL occurs during puberty. Eighty-eight percent of the increase in HDL associated with estrogen replacement in postmenopausal women occurred within HDL3a and HDL2a. Reported alcohol intake in adult men correlated with two HDL regions: one within the HDL2b region and a second within the HDL3a/2a region, whereas in women the positive correlation between alcohol and HDL levels was within the HDL2b region only. In both men and premenopausal adult women, increasing levels of body mass index were associated with higher levels of HDL3b and lower levels of HDL2b.(ABSTRACT TRUNCATED AT 250 WORDS)

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**Differential effects of estrogen on low-density lipoprotein subclasses in healthy postmenopausal women.**

*Metabolism: Clinical & Experimental* 1993 Sep;42(9):1153-8

The use of estrogen by postmenopausal women decreases plasma low-density lipoprotein (LDL) cholesterol levels. To determine whether LDL subclass profiles influence this response, we studied 31 healthy postmenopausal women who were administered two doses (0.625 and 1.25 mg/d) of conjugated equine estrogen in a placebo-controlled double-blind crossover study. Lipid-stained gradient gels were used to categorize LDL subclass patterns. All women were classified as LDL subclass pattern A (predominant LDL peak < 260 Å). Within the pattern A classification, there were 12 women during placebo treatment with LDL subclass I pattern (predominant LDL peak > 271 Å) and 19 women with LDL subclass II pattern (predominant LDL peak 271 and 260 Å). Postmenopausal women with LDL subclass I on placebo treatment had significantly lower LDL cholesterol levels compared with women having LDL subclass II ( $126 \pm 28$  v  $147 \pm 23$  mg/dL,  $P < .03$ ). Postmenopausal women with LDL subclass I also had significantly ( $P < .05$ ) lower very-low-density lipoprotein (VLDL) cholesterol, VLDL triglyceride, and VLDL apo B levels and significantly higher ( $P < .05$ ) high-density lipoprotein 2 (HDL2) cholesterol, HDL3 cholesterol, and HDL2 apo A-I levels. Estrogen replacement significantly ( $P < .05$ ) decreased LDL cholesterol levels and increased VLDL and LDL triglyceride, HDL2 and HDL3 cholesterol and apo A-I, and HDL2 apo A-II levels to a similar extent in postmenopausal women with LDL I or II subclass patterns.(ABSTRACT TRUNCATED AT 250 WORDS)

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**LDL subclass phenotypes and the insulin resistance syndrome in women.**

*Circulation* 1993 Aug;88(2):381-7

**BACKGROUND.** Low-density lipoprotein (LDL) subclass phenotype B, characterized by predominance of small, dense LDL particles, is associated with elevated plasma triglycerides and apolipoprotein B and with lower high-density lipoprotein (HDL) cholesterol and apolipoprotein A-I. Because these abnormalities resemble the dyslipidemia of insulin resistance, we examined associations of LDL subclass phenotype with plasma insulin levels and with other aspects of the insulin resistance syndrome. **METHODS AND RESULTS.** LDL subclass phenotypes were determined by gradient gel electrophoresis in 682 female twins aged 30 to 91 years who participated in the second examination of the Kaiser Permanente Women Twins Study. Prevalence of phenotype B and the intermediate phenotype (I) increased strongly with age, obesity, and non-insulin-dependent diabetes. In multivariate analysis of nondiabetic women, phenotype B or I was independently associated with each aspect of the insulin resistance syndrome, including higher plasma triglycerides, waist-hip ratio, fasting and postload insulin levels, and systolic blood pressure and lower HDL cholesterol levels after adjustment for age and body mass index. The prevalence of phenotype B or I rose progressively from 5.6% in women with no manifestations of the insulin resistance syndrome to 100% in women with four syndrome components. In 25 nondiabetic, monozygotic twin pairs discordant for subclass phenotype, the twins with phenotype B (or I) had significantly higher levels of body mass index, waist-hip ratio, and systolic blood pressure than their twins with phenotype A. Thus, nongenetic variation in these risk factors is important in

explaining their associations with LDL subclass phenotype. CONCLUSIONS. Small, dense LDL is an integral feature of the insulin resistance syndrome. Nongenetic (ie, behavioral or environmental) factors are important for the expression of the phenotype and for its association with other heart disease risk factors.

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### **Insulin resistance and hyperinsulinemia in individuals with small, dense low density lipoprotein particles [see comments].**

Comment in: *J Clin Invest* 1993 Jul;92(1):3  
*Journal of Clinical Investigation* 1993 Jul;92(1):141-6

Subjects characterized by a predominance of small LDL particles (pattern B) have changes in plasma triglyceride (TG) and HDL-cholesterol concentrations consistent with the presence of resistance to insulin-mediated glucose uptake. To pursue this issue, plasma glucose and insulin responses to oral glucose, insulin-mediated glucose disposal, and lipoprotein concentrations were measured in subjects categorized on the basis of LDL peak diameter measured by gradient gel electrophoresis. Subjects with pattern B had higher ( $P < 0.05$ - $0.001$ ) total integrated plasma glucose ( $20.7 \pm 1.0$  mmol/liter.h) and insulin ( $1,743 \pm 293$  pmol/liter.h) responses to oral glucose compared with glucose ( $16.3 \pm 0.4$  and  $19.2 \pm 0.8$  mmol/liter.h) and insulin ( $856 \pm 60$  and  $1,222 \pm 168$  pmol/liter.h) responses in those with either pattern A or an intermediate pattern. Pattern B individuals were shown to be more insulin resistant on the basis of higher steady state plasma glucose concentrations (SSPG,  $10.4 \pm 1.0$ ,  $P < 0.002$ , vs.  $7.5 \pm 0.7$  and  $6.0 \pm 0.4$  mmol/liter) after a constant infusion of somatostatin, glucose, and insulin than those with either the intermediate or pattern A subclass. Pattern B subjects also had higher concentrations of ( $P < 0.001$ ) TG ( $1.98 \pm 0.15$  vs.  $1.33 \pm 0.17$  and  $0.77 \pm 0.05$  mmol/liter) and lower ( $P < 0.01$ - $0.001$ ) HDL cholesterol ( $1.12 \pm 0.06$  vs.  $1.34 \pm 0.05$  vs.  $1.45 \pm 0.05$  mmol/liter) than those with either the intermediate or pattern A. Finally, significant ( $P < 0.001$ ) correlation coefficients existed between LDL diameter and SSPG ( $r = -0.44$ ); glucose ( $r = -0.41$ ) and insulin ( $r = -0.38$ ) responses; TG ( $r = -0.65$ ) and HDL-cholesterol ( $r = 0.42$ ) concentrations; and systolic ( $r = -0.34$ ) and diastolic ( $r = -0.34$ ) blood pressure. Thus, pattern B subjects are insulin resistant, have higher glucose, insulin, and TG, lower HDL-cholesterol levels, and higher blood pressure than those with pattern A or intermediate.

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### **The hypertriglyceridemia of acquired immunodeficiency syndrome is associated with an increased prevalence of low density lipoprotein subclass pattern B.**

*Journal of Clinical Endocrinology & Metabolism* 1993 Jun;76(6):1423-7

Plasma low density lipoproteins (LDL) comprise multiple discrete subclasses, differing in size, density, and chemical composition. Gradient gel electrophoresis of LDL has demonstrated three common subclass patterns based on the predominant LDL subclass: large LDL, designated subclass pattern A; small LDL particles, designated subclass pattern B; and an intermediate pattern. Genetic studies have demonstrated that these patterns are inherited, but several lines of evidence suggest that environmental factors are important in the phenotypic expression of the pattern. The LDL B pattern is associated with increased levels of plasma triglyceride, reduced high density lipoprotein (HDL), and obesity. To better define the role of environmental factors on LDL phenotypic expression, we determined LDL patterns in patients with the acquired immunodeficiency syndrome (AIDS), an infection characterized by hypertriglyceridemia and weight loss. Similar to previous studies, plasma triglyceride levels were increased, whereas plasma cholesterol, LDL cholesterol, and HDL cholesterol levels were decreased in the AIDS subjects compared to those in age-matched controls. The percentage of AIDS subjects with the LDL B phenotype was increased 2.5-fold, demonstrating an increased prevalence of the LDL B phenotype in an acquired form of hypertriglyceridemia. For each LDL phenotype in AIDS, serum triglyceride levels were higher than the same phenotypic pattern in controls, with the most marked elevations in triglycerides found in AIDS

subjects with the LDL B phenotype. In contrast to what was observed in controls, HDL cholesterol levels were decreased in all AIDS subjects and were unrelated to LDL pattern. Total and LDL cholesterol levels were higher in controls with the LDL B phenotype than in those with the LDL A phenotype, but there was no difference in total and LDL cholesterol in AIDS subjects with LDL B compared to A. On multiple regression analysis in subjects with AIDS, plasma triglyceride levels, age, and HDL cholesterol all contribute to the occurrence of the LDL B phenotype, but elevations in plasma triglyceride levels are the strongest independent predictor. Body mass index was not a predictor of LDL B phenotype in AIDS. These results suggest that disturbances in triglyceride metabolism that are caused by AIDS lead to the appearance of the LDL subclass B phenotype and provide further evidence that environmental or disease states that perturb lipid metabolism can produce an increased prevalence of the LDL B phenotype.

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### **Genetics of LDL subclass phenotypes in women twins. Concordance, heritability, and commingling analysis.**

*Arteriosclerosis & Thrombosis* 1993 May;13(5):687-95

Low density lipoprotein (LDL) subclass phenotype B, characterized by a predominance of small LDL as determined by gradient gel electrophoresis, has been associated with increased risk of coronary heart disease and an atherogenic lipoprotein profile. Previous studies employing complex segregation analysis have demonstrated a major, single gene effect on the inheritance of this phenotype in families. Recently, linkage between this phenotype and variation at the LDL receptor locus on chromosome 19 has been reported. However, variation in LDL subclass phenotypes has also been associated with age, gender, diabetes status, beta-blocker medication, and diet. The present study further evaluates the relative importance of genetic and nongenetic influences on LDL subclass phenotypes and on LDL peak particle diameter (as a reflection of the size of the major LDL subclass) in monozygotic and dizygotic women twin pairs. The analysis is based on 203 monozygotic and 145 dizygotic pairs of adult female twins who participated in the second examination of the Kaiser Permanente Women Twins Study. The average age was 51 years at this exam and 90% were white. Concordance analysis revealed that monozygotic cotwins shared LDL subclass phenotypes more frequently than dizygotic cotwins, and this was confirmed using logistic regression analysis after controlling for potential confounding factors. Heritability analyses suggested that approximately one third to one half of the variation in LDL peak particle diameter, a continuous variable reflecting LDL size, could be attributed to genetic influences. Commingling analysis of the frequency distribution of LDL peak particle diameter identified three distinct subgroups of subjects, one of which corresponded to those subjects with LDL subclass phenotype B. (ABSTRACT TRUNCATED AT 250 WORDS)