Technology Assessment

Low Density Lipoprotein Subfractions: Systematic Review of Measurement Methods and Association with Cardiovascular Outcomes

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Low Density Lipoprotein Subfractions: Systematic Review of Measurement Methods and Association with Cardiovascular Outcomes

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Tufts Evidence-based Practice Center

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Tables and Figures

Abbreviations

Abbreviation	Definition
Adj	adjusted for cardiovascular risk factors, including lipids and/or triglycerides
AHRQ	Agency for Healthcare Research and Quality
ApoB	apoprotein B
Assns	associations
ATP III	Adult Treatment Panel III (3rd report of the National Cholesterol Education Program Expert Panel)
AUC	area under the curve
BMI	body mass index
CABG	coronary artery bypass graft
CAC	coronary artery calcification
CAD	coronary artery disease
CC	case control studies
CDC	Centers for Disease Control and Prevention
CerebroVD	cerebrovascular disease
CHD	coronary heart disease
CI	confidence interval
CITP	capillary isotachophoretic method
CK	creatine kinase
CLIA	Clinical Laboratory Improvement Act
CMS	Centers for Medicare and Medicaid Services
hsCRP	high sensitivity C reactive protein
CV	coefficient of variation
CVD	cardiovascular heart disease
DBP	diastolic blood pressure
DGUC	density gradient ultracentrifugation
DM	diabetes mellitus
ECG	electrocardiogram
EPC	Evidence-based Practice Center
ESRD	end stage renal disease
FH	familial (hereditary) hypercholesterolemia (homozygous or heterozygous)
FHx	family history
FPG	fasting plasma glucose
Fram Sc	Framingham score
GE	gel electrophoresis
HDL	high density lipoprotein
HDL-c	HDL cholesterol
HPLC	high performance gel filtration (liquid) chromatography
HTN	hypertension
IMT	intima-media thickness
Inter	intermediate
JNC 7	The 7th Report of the Joint National Committee on Prevention, Detection,

Abbreviation	**Definition**
	Evaluation, and Treatment of High Blood Pressure
LDL	low density lipoprotein
LDL-c	LDL cholesterol
LDLSF score	LDL subfraction score
LOA	Bland-and-Altman limits of agreement
MI	myocardial infarction
MLD	minimum lumen diameter (coronary arteries)
MRI	magnetic resonance imaging
N	sample size
nCC	nested case control studies
nd	no data
NMR	nuclear magnetic resonance
No.	number
OR	odds ratio
P btw	P value for difference between treatment and control
P Cohort	prospective cohort (cross-sectional) studies
P Long	prospective longitudinal studies
PTCA	percutaneous transluminal coronary angioplasty
py	pack-year
r	correlation coefficient
RCT	randomized controlled trial
Ref Std	reference standard
Rf	ratio of distance moved by band relative to marker
RR	relative risk
SBP	systolic blood pressure
SD	standard deviation
sd LDL	small dense LDL
TC	total cholesterol
Tg	triglycerides
TIA	transient ischemic attacks
UC	ultracentrifugation
UI	Medline unique identifier
Unadj	unadjusted for lipids and/or triglycerides
VAP	Vertical Auto Profile
WHO	World Health Organization
XS	cross-sectional

Chapter 1. Introduction

Cardiovascular disease (CVD) is the leading cause of disability and death in the US.[1] Identifying individuals at high risk for CVD and aggressively treating them is a critical component to lower the population-wide disease burden. The Adult Treatment Panel III (ATP III) of the Expert Panel of the National Cholesterol Education Program has identified a group of risk factors associated with CVD. Risk factor assessment is used to estimate individual risk and inform decisions on course of treatment and target goals for the efficacy of treatment once it has been initiated. Cardiovascular risk factors addressed by ATP III (in addition to elevated LDL cholesterol) include cigarette smoking, hypertension (blood pressure > 140/90 mm Hg or on antihypertensive medication), low concentration of high density lipoprotein (HDL) cholesterol (< 40 gm/dL), family history of premature coronary heart disease (CHD), and older age (women > 55 years; men > 45 years).[2] For individuals with two or more risk factors, ATP III recommends estimating a 10-year CHD risk score (Framingham Score) and making treatment recommendations and setting LDL cholesterol goals on the basis of this score.[3]

Recently, questions have been raised as to how well the standard ATP III criteria for estimating CVD risk identifies high-risk individuals and whether additional diagnostic criteria are needed to adequately estimate CVD risk.[4-7] For the most part, this controversy has centered on the incremental value of additional risk factors to those currently used. Some additional candidate risk factors include high sensitivity C-reactive protein, lipoprotein-associated phospholipase A2, N-terminal pro-atrial natriuretic peptide, aldosterone, renin, fibrinogen, D-dimer, plasminogen-activator inhibitor type 1, homocysteine, urinary albumin-to-creatinine ratio, hemoglobin A1c, lipoprotein (a) [Lp(a)], apoprotein (apo) A-I, apo B and LDL particle size.

It has been suggested that determining LDL particle size distribution provides additional predictive power to LDL cholesterol measurement alone to estimate an individual's CVD risk.[2] On the basis of particle density, small dense LDL particles are thought to confer a higher level of risk than larger less dense LDL particles.[8,9] In vitro, small dense LDL particles are taken up more avidly by macrophages than larger less dense LDL particles.[10] This may be related to small dense LDL being more susceptible to oxidative modification or having a greater binding potential to arterial wall proteoglycans than the larger less dense LDL particles. Higher plasma concentrations of small dense LDL tend to be associated with higher concentrations of triglyceride and apo B-100, and lower concentrations of HDL cholesterol and apo A-I, each of which has independently been associated with increased CVD risk.[11]

The American Diabetes Association, together with the American College of Cardiology Foundation, convened a panel of experts to develop a consensus position for patients with "cardiometabolic risk."[12] In their opinion, LDL particle number, as measured by nuclear magnetic resonance (NMR) may be a better discriminator of risk than LDL cholesterol and that both LDL particle concentration and LDL size are "important predictors of CVD;" though several limitations, including availability and accuracy of the method, were noted. Despite this consensus piece, it has yet to be determined whether CVD risk assessment and treatment decisions would be improved if LDL subfraction measurements were available to clinicians and were factored into the decision making process. Furthermore, there are numerous disparate systems currently available to estimate LDL subfractions, though most are labor-intensive and/or require long assay turnaround times, making them impractical for routine use by clinical laboratories. Were LDL subfractions associated with altered CVD risk, it is unclear whether the different characteristics of the LDL subfractions assessed by the different methods would result in similar predictive qualities for estimating CVD risk. It is also unclear whether measuring LDL subfractions would be of incremental benefit over measurement and treatment of traditional cardiovascular risk factors.

Multiple terms are used in the literature to describe LDL subfractions and related features of LDL, including LDL subclasses, particles, particle concentration, particle numbers, and patterns. These terms describe separate, but sometimes overlapping features of LDL. For simplicity, this report uses what we believe is the most generic term, subfractions, except where specific measurements are being described. We acknowledge that this term does not completely describe all measured features of LDL, be we determined it was a reasonable compromise to reduce the burden of repeatedly listing terms. LDL subfraction clearly has deficiencies as a generic term, and our use of the term is not meant as a recommendation that this term be adopted by the research community. We also do not mean to suggest that that the disparate methods for analyzing LDL can be fully subsumed in a single concept.

In December 2006 the Food and Drug Administration (FDA) held a public hearing on lipoprotein subfractions (www.fda.gov/OHRMS/DOCKETS/ac/06/transcripts/2006-4263t1-01t.pdf, accessed Feb 19, 2008). Several questions were formulated from this meeting regarding the use of LDL (and HDL) subfractions for clinical decision making. Based on this hearing, the Centers for Medicare & Medicaid Services (CMS) requested a review of the literature on LDL subfractions and the risk of CVD. After an early overview of the potentially relevant literature by the Tufts Evidence-based Practice Center (Tufts EPC), the questions of interest for this report were restricted to a description of the measurement methods that potentially could be routinely used by clinical laboratories, comparisons of the different measurement methods, a review of the evidence regarding the association between LDL subfractions and CVD, and a review of studies that evaluated an intervention that may "improve" LDL subfraction profiles and also evaluated cardiovascular outcomes. The primary population of interest for this review is the over age 65 Medicare population; however, data from all adults are also of interest to CMS.

Key questions to be addressed

1. What are the methods that have been proposed to be used routinely to measure LDL subfractions? Is there a method that is considered the reference standard?

2. How do different methods of measuring LDL subfractions compare in terms of test performance?

3.1 How much variability is there in measures of LDL subfractions from day to day?

3.2 How much variability is there in measures of LDL subfractions within the same individual (measure to measure)?

4.1 What is the relationship between LDL subfractions and outcome measures related to CVD?

4.2 If these tests are used in combination with other cardiovascular risk assessment technologies, what is the incremental increase of diagnostic performance?

4.3 If there is a relationship between LDL subfractions and CVD, how strong is it relative to other risk factors?

4.4 What do studies report regarding the link between therapies to alter LDL subfractions and CVD outcomes?

Chapter 2. Methods

This report on the low density lipoprotein (LDL) subfractions and associations with CVD is based on a systematic review of the literature, selected review articles, and Food and Drug Administration (FDA) documents.

Search Strategy

A comprehensive search of the scientific literature was conducted to identify relevant studies addressing the key questions. Our final search was conducted on August 22 2007. We searched MEDLINE (from 1950 to present), CAB Abstracts (1973 to present), the Cochrane Clinical Trial Registry (3rd quarter 2007), and the Cochrane Database of Systematic Reviews (3rd quarter 2007) to identify articles relevant to each key question. In electronic searches, we used various terms for LDL, particle size/subfractions, and test methodologies, limited to humans and English language (see **Appendix A** for complete search strategy). The same literature data set was used for all key questions. We did not systematically search for unpublished data with the exception of FDA documents, as described below.

Classification of LDL Subfraction Methods (Tests)

For the purposes of our analyses, we divided the researched methods into different categories:

- Nuclear Magnetic Resonance (NMR). This method uses NMR techniques to measure amplitudes of spectral signals emitted by lipoprotein subfractions of different sizes. This method is available for clinical use via a small number of medical laboratories.
- LipoPrint™. This is a measurement technique clinically available that uses a standardized method for using linear polyacrylamide gel electrophoresis to separate LDL particles on the basis of size and to a lesser extent charge. The kit and instrument for this method are marketed by Quantimetrix.
- Berkeley HeartLab® gradient gel electrophoresis. This is a standardized system using a specific gradient GE to provide LDL subfraction patterns. The standardized version of this system is performed only at the Berkeley HeartLab®, but is clinically available.
- Gel electrophoresis (GE) (Bench). This covers a wide range of methods using GE. These methods are either not standardized or, if standardized, are not routinely used by clinical laboratories. In general, researchers prepare their own gels and use their own methods for running the analyses. Different compounds are used to create the gels, though polyacrylamide is most common, and different distributions of gel densities are used. These methods are time- and resource-intensive.
- Ultracentrifugation. This covers a wide range of methods that separate lipoprotein particles on the basis of density, either sequentially and continuously, prior to lipid or apoprotein analysis. These methods are time- and resource-intensive.
- Other methods that were considered include high pressure liquid chromatography (HPLC), capillary isotachophoresis (CITP), Lipophor™ (another GE method developed by Quantimetrix), and other techniques. In addition, other clinically available methods for

measuring LDL subfractions include an ultracentrifugation technique performed at the University of Washington's Northwest Lipid Research Laboratory and the Vertical Auto Profile®. However, as described in the results section for Question 4, no studies eligible for the clinical associations portions of this review used these latter two methods.

Study Selection

We assessed titles and/or abstracts of citations identified from literature searches for inclusion, using the criteria described below. For studies that potentially met the criteria, the full text articles were retrieved and a second review was conducted to determine inclusion by reapplying the eligibility criteria. A low threshold was used to retrieve articles for full rescreening.

Eligibility criteria for key question 1 (routinely used measurement methods and reference standards)

General approach: Discussion regarding methods (tests) that are available for routine use or that may be used as a reference standard. The term "routine" was operationalized to mean that the method could be suitable for use by a commercial or institutional clinical laboratory for measuring LDL subfractions, as ordered by clinicians.

Study design: Narrative or systematic review, editorial or letter with references. English language. Published since 2001.

Intervention: Methods (tests) for the measurement of LDL subfraction distribution.

In addition, to identify methods submitted to the Food and Drug Administration (FDA) for clearance to proceed to market, the FDA Clinical Laboratory Improvement Act (CLIA) database was searched for all listed analyte names with "lipoprotein fractions" and for "nuclear magnetic resonance/NMR" test systems or specific manufacturers. All documents and internet links associated with the FDA CLIA records were examined for the relevance to the methods of measuring or separating LDL subfractions.

Eligibility criteria for key questions 2 and 3 (test performance)

Population: Human serum samples. If information is provided on the individuals, then they must be at least 18 years old.

Intervention: Any method to measure LDL subfraction distribution.

Comparators: For question 2, studies must have compared methods from two or more different categories of methods (as described above). Exclude studies that evaluated only incremental or technical changes to the methods. For question 3.1, studies must have drawn serum samples from the same volunteers on multiple days within a short period of time (we did not set a strict upper limit on the time frame). For question 3.2, studies must have measured the same serum samples using the same methods at least twice.

Outcomes: We allowed any method of comparing test accuracy, validity, or consistency, including sensitivity/specificity, Bland-Altman plots, correlation (r), or measures of variability.

Design: Articles must report original data; review articles were excluded. Articles must have been peer reviewed; letters and abstracts were excluded. The dataset must include serum samples from at least 10 individuals for each method.

Eligibility criteria for key questions 4.1-4.3 (association with CVD)

Population: Adult humans (≥18 years old). Excluded highly atypical populations on a case by case basis (eg, a study of people with hypopituitary growth hormone deficiency was excluded).

Predictors: LDL subfraction information, including size, concentration, or subclass pattern, using any method (test). Serum (or plasma) samples must be drawn prior to outcomes (for incidence studies) or at least 1 month after a cardiovascular event (for prevalence studies) to allow time for stabilization of lipoproteins after the event. Studies were excluded if they used a measurement method that was determined to be outdated to the extent that there is little comparability to modern methods. For question 4.2, studies must report the incremental change in diagnostic performance over other cardiovascular risk assessment tools. For question 4.3, studies must report complete results of multivariable analyses that included both LDL subfractions and other cardiovascular risk factors (though not exclusively other lipoprotein subfractions). For all questions we did not evaluate differences in constituents of LDL, such as percent total protein, apo B, cholesteryl esters, or triglycerides.

Outcomes: Clinical or selected surrogate cardiovascular outcomes, including cardiovascular events, clinical CVD status (eg, diagnosis or prevalence of CVD, stage or severity of CVD), intima-media thickness (IMT, Doppler ultrasonography measurement of degree of arterial atherosclerosis), or electron beam computerized tomography (EBCT, a measurement of calcium deposits in the coronary vessels).

Design: Prospective or retrospective, cross-sectional (for prevalence) or longitudinal (for incidence). Single or parallel cohort studies, case control or nested case control studies. Data set must include at least 10 subjects per study group. Studies must report sufficient data or analyses to assess the association between LDL subfractions and cardiovascular outcomes. No minimum duration for longitudinal studies.

Eligibility criteria for key question 4.4 (therapy, LDL subfraction, & CVD)

Population: Adult humans (≥18 years old). Excluded highly atypical populations on a case by case basis.

Interventions: Pharmaceutical or other intervention hypothesized to beneficially affect LDL subfractions.

Comparators: Other interventions that may affect LDL subfractions, or placebo, usual care, or no treatment.

Outcomes: Clinical or selected surrogate cardiovascular outcomes, including cardiovascular events, clinical CVD status (eg, diagnosis or prevalence of CVD, stage or severity of CVD), IMT (Doppler ultrasonography measurement of degree of arterial atherosclerosis), or electron beam computerized tomography (EBCT, a measurement of calcium deposits in the coronary vessels).

Analyses: At a minimum, the studies must have reported how the baseline or on-trial LDL subfractions were associated with CVD outcomes, stratified by intervention (i.e., the associations in both the intervention and the control arms), or they must have reported how the change in LDL subfractions from baseline to on-trial was associated with CVD outcomes. Studies were excluded (for this question) if they reported associations between baseline or on-trial LDL subfractions and CVD outcomes if they pooled interventions, even if they adjusted for intervention in a multivariable model.

Design: Randomized controlled trials (RCTs) or nested case control studies within an RCT. Dataset must include at least 10 subjects per arm (or original arm of the RCT). No minimum duration.

Data Extraction

Separate data extraction forms were designed for questions 2 & 3 and for question 4. For studies that met criteria for questions 4.1-4.4, full data extraction was completed only for studies that used specific methods or kits that are currently available for clinical use or had the samples analyzed by laboratories that also perform LDL subfraction analyses for clinical use (using the same methods that are currently used for clinical samples). We used the best information available to us from CMS, FDA, domain experts, the reviewed studies, internet searches, invited reviewers, and conversations with several laboratories to determine which methods are available for clinical use. We also used the best available information to determine whether the specific methods used by investigators are similar to the methods used by clinical laboratories; however, we did not contact investigators. Because the methods used in other studies are not clinically available in the US, data from studies that used these other methods were summarized only briefly (see below for more details). For eligible studies we extracted data on study year, country, setting, funding source, study design, timing of endpoints (if applicable), eligibility criteria, measurement method, comparator (if applicable), definitions of outcomes, subject characteristics (if applicable), and baseline, final, or correlation results for outcomes of interest (as applicable).

For question 4.1-4 we focused on two types of analyses: adjusted analyses (multivariable analyses where the association between LDL subfraction and CVD outcomes were adjusted for LDL cholesterol, HDL cholesterol, non-HDL cholesterol, and/or triglycerides); and unadjusted analyses (whether completely unadjusted or, if these data are not reported, adjusted only for variables not included in the adjusted list, such as other lipoprotein subfractions, clinical history, demographics, or blood pressure).

For questions 4.1-4.4 studies of “other” methods, data were extracted directly into summary tables.

Quality Assessment

We assessed the methodological quality of each fully extracted study (and all question 4.4 studies) based on predefined criteria. We used a 3-category grading system (A, B, C) to denote the methodological quality of each study. This grading system has been used in most of the previous evidence reports from the Tufts EPC as well as in evidence-based clinical practice guidelines. This system defines a generic grading system that is applicable to varying study designs including randomized and nonrandomized comparative trials, cohort, and case-control studies. Studies were not rejected due to poor quality.

A (good)
Good quality studies are likely to have the least bias and results are considered valid. They include studies that adhere most closely to the commonly held concepts of high quality including the following: a formal randomized controlled study; clear description of the population, setting, interventions, and comparison groups; appropriate measurement of outcomes; appropriate statistical and analytic methods and reporting; no reporting errors; clear reporting of dropouts; and no obvious bias. For studies evaluating associations between LDL subfractions and CVD outcomes, only those that evaluated incidence or progression of disease in longitudinal studies were eligible to be a grade A study. The association between LDL subfractions and prevalent CVD was not deemed to be a clinically high quality analysis.

B (fair)
Fair quality studies are susceptible to some bias, but not sufficient to invalidate the results. They do not meet all the criteria in category A because they have some deficiencies, but none likely to cause major bias. The study may be missing information, making it difficult to assess limitations and potential problems.

C (poor)
Poor quality studies have substantial bias that may invalidate the results. These studies have serious problems in design, analysis, or reporting; have large amounts of missing information, large dropout rates, discrepancies in reporting, lack of proper adjustments for relevant variables, or other major sources of bias.

Applicability Assessment

Applicability addresses the relevance of a given study to a population of interest. Every study applies certain eligibility criteria when selecting study subjects. Most of these criteria are explicitly stated (eg, disease status, age, comorbidities). Some may be implicit or due to unintentional biases, such as those related to location (eg, multicenter vs. single center, intensive care vs. all inpatients), year of procedure, and other issues. The applicability of a study is dictated by the key questions, the populations, and the interventions that are of interest to this review, as opposed to those of interest to the original investigators.

We categorized studies within a target population into 1 of 3 levels of applicability that are defined as follows:

High Sample is representative of Medicare population in relevant settings. Patients' age (older adult), gender, spectrum of disease severity and type, etc. are representative of population of interest. No substantial exclusion criteria that would make the sample atypical of Medicare patients for whom LDL subfraction testing might be considered.

Moderate Sample is an important subgroup of population of interest. Possibly limited by a narrow or young age range, type of disease, gender, restrictive eligibility criteria, etc.

Low Sample represents only a narrow, atypical subgroup of population of interest.

Summary Tables

For each question we summarized data in summary tables which include data on study design characteristics, subject characteristics, test method, number of subjects (or samples) analyzed, results data, and for most questions, quality, and applicability.

Most tables include details of the outcome data as reported by the study authors. However, because of the large number of studies that used methods that are not available for clinical use and because of the limited applicability of these studies to clinical practice (given the lack of standardization of LDL subfraction measurement or reporting), we report only qualitative results for each of these studies that addressed questions 4.1-4.3. Symbols were used to denote statistically significant positive or negative associations between LDL subfractions and CVD outcomes, lack of association, or in a few cases what the authors reported as substantial associations but where statistical analysis was not reported. See Tables 9 and following for the symbols and their definitions. Analyses that were adjusted or unadjusted for lipoproteins are presented in separate columns, with differently shaded symbols. For these tables, we distinguished between measurements of "size" (diameter in angstroms) and measurements of "pattern." Pattern covered all the different measurements of specific subfraction concentrations (or other levels), proportions (compared to overall or other subfractions), and other measurements describing the distribution of subfractions.

For question 4.1, grand summary tables (Tables 14-16) were also created, presenting clinically available methods and other methods together. These describe the number and type of studies that found positive, negative or no associations with CVD outcomes in both unadjusted and adjusted analyses. The final table (Table 16) also summarizes those studies that reported both unadjusted and adjusted analyses, to evaluate the effect of adjusting for lipoproteins.

For questions 4.2 and 4.3, separate summary tables were created for incident and prevalent CVD, each clinically available method, and univariable and multivariable analyses. Univariable and multivariable sets of data that do not include LDL subfraction are not included. Each table includes the same list of potential cardiovascular risk factors. This list was derived from the evaluated studies. It includes, in order, the LDL subfraction measures, the lipoprotein cholesterol and triglyceride concentrations, the risk factors used by ATP III[2] and JNC 7,[13] and other potential risk factors. The other lipoprotein subfractions are omitted from analysis. The primary purpose of the evaluation of the multivariable analyses for this report is to determine whether any measures of LDL subfractions are predictors of outcomes independent of other known or commonly measured predictors of or risk factors for CVD used in clinical practice. The approach used, and this report in general, is not meant to evaluate etiology of any associations. The tables are designed to describe the relative strengths of the associations between the risk factors and the CVD outcomes, not to describe each model created by individual studies or the value, per se, of each risk factor. Therefore, to maintain simplicity and readability, the measurement units for each risk factor are not included in the tables (except for percent of subjects. Other tables provide the more detailed data for the LDL subfractions. The original papers should be read for other detailed data. Unadjusted risk factors were ranked based on the statistical significance of their association with the outcome. From adjusted, multivariable models, the risk factors with the strongest associations with the CVD outcomes (eg, largest OR) are tabulated.

For question 4.4, tables were created based on the different potential analyses described under *Eligibility criteria key question 4.4*, above. Separate tables were created for data on the association between changes in LDL subfraction and CVD outcomes, between baseline LDL subfraction and outcomes stratified by intervention, and between on-trial LDL subfraction and outcomes stratified by intervention. Results are given for analyses both unadjusted and adjusted for lipoprotein concentrations.

Chapter 3. Results

Literature Search

The literature search yielded 6373 unique citations from Medline (n=5996), CAB Abstracts (n=326), the Cochrane clinical trial registry (n=47), and the Cochrane database of systematic reviews (n=4). Of these, 457 full text articles were retrieved. As described further below, 9 articles provided information for Question 1, 9 studies were eligible for Question 2, 5 studies were eligible for Question 3 (two of which were also eligible for Question 2), and 65 studies were eligible for Question 4 (one of which was also eligible for Question 2).

Among the 374 rejected articles, 270 evaluated possible treatments for abnormal LDL subfractions but did not evaluate CVD outcomes (see **Appendix C**). Among the remaining rejected articles 54 had no relevant information, 12 did not evaluate LDL subfractions, 9 did not evaluate a clinical CVD outcome (for Question 4), 7 were duplicate publications, and 22 were rejected for other reasons (see **Appendix B**).

Question 1

What are the methods that have been proposed to be used routinely to measure LDL subfractions? Is there a method that is considered the reference standard?

Among the studies evaluated below for Question 4, four general methods for separating and measuring LDL subfractions were identified: gel electrophoresis (GE), nuclear magnetic resonance (NMR), ultracentrifugation, and high pressure liquid chromatography (HPLC). The most common methods reported for measuring LDL subfractions involve either GE or ultracentrifugation methods.

Gel electrophoresis

The large majority of studies that have implemented GE used specific methods that were particular to the research laboratories. The researchers created their own gels and used techniques that may have been based on previous researcher's work, but were not standardized. They also tended to use definitions of LDL subfractions that were unique to their laboratories or were otherwise not standardized. LDL subfractions quantified by GE are frequently classified into either pattern A, pattern B, or an intermediate pattern. Investigators may also use different algorithms for the classifications of LDL patterns. GE can also determine the LDL subfraction sizes by comparisons with calibrators that included particles and/or LDL lyophilized standards with known sizes. A drawback of these methods is the lengthy labor intensive nature of the experimental procedures some of which require more than a day for sample analysis. There is also a practical restriction to the number of samples that can be analyzed at any one time, which may limit applications of these methods for routine clinical use.[14] Another limitation of these GE methods was due to limits in thickness of the gradient gels as related to ensuring reproducibility of the gradient gels, which in turn may affect comparability of LDL particle separation from laboratory to laboratory. Moreover, the visualization of the protein bands requires the removal of the gel from its casing, incubation with a staining solution followed by destaining and scanning, again, a labor intensive process that can introduce variability.

LipoPrint® (Quantimetrix Corp.) is a GE system that is available to clinical laboratories for testing of LDL subfractions in patients. The system includes specific equipment and reagents and a standard method for defining LDL subfractions. It uses a loading gel that is polymerized with fluorescent light. This method permits separation of LDL into seven subfractions within 60 minutes. Multiple samples can be run simultaneously. Because the gels are prepared by the company, it is technically simpler, less expensive, and more conducive to routine laboratory testing than traditional GE.[15] LDL particles are separated by size and to a lesser extent charge, and migration distance is quantified by densitometric scanning. According to LipoPrint® product insert describing the manufacturer's instruction for the analytic procedure and producing quantitative results (http://www.4qc.com/products/lipoprint/index.html), a typical Lipoprint® profile consists of 1 VLDL band, 3 midbands (comprising primarily IDL), up to 7 LDL bands, and 1 HDL band. After the electrophoresis is completed, the various stained lipoprotein fractions (bands) present in the sample are identified by their mobility (Rf) using VLDL as the starting reference point (VLDL=0) and HDL as the leading reference point (HDL=1). The relative area for each lipoprotein band is determined and multiplied by the total cholesterol concentration of the sample to yield the amount of cholesterol for each band in mg/dL. The lipoprotein subfraction profiles can also be classified into Type A (normal) and Type B (abnormal) based on the average particle size of the LDL particles described in a paper by Austin and associates.[16] Use of the Lipoprint® to determine particle sizes or LDL scores or any other form of classification is not recommended by the manufacturer of the kit. However, as will be noted below, research laboratories using Lipoprint® frequently have not used the recommended LDL subfraction definitions.

Berkeley HeartLab® uses LDL Segmented Gradient Gel Electrophoresis (LDL-S_3GGE™).[17] This technique separates LDL particles into 7 LDL subfractions (LDL I, IIa, IIb, IIIa, IIIb, IVa, and IVb) based on particle size and shape. LDL-S_3GGE™ gel kit provides a method (a computer algorithm) for calculating the number of particles in an LDL subfraction.[18] The LDL particle number is determined by assuming a physiological 1:1 ratio between apo B and LDL particles. In published literature, investigators use the S_3GGE™ gel kit for classifying LDL subfractions as pattern A, AB, or B based on LDL size cutoffs.[17]

Ultracentrifugation

Similar to GE, the studies that have implemented ultracentrifugation used a variety of instruments, specific methods, and definitions of LDL subfractions in their laboratories. Ultracentrifugation is likewise labor intensive, particularly sequential flotation, which may require more than a day for sample analysis. An arbitrary selection of density ranges is often used. LDL subfractions quantified by ultracentrifugation are frequently classified into either pattern A, pattern B, or an intermediate pattern. Investigators also used different algorithms for the classifications of LDL subfractions.

As best we could determine, the University of Washington's Northwest Lipid Research Lab (http://depts.washington.edu/nwlrl/) uses an ultracentrifugation method and is available to run clinical samples.

Nuclear magnetic resonance

The NMR method measures the signal from the aggregate number of terminal methyl groups in the lipid within the particle. The number of methyl groups is reflected in the amplitude of the methyl NMR signal. The amplitude of each lipoprotein particle signal serves as a measure of the concentration of that lipoprotein. Using standard assumptions concerning lipoprotein diameter and lipid content, the NMR data can be transformed (through calculations) into subfraction concentrations. Other quantitative subfraction information, such as LDL size and patterns, can also be derived through additional calculations.[19] NMR is available to patients and clinicians by sending samples to a small number of clinical laboratories that have the equipment.

The concept of using proton NMR spectroscopy to measure plasma lipoprotein particle concentrations was introduced in the early 1990s and was commercialized for clinical research in 1997.[19] NMR can quantify the numbers of lipoprotein subclass particles based on two phenomena. First VLDL, LDL, and HDL subclasses of different sizes in plasma simultaneously emit distinctive NMR signals whose individual amplitudes can be accurately and reproducibly measured. Second, the measured subclass signal amplitudes are directly proportional to the numbers of subclass particles emitting the signal, irrespective of variation in particle lipid composition. Therefore, NMR spectroscopy can provide simultaneous measurements of LDL particle number and size (through calculations), as well as measurement of high density and very low density lipoprotein (HDL and VLDL) subfractions. There are, however, several assumptions for NMR measurements of lipoprotein subfractions. The NMR method is calibrated by its library of over 30 signal envelopes from size-characterized purified fractions. It is assumed that every sample analyzed and the NMR spectra deconvoluted by the NMR method software has components encompassed closely enough by this calibration library, and all NMR spectral components of a given sample are unique to lipoproteins (ie, no spectral interferences). There are many layers of assumptions within the NMR software, which is proprietary. Some of the unknown assumptions, calibration and validation issues have been addressed[19] but some remain to be fully evaluated.

High performance liquid chromatography (HPLC)

The original HPLC method for measuring LDL size monitors the column effluent at 280 nm of the isolated LDL subfraction by ultracentrifugation. The retention time of the LDL peak is then used to calculate the LDL diameter.[20] A drawback of this method is the necessity of LDL isolation by ultracentrifugation prior to chromatography. A modified HPLC method that is based on selective detection of lipoproteins by postcolumn labeling with parinaric acid (a fluorescent lipid probe) permits direct measurement of LDL size in whole plasma or serum.[21] Notably, though, despite a positive report of the method's comparability to GE, as described below, the method has only rarely been used over the past decade by researchers of LDL subfractions and CVD risk.

Methods submitted to the Food & Drug Administration (FDA)

The CLIA database contains the marketed in vitro test systems categorized by the FDA since January 31, 2000 and tests categorized by the Centers for Disease Control and Prevention (CDC) prior to that date. A search on the CLIA database for all listed analyte names with "lipoprotein fractions" returned a total 31 records meeting this search criterion. All documents and internet links associated with these 31 records were examined for the relevance to the methods of measuring or separating LDL subfractions. Seven devices were identified: Helena Laboratories REP HDL/LDL-30 Electrophoresis System, Helena Laboratories REP Ultra HDL, VLDL/LDL Cholesterol System, Isolab LDL–Direct, Isolab LDL–Direct Plus, LipoPrint®, LFS Lipogel System® (Zaxis, Inc.), and Hydragel K20 System with HYDRASYS® (Sebia, Inc). These devices are also categorized under the device classification name of "electrophoretic separation, lipoproteins". The first four devices (by Helena Laboratories and Isolab) did not have any other associated documents posted on the CLIA database except for the standard report from the database search. There are summaries/statements of the 510(k) notification in concordance of the Safe Medical Devices Act (SMDA) posted for the latter three devices (LipoPrint®, LFS Lipogel System®, and Hydragel K20 System).

From evaluation of the summaries/statements of the 510(k) notification, we concluded that, all of these devices that have been used to measure LDL subfractions or sizes in the literature were cleared by FDA for the use of separating or measuring LDL fraction (ie, separating LDL cholesterol from other cholesterol-containing lipoprotein particles), not for the use of measuring LDL subfractions or sizes. According to the summary/statement of the 510(k) notification, Quantimetrix LipoPrint® System classifies LDL subfractions as Mid-C, Mid-B, Mid-A, and LDL-1 through 7. The sum of all subfractions constitutes total LDL cholesterol. The intended use of Quantimetrix LipoPrint® System declared in the summary/statement of the 510(k) notification of FDA was "to measure lipoprotein cholesterol (for lipoprotein fractions and subfractions from VLDL to HDL) in fasting serum or plasma with a total cholesterol concentration ≥ 100 mg/dL." The performance characteristics comparing Quantimetrix LipoPrint® System to direct HDL or LDL cholesterol methods were also provided. These data confirmed that the LipoPrint® LDL Test System performs comparably to the direct HDL or LDL cholesterol methods in a clinical setting. The device was therefore found to be substantially equivalent to legally marketed devices by FDA, and was permitted to proceed to the market.

The search of the FDA CLIA database for "nuclear magnetic resonance/NMR" test systems or LipoScience/LipoMed manufacturer resulted in no records found. According to the information provided by LipoScience, Inc., all tests are performed using FDA cleared reagents and methods. The current FDA cleared LDL cholesterol measurement system of NMR LipoProfile® is Beckman Synchron CX 4 system (reagents and methods) measuring LDL cholesterol in human serum or plasma. A search of the FDA CLIA database for Beckman Synchron CX 4 resulted in eight records with effective dates from 1995 to 2000.

We did not identify any federal documents by FDA or other government agencies that discuss possible reference standards for measuring LDL subfractions.

Narrative reviews

To provide greater insight into what methods may be used routinely to measure LDL subfractions and whether any method is considered a reference standard, we systematically searched for review articles and editorials that discussed potential routine use of any method or suggested a reference standard. An important caveat is that some of those reviews were written by authors who were actively involved in bench research of LDL subfractions and had either a professional or financial stake in the use of a given methodology.

Ultracentrifugation has been described as the "original gold standard" to which subsequent methods have been calibrated and validated.[11] James Otvos and Elias Jeyarajah, who with others developed NMR analysis of LDL subfractions, also described that the NMR measures were calibrated against ultracentrifugation-derived reference data on isolated lipid subfractions.[22] However, ultracentrifugation is time-consuming and available only at some research laboratories.[11]

As described by several review articles, and also as evidenced by the studies eligible for Question 4 below, GE is the most commonly used procedure in research laboratories.[23,24] Researchers use many different specific measurements or methods of analyzing the data from GE; however, among the more consistent measurements is the assignment of phenotypes into larger more buoyant LDL phenotype A and smaller dense LDL phenotype B (and A/B or intermediate phenotypes). However, as we also found in our review for Question 4, there is not complete consistency in the definition of the particle diameter threshold to distinguish phenotypes A and B, or how to analyze those with intermediate phenotypes. Quantimetrix has commercialized the LipoPrint® GE method for LDL subfractionation. However as we describe below (Questions 2 and 4) and as commentators have noted,[25] there does not appear to be harmonization by researchers of the measurements derived from the test or clear validation of the method against other methods.

NMR measurement of LDL subfractions has been commercialized and has been described as the most rapid and convenient method for determining LDL size and subfraction concentration, though questions remain about its calibration and validation.[11] Despite its commercial availability, it has been described as not being a popular measurement method due to the requirement for expensive specialized laboratory equipment which is "too difficult to use in daily clinical practice."[23] Nevertheless, an advantage ascribed to NMR (by Drs Jeyarajah and Otvos) is that it has the "unique ability to quantify lipoprotein particle numbers, even in the face of significant variation in the cholesterol composition of subfraction particles among individuals."[19]

Summary

There is currently no generally accepted reference standard for measuring LDL subfractions. The most common methods for measuring LDL subfractions involve either GE or ultracentrifugation methods. However the lengthy experimental procedures and heterogeneity in the algorithms to classify LDL patterns or sizes limit their application for routine clinical practice. Furthermore, all current gel electrophoresis devices in the FDA database have been used to measure LDL subfractions or sizes in the literature were based on substantial equivalence to legally marketed devices for measuring LDL cholesterol, not LDL subfractions. LipoPrint® is the only FDA-cleared devices that declared its intent to measure LDL subfractions as the primary use. NMR measurement of LDL subfractions has been commercialized and has been described as the most rapid and convenient method for determining LDL size and subfraction concentration, though questions remain about its calibration and validation. HPLC has rarely been reported in research studies over the past decade.

Question 2

How do different methods of measuring LDL subfractions compare in terms of test performance?

In this section, we review primary studies that compared different methods of measuring LDL subfractions. The methods examined include NMR, LipoPrint® GE, other GE methods (bench methods), ultracentrifugation, and others. Studies had to use adult serum samples from at least 10 individuals for each method. We excluded studies that evaluated only incremental or technical changes to the methods (eg, comparison of LDL particle size determination by GE using two different approaches; comparison of LDL particle size by HPLC with ultraviolet light detection to a modified method based on selective detection of lipoproteins by postcolumn labeling with a fluorescent lipid probe).

We allowed any method of comparing test performance, including sensitivity/specificity, Bland-Altman plot (or bias and limit of agreement), correlation (r), or measures of concordance or agreement between tests. We reviewed all statistical approaches acknowledging that different methods of comparing test performance make different statistical assumptions and have different interpretations of the results:

- Sensitivity and specificity measure the clinical diagnostic test performance. Their calculations require a "gold" or "reference standard" that is presumed to have no measurement errors. Sensitivity is the proportion of people with the "disease" (or a positive reference standard) who are identified by the test. Specificity is the proportion of people with a negative reference standard who also have a negative test result.

- Correlation coefficient (*r*) measures the correlation of one diagnostic test to another, but does not provide any information about the clinical utility of the test. Correlation coefficient is inadequate for comparing a new method of measuring LDL subfractions with an established one for several reasons: First, *r* measures the strength of a relation between two variables, not the agreement between them. Two variables are in perfect agreement not only if the points from the scatter plot lie along the line of equality (the diagonal line of a scatter plot), but also if the points lie along any straight line. Second, *r* depends on the range of values in the sample. If the range is wide, the correlation is likely to be greater than if it is narrow. Third, correlation ignores bias (or the systematic difference between methods) and it measures relative rather than absolute agreement.[26] Thus, interpretations of test accuracy using correlation coefficients may be misleading. A high correlation does not necessarily imply that there is good agreement between the two methods.

- Bland-Altman bias and limits of agreement measure the absolute agreement between two tests, assuming there are measurement errors in both tests (ie, neither test is a "gold standard").[26] Bland-Altman bias and limits of agreement do not provide any information about the clinical utility of the diagnostic test. A Bland-Altman plot plots the mean of the results from the compared tests (x-axis) against the difference between the two tests (y-axis). The accuracy is assessed by evaluating how close the data points are to zero on the y-axis (difference between tests; the limits of agreement) and whether there is a trend as the value on the x-axis (mean value) increases (or bias). Zero bias and narrow limits of agreement indicate a good agreement between the two methods. In addition, ideal tests would have consistent limits of agreement across wide range of testing populations.

- Kappa is a measure of agreement between two tests taking into account agreement that could occur by chance. Kappa does not provide any information about the clinical utility of the diagnostic test. A kappa value of one indicates the two tests have perfect agreement, and a kappa value of zero indicates the two tests have no agreement.

Nine articles provided data on the comparison of different methods.[15,17,20,27-31] Four articles reported five comparisons of NMR and GE, three articles compared LipoPrint® and other GE, four articles reported five comparisons of ultracentrifugation and GE, and one article compared HPLC and GE (Table 1). Only one study (Witte 2004) used a random sample of the study populations and blinding of the investigators for the alternate test results.[31] Therefore, this was the only good quality study. All other studies used convenience samples; half reported that the test results were assessed in blinded fashion in relation to alternate test results. Seven studies were of fair quality. The one poor quality study gave an inadequate description of the tests compared.[28]

Nuclear magnetic resonance (NMR) vs. gel electrophoresis (GE)

Four articles reported five comparisons of NMR and GE involving 436 subjects (Table 1, NMR vs. GE).[17,27,30,31] Witte 2004 randomly selected patients with type 1 diabetes and people without diabetes from the general population. Ensign 2006 and Blake 2002 enrolled a convenience sample of healthy people. Hoefner 2001 did not describe how the study population was selected. The lipid profiles of all 436 subjects across studies were heterogeneous.

Although there was a good correlation between NMR-assessed and GE-assessed LDL particle sizes in 21 apparently healthy men ($r=0.89$, $P<.001$),[27] Bland-Altman limits of agreement analyses in 324 men and women with and without type 1 diabetes showed that the mean difference between measured LDL size on NMR and peak LDL size on GE was 53.8 Å (with NMR being smaller).[31] The 95 percent limits of agreement were 39.7 and 67.9 Å, indicating the 95 percent of the differences between the two methods can be expected to fall within this range. The difference and the strength of the relation between LDL size according to NMR and GE were also different across different subgroups of the study population, suggesting inconsistent agreements between NMR and GE across populations. The mean difference was larger for patients with type 1 diabetes, women, and those with lower triglyceride concentrations.

Two studies involving a total of 90 subjects showed a fair to good concordance or agreement between NMR-assessed and LipoPrint® or nonstandardized GE-assessed LDL patterns (ranging from 51 to 94 percent).[17,30] The wide range of agreement may be partly explained by the heterogeneity in the classifications of LDL patterns between the different methods. Studies using NMR classified LDL patterns (ie, pattern A, intermediate, or pattern B) based on absolute size cutoffs. Studies that utilized GE to measure LDL subfractions used the same classification scheme but different cutoffs were chosen. Studies using LipoPrint® classified LDL patterns based on complicated LDL scores using area under the curve at various predefined electrophoretic mobility (R_f) values. The concordance rates between NMR-assessed and GE-assessed LDL patterns also varied according to the LDL phenotypes.

LipoPrint® GE vs. other GE methods

Three articles compared LipoPrint® and other methods of GE LDL subfraction separation involving a total of 188 subjects (Table 1, LipoPrint® vs. GE).[15,17,30] Ensign 2006 enrolled a convenience sample of healthy people. Hirany 2003 and Hoefner 2001 did not describe how the study populations were selected. The lipid profiles of all 188 subjects across studies were heterogeneous.

All three studies evaluated the concordance or agreement between LipoPrint®-assessed and GE-assessed LDL patterns.[15,17,30] However, the Lipoprint® kit was not used according to the manufacturer's instructions. Each of the three investigators created its own criteria to evaluate and classify the results of the Lipoprint® test. Hirany 2003 classified LDL subfractions into small, intermediate or large based on electrophoretic mobility (Rf) cutoffs (ie, small LDL: Rf>0.40, intermediate LDL: Rf=0.38-0.40, large LDL: Rf<0.38). Ensign 2006 classified LDL subfractions into pattern A, AB, and B based on the LDL subfraction score (LDLFS) that was developed and used in Hoefner 2001 paper (ie, normal or pattern A: LDLSF score <5.5, Intermediate or pattern AB: 5.5-8.5; atherogenic or pattern B: >8.5) The concordance rates between LipoPrint®-assessed and GE-assessed LDL patterns varied according to the LDL phenotypes.

Hirany 2003 reported a good agreement between LipoPrint® and an alternate GE method after evaluating the data using kappa statistics (weighted kappa = 0.78; 95% CI, 0.68-0.87). LipoPrint® had an agreement of 92 percent concordance for classification of the small LDL subfraction compared with GE. For large LDL subfraction, LipoPrint® had an agreement of 77 percent concordance compared with GE.

Hoefner 2001 reported 84, 64, and 24 percent agreement for classification of the small, intermediate, and large LDL subfraction, respectively, for LipoPrint® and GE. Ensign 2006 showed only 40 percent agreement in the classification of LDL patterns between LipoPrint® and GE.

Ultracentrifugation vs. GE

Four articles reported five comparisons of ultracentrifugation and GE methods involving a total of 152 subjects (Table 1, Ultracentrifugation vs. GE).[17,28,29,32] Dormans 2001, Ensign 2006, and Davies 2003 enrolled a convenience sample of healthy people. O'Neal 1998 enrolled a convenience sample of patients with type 2 diabetes (26 percent) or from the general population. The lipid profiles of these 152 subjects across studies varied greatly although the data were incompletely reported in most studies.

There was no uniform ultracentrifugation or GE methodology across studies. Therefore, the results from these five comparisons are evaluated individually.

Dormans 2001 showed that migration distance of the predominant LDL subfraction from GE correlated strongly with the density of the predominant LDL band from ultracentrifugation (r=0.85, P<.0001) in 41 healthy individuals.

Ensign 2006 reported 41 percent agreement for classification of LDL patterns between ultracentrifugation vertical auto profile and GE, and 11 percent agreement for classification of LDL patterns between ultracentrifugation vertical auto profile and LipoPrint®.

O'Neal 1998 showed a good correlation (r=0.78, P<.0001) when comparing vertical ultracentrifugation and light-scattering methodology with GE for determining LDL particle size. However, the mean LDL size obtained by vertical ultracentrifugation was smaller than those obtained by GE (231 vs. 261 Å, P<0.0001).

Davies 2003 examined the diagnostic test performance of an LDL peak density of >1.025 kg/L and area under the LDL profile (>1.028 kg/L) by iodixanol ultracentrifugation in predicting a predominance of small dense LDL III (pattern B) as determined by GE or salt ultracentrifugation. This study was graded poor due to inadequate description of the reference standard. An area under the LDL profile of over 51 percent (density>1.028 kg/L) was shown to give 100 percent specificity and sensitivity in differentiating a predominance of small dense LDL III (pattern B). This was reported to be "marginally better" as a predictor of small dense LDL III than the cutoff density of 1.028 kg/L alone (94 percent sensitivity; 92 percent specificity).

High performance gel filtration chromatography (HPLC) vs. GE

One article compared HPLC with GE involved 60 patients with type 2 diabetes (Table 1, HPLC vs. GE).[20] The total cholesterol and triglyceride concentrations ranged from 135 to 315 mg/dL and 45 to 509 mg/dL, respectively.

LDL size as measured by HPLC and GE was highly correlated (r=0.88, P<.0001). Bland-Altman limits of agreement analyses showed that the mean difference between LDL size on

HPLC and on GE was 2.5 Å (with HPLC being larger). The 95 percent limits of agreement were –6 and +10 Å, indicating that 95 percent of the differences between the two methods can be expected to fall within this range.

Summary

A wide range of agreement (described as fair to good agreement) was reported for the comparison of NMR-assessed with GE-assessed LDL patterns and for Lipoprint®-assessed versus other GE-assessed LDL patterns. The differences between the methods, though, varied across different prespecified populations. One study found that NMR measurements of LDL size are on average about 54 Å smaller than measurements based on GE, with wide limits of agreement, implying that size measurements made with the different methods are not interchangeable. The measured size difference was larger for patients with type 1 diabetes, women, and those with lower triglyceride concentrations, suggesting inconsistent limits of agreements between NMR and GE across testing populations. The studies comparing ultracentrifugation and GE methods used different techniques and measurements; therefore the agreements between ultracentrifugation and GE methods for assessing LDL patterns are each unique to the individual study. One study compared HPLC and GE; it found good agreement between HPLC-assessed and GE-assessed LDL sizes but, on average, HPLC measurement of LDL sizes are 2.5 Å larger than measurements based on GE, implying that size measurements made with the different methods are not interchangeable.

Question 3

Question 3.1
How much variability is there in measures of LDL subfractions from day to day?

To answer this question, studies must have drawn serum samples from the same volunteers on multiple days within a short period of time (we did not set a strict upper limit on the time frame). No study addressed this question.

Question 3.2
How much variability is there in measures of LDL subfractions within the same individual (measure to measure)?

For Question 3.2, studies must have measured the same serum samples using the same method at least twice. Five studies reported data on the intraassay variability (or the reproducibility) and/or the interassay variability (or the imprecision of test by analyzing stored samples on different days) using repeated measures by the same test (Table 2).[19-21,30,33] No study described how the subsample was selected from the study population and none was primarily designed to address this question.

Hoefner 2001 took two plasma samples from the study population and measured their LDL subfraction scores using LipoPrint® GE. The intraassay coefficients of variations for patient samples analyzed 10 times in duplicate were 4.6 and 4.3 percent at LDL subfraction scores of 3.4 and 13.3, respectively. Interassay precision was determined using plasma from 19 subjects with

LDL scores ranging from 2.9 to 16.5 assayed on 3 days over a 1 week period. The mean interassay coefficient of variation (CV) was 13 percent, although how the blood samples were stored during the 1 week period was not reported.

Scheffer 1997 took a subset of the study population and measured their LDL size using GE and HPLC methods. Between-run reproducibility for particle diameter was determined by repeatedly analyzing an isolated LDL sample stored in aliquots at –70° C. GE and HPLC reproducibility, expressed as coefficients of variation (CV) determined over an 8 week period, were 0.6 percent (n=14) and 0.2 percent (n=12), respectively. Within-run reproducibility for LDL size measurements was assessed only for the HPLC method. For the sample of 10 patients with type 2 diabetes, the CV for two different LDL samples was less than 0.1 percent. In a subsequent study, Scheffer et al. modified the HPLC method and compared the test performance of the modified method to the original HPLC method for measuring LDL sizes. Using isolated LDL and whole plasma samples from 10 subjects, Scheffer 1998 reported that the within-run CV of the modified HPLC method were 0.14 and 0.22 percent, respectively. Using isolated LDL samples stored in aliquots at –86° C they reported that between-run CVs calculated from measurements performed on different days was 0.21 percent.

Adler 2000 compared LDL particle size determination by GE with two additional methods for LDL fractionation: ultracentrifugation using a density range of 1.019 and 1.063 g/mL, and precipitation of apo B-containing lipoproteins from plasma. This study was graded poor quality due to inadequate reporting of the study population and statistical analyses for the test variability. Peak particle diameter was reproducible with a CV of 1.2 percent for LDL samples separated by ultracentrifugation and 1.4 percent for LDL samples separated by apo B precipitation in six separate gels. It was also reported that "the intraassay variation (within a single gel) was 0.2 percent", although it was unclear how many samples and which separation method were used for this calculation.

In a review article by Jeyarajah 2006,[19] data on the intraassay and interassay precision of NMR lipoprotein measurements were reported. This study was graded poor quality due to inadequate reporting of the study population and methods for sample handling (although the authors stated that all procedures were following "standard protocol"). Two plasma pools were used, one with nominally "high triglycerides and low HDL" and the other with "low triglycerides and high HDL." For the plasma pool with "high triglycerides and low HDL", the intraassay and the interassay precision for total LDL particle concentration were 2.4 percent CV and 2.1 percent CV, respectively. For the same plasma pool, the intraassay and the interassay precision for LDL size were 0.4 percent CV and 0.5 percent CV, respectively. For the other plasma pool with "low triglycerides and high HDL", the intraassay and the interassay precision for total LDL particle concentration were 4.0 percent CV and 4.3 percent CV, respectively. For the same plasma pool, the intraassay and the interassay precision for LDL size were 0.5 percent CV and 0.6 percent CV, respectively.

Summary

The test variability is substantially greater when analyzing LDL patterns (ie, pattern A, intermediate, or pattern B) than when analyzing LDL sizes. The intraassay variability was relatively small (ranging from <0.1 to 0.22 percent) compared to the interassay variability (ranging from 0.2 to 1.4 percent in four studies, with a fifth study having 13 percent variability) within the same method for measuring LDL sizes. In one study, it was shown that the intraassay variability was greater, as assessed by HPLC, when whole plasma was used compared to isolated LDL (0.22 vs. 0.14 percent).

Table 1. Comparison of different methods for measuring LDL subfractions

Author, Year Country UI	N	Mean (range), mg/dL			Population	Tests		Concordance or Agreement		Quality
		LDL-c	TC	Tg		Test 1 (Metric)	Test 2 or "Ref Std" (Metric)	r (P Value)	LOA (95%CI) or Other Results	
NMR vs. GE										
Witte, 2004[31] Netherlands 14993238	324	nd	nd	≤545	Case: diabetes (type 1) Control: general	NMR (size, nm)	GE (size, nm)	nd	All (n=324): -5.38 (-6.79, -3.97) Type 1 DM (n=152): -5.49 (-7.31, -3.68) No DM (n=172): -5.27 (-6.96, -3.60) Men (n=156): -5.20 (-6.86, -3.53) Women (n=168): -5.55 (-7.41, -3.68) Tg<79 mg/dL (n=108): -5.73 (-7.54, -3.92) Tg 79-118 (n=109): -5.41 (-6.94, -3.89) Tg>118 (n=107): -4.99 (-6.61, -3.37)	A
Hoefner, 2001[30] US 11159775	51	120	213	217	nd	NMR (pattern A, intermediate, pattern B based on absolute size cutoffs)[A]	LipoPrint™ (pattern A, intermediate, pattern B based on LDLSF score)[B]	-0.67 (<.001)	Concordance: Pattern A=94% Intermediate=7% Pattern B=67%	B
Ensign, 2006[17] US 16740651	37-40	(58-820)	nd	(37-479)	General	NMR (pattern A or B)	GE (pattern A, AB, or B)[C]	Agreement = 70% (28/40) Agreement when GE pattern AB combined with pattern A =80% (32/40)		B
							LipoPrint™ (pattern A, AB, or B)[D]	Agreement = 51% (19/37) Agreement when GE pattern AB combined with pattern A =54% (20/37)		
Blake, 2002[27] US 12370215	21	111	117	164	General	NMR (size, nm)	GE (size, nm)	0.89 (<.001)	nd	C

continued

Table 1. Continued

Author, Year Country UI	N	Mean (range), mg/dL LDL-c	TC	Tg	Population	Tests: Test 1 (Metric)	Test 2 or "Ref Std" (Metric)	Concordance or Agreement: r (P Value)	LOA (95%CI) or Other Results	Quality
LipoPrint® vs. other GE										
Hirany, 2003[15] US 12669713	102	125 (42-452)	219 (113-563)	270 (61-617)	nd	LipoPrint® GE (small, intermediate; large based on Rf cutoff values) [E]	GE (small, intermediate; large based on absolute size cutoffs) [F]	Weighted kappa= 0.78 (0.68-0.87) Concordance: Small=92% Intermediate=33% Large=77%		B
Hoefner, 2001[30] US 11159775	51	120	213	217	nd	LipoPrint® GE (pattern A, intermediate, pattern B based on LDLSF score) [B]	GE-Zaxis (pattern A & B per Berkley HeartLab cutpoints)	Concordance: Pattern A=88% Intermediate=64% Pattern B=24%		B
Ensign, 2006[17] US 16740651	35	(58-820)	nd	(37-479)	General	LipoPrint® GE (pattern A, AB, or B) [D]	GE (pattern A, AB, or B) [C]	Agreement = 40% (14/35)		B
Ultracentrifugation vs. GE										
Dormans, 2001[29] Netherlands 2049850	41	nd	213	143	General	DGUC (LDL-1, LDL-2 or LDL-3, g/mL)	GE (migration distance, mm)	0.85 (<.001)	nd	B
Ensign, 2006[17] US 16740651	37	(58-820)	nd	(37-479)	General	UC-VAP-II (pattern A, AB, or B) [G]	GE (pattern A, AB, or B) [C]	Agreement = 41% (15/37)		B
							LipoPrint™ (pattern A, AB, or B) [D]	Agreement = 11% (4/37)		
O'Neal, 1998[32] Australia 9788255	27	nd	nd	(61-213)	Both general & diabetes (type 2)	Vertical DGUC with light-scattering methodology (size, nm)	GE (size, nm)	0.78 (<.0001)	Mean size obtained by vertical DGUC were smaller (23.1 vs. 26.1 nm, p<0.0001) than those obtained by GE	B
Davies, 2003[28] UK 14578318	47	nd	nd	nd	General	Iodixanol DGUC (>51% AUC LDL density >1.028 kg/L)	GE or salt DGUC [H] (sd LDL-III, or LDL subfraction pattern B)	Sensitivity = 100% Specificity = 100%		C
						Iodixanol DGUC (peak density >1.028 kg/L)		Sensitivity = 94% Specificity = 92%		

continued

Table 1. Continued

Author, Year Country UI	N	Mean (range), mg/dL			Population	Tests		Concordance or Agreement		Quality
		LDL-c	TC	Tg		Test 1 (Metric)	Test 2 or "Ref Std" (Metric)	r (P Value)	LOA (95%CI) or Other Results	
HPLC vs. GE										
Scheffer, 1997[20] Netherlands 9342011	60	nd	231 (135-315)	209 (45-509)	Diabetes (type 2)	HPLC (size, nm)	GE (size, nm)	0.88 (<.001)	0.25 (-0.6 to 1.0)	B

[A] Pattern A: 20.6-22.0 nm; Intermediate: 20.4-20.5; Pattern B: 19.0-20.3 nm
[B] Pattern A: LDLSF score <5.5; Intermediate: 5.5-8.5; Pattern B: >8.5
[C] Large LDL (pattern A): 26.35-28.5 nm; Intermediate LDL (pattern AB): 25.75-26.34 nm; Small LDL (pattern B): 22.0-25.74 nm
[D] Normal (pattern A): LDLSF score <5.5, Intermediate (pattern AB): 5.5-8.5, Atherogenic (pattern B): >8.5
[E] Small LDL: Rf>0.40, Intermediate LDL: Rf=0.38-0.40, Large LDL: Rf<0.38
[F] Small LDL: <25.8 nm, Intermediate LDL: 25.8-26.3 nm, Large LDL: >26.3 nm
[G] LDL1 (most buoyant) through LDL 6 (most dense): LDL1 and LDL2 comprise pattern A; LDL3 and LDL4 comprise pattern B
[H] The authors used both salt DGUC and GE as the reference standard in the calculation of the test (iodixanol DGUC) performance

Table 2. Test Variability (or Imprecision)

Author, Year Country UI	N	Population	Tests	N repeated measurements per patient (N selected samples)	Test Variability		Quality
					How much variability is there in measures of LDL subfractions from day to day?	How much variability is there in measures of LDL subfractions within the same individual (measure to measure)?	
Hoefner, 2001[30] US 11159775	51	nd	LipoPrint™ (pattern A, intermediate, pattern B based on LDLSF score)[B]	nd (19)[A]		Mean CV = 13%	B
				10[C] (2)		Intraassay CVs of 4.6% and 4.3% at LDLSF scores of 3.4 and 13.3, respectively	
Scheffer, 1997[20] Netherlands 9342011	60	Diabetes (type 2)	GE	n/a (14)[D]	nd	Between-run CV (or reproducibility), over an 8-week period = 0.6%	B
			HPLC (size, nm)	n/a (12)[D]	nd	Between-run CV (or reproducibility), over an 8-week period = 0.2%	
				2 (10)		Within-run CV <0.1%	
Scheffer, 1998[21] Netherlands 9761248[G]	56	Both general & diabetes (type 2)	HPLC-isolated LDL samples (size, nm)	nd (10)	nd	Between-run CV (or reproducibility) within 4 days[E] = 0.21%	B
						Within-run CV = 0.14%	
			HPLC-whole plasma samples (size, nm)	nd (10)	nd	Within-run CV = 0.22%	
Adler, 2000[33] Canada 10913516[G]	41	nd	GE-samples separated by UC (peak particle diameter, nm)	n/a (6)	nd	Between-run CV (or reproducibility) = 1.2%	C
						"The intra-assay variation (within a single gel) was 0.2%"[F]	
			GE-samples separated by Apo B precipitation (peak particle diameter, nm)	n/a (6)	nd	Between-run CV (or reproducibility) = 1.4%	
						"The intra-assay variation (within a single gel) was 0.2%"[F]	

continued

Table 2. Continued

Author, Year Country UI	N	Population	Tests	N repeated measurements per patient (N selected samples)	Test Variability: How much variability is there in measures of LDL subfractions from day to day?	Test Variability: How much variability is there in measures of LDL subfractions within the same individual (measure to measure)?	Quality
Jeyarajah, 2006[19] US 17110242	nd	Plasma pool A: "high Tg and low HDL" Plasma pool B: "low Tg and high HDL" (pool B)	NMR- aliquoted and frozen samples (LDL concentrations, nmol/L)	Inter-assay precision: 20 consecutive days across 6 different NMR analyzers Intra-assay precision: thawing and analyzing 20 replicates on 1 NMR analyzer	nd	Plasma pool A - total LDL particles (nmol/L): Intra-assay CV = 2.4% Inter-assay CV = 2.1%	C
						Plasma pool B - total LDL particles (nmol/L): Intra-assay CV = 4.0% Inter-assay CV = 4.3%	
			NMR- aliquoted and frozen samples (LDL sizes, nm)		nd	Plasma pool A - LDL size (nm): Intra-assay CV = 0.5% Inter-assay CV = 0.4%	
						Plasma pool B - LDL size (nm): Intra-assay CV = 0.5% Inter-assay CV = 0.6%	

A 19 subjects with LDL scores ranged from 2.9 to 16.5, assayed on 3 days over a 1-week period

B Pattern A: LDLSF score <5.5; Intermediate: 5.5-8.5; Pattern B: >8.5

C Samples were analyzed in duplication of 10

D Between-run reproducibility for particle diameter was determined by repeatedly analyzing an isolated LDL sample stored in aliquots at –70° C over an 8-week period

E Between-run CVs calculated from measurements performed on different days (not defined but all samples were analyzed within 4 days), using isolated LDL sample stored in aliquots at –86° C

F Unclear how many samples and which separation method were used for the intra-assay variation

G The studies only included for the questions on test variability, not for the comparison of different methods for measuring LDL subfractions

Question 4

Question 4.1
What is the relationship between LDL subfractions and outcome measures related to CVD?

We evaluated all studies that analyzed the association between LDL subfractions and cardiovascular outcomes. We performed detailed analysis of the studies that used the methods available for clinical use for measuring LDL subfractions. For this section, we searched for and, where available, included eligible studies that used NMR; a specific kit for GE that is available for clinical use (LipoPrint®); a specific gradient GE method used at the Berkeley HeartLab®; the current method used at the Northwest Lipid Research Lab; and the Vertical Auto Profile® method used by Atherotech.

Ten studies examined the relationships between NMR measured LDL subfractions and cardiovascular outcomes.[27,34-42] All NMR studies had their samples run by a single set of researchers at LipoScience® or its precursors. (We do not repeatedly name this company, as is necessary to distinguish the proprietary GE tests, since "NMR" is sufficiently descriptive.)

Eight studies examined the relationships between LipoPrint® GE measured LDL subfractions and cardiovascular outcomes.[43-50]

One study had their samples analyzed by the Berkeley HeartLab® using what we concluded were the same methods that are available clinically.[51]

No study that met eligibility criteria used the Vertical Auto Profile®. We concluded that none of the studies had their samples performed at the University of Washington's Northwest Lipid Research Laboratory using the currently clinically available methods.

NMR measured LDL subfractions

Five nested case-control studies,[27,35,37,40,42] four cross-sectional studies,[34,36,38,39] and one prospective longitudinal study[41] reported on the association between NMR measured LDL subfractions and cardiovascular outcomes. Four studies were of good methodological quality and six were of fair methodological quality. The number of subjects in these studies ranged from 118 to 5538. Many of the studies have slightly different definitions of the LDL size subfractions (eg, one study defined small LDL as 18.3 to 19.7 nm,[27] while another study defined small LDL as 18.0 to 21.2 nm[40]). Some studies enrolled only women (eg, Women's Health Study[27]) and some studies enrolled only men (eg, VA HDL Intervention Tria l[40]). Some studies enrolled healthy subjects at baseline and some studies enrolled only patients with diabetes or low HDL cholesterol concentrations. Half of the studies enrolled 40 percent or more patients older than 65 years.

Incidence or progression of CVD

Five studies evaluated the association between NMR-measured LDL subfractions and incident CVD or progression of CVD (Tables 3a & 3b).[27,35,37,40,42]

Fatal or nonfatal CVD events

Both good quality nested case-control studies found that LDL particle number was associated with the risk of incident fatal or nonfatal coronary artery disease, or stroke (Blake

2002: adjusted OR 4^{th} quartile compared to 1^{st} quartile = 2.90 (1.16-7.30), P=0.03; El Harchaoui 2007: adjusted OR 4^{th} quartile compared to 1^{st} quartile = 1.37 (1.04-1.83), P=0.02). While the LDL particle size showed unadjusted significant differences between cases and control in these studies, the relative risk comparing different quartiles of particle size failed to demonstrate statistical significance after adjustment for baseline lipid variables. One fair quality study found statistically significant differences between cases (incident myocardial infarction or angina) and control in LDL particle concentration and size in women, but not in men.[37] After a bivariate analysis including LDL-cholesterol in the calculation, LDL particle concentration (OR 1.11 per 100 nmol/L, 1.03-1.09) remained significantly different between case and control. The other fair quality study found similar relationships between LDL particle number and the risk of incident myocardial infarction or deaths from coronary artery disease (OR 1.20 (95% CI 1.05-1.37) per 1-SD increment of LDL particle number) in men. The authors reported that adjustment for baseline lipid variables did not "appreciably change these relations" but the actual data were not shown.[40]

Diagnosis of CVD

One fair quality study reported unadjusted significant differences between cases (incident coronary artery disease) and controls in LDL particle size, medium and small LDL. Small and medium size LDL failed to predict incident coronary artery disease in multivariate analysis.[42]

Change in minimum lumen diameter

One fair quality prospective study reported an association between LDL particle size and small LDL with worsening in minimum lumen diameter (Table 4).[41] The study reported adjusted ORs of 0.2 (95% CI 0.1- 0.9) for particle size (above vs. below median size) and 9.1 (95% CI 2.1-39) for small LDL (above vs. below median concentration).[41]

Prevalent CVD

Four studies evaluated the association between NMR-measured LDL subfractions and prevalence of CVD (Tables 5a & 5b).[34,36,38,39]

Diagnosis of CVD

One poor quality study found a statistically significant difference between healthy subjects and subjects with CVD in the proportion of large LDL particle (66.5% vs. 43.3%, P=0.001) and particle size (21.4 nm vs. 20.8 nm, P=0.001).[34] This study did not report adjustment for differences in baseline lipid measurements.

Intermediate markers of CVD

Three fair quality cross-sectional studies analyzed the relationships between LDL subfractions and intermediate markers of prevalent CVD. The first study found that large and small LDL particles were associated with carotid IMT (Change in IMT in microns per one SD = 30.3 for large, and 34.8 for small, both P = 0.001).[39] The second study found that there was no association between small LDL with reduction in lumen diameter.[36] The third study found that LDL particle number, size, and small LDL were associated with coronary calcification (adjusted OR 1.44 (95% CI 1.04-1.99); 0.55 (95% CI 0.31- 0.99); 1.36 (95% CI 1.04-1.77); respectively, per 1-SD increase in lipoprotein subclass).[38]

Summary

Results from the good and fair quality case-control studies suggest that LDL particle concentration and particle number (as measured by NMR spectroscopy) are associated with incident cardiovascular outcomes. But the association between LDL particle size and incident cardiovascular outcomes is inconsistent; two good and one fair quality case-control studies did not find associations while one fair quality study reported an association in women, but not men.

Two fair quality cross-sectional studies with a total of 5696 patients suggest that small LDL particles are associated with intermediate markers of prevalent CVD while one fair quality study that analyzed 158 patients did not find this association. One fair quality longitudinal study did find an association between small LDL and changes in minimum lumen diameter.

LipoPrint® GE-measured LDL subfractions

It is important to note that the intended use for the Lipoprint® test as stated in the manufacturer's product insert is to measure the amount of cholesterol in each of the large buoyant and small dense LDL subfractions. Use of the Lipoprint® kit to determine particle sizes or LDL scores or any other form of classification is not recommended by the manufacturer of the kit. Despite this disclaimer from the manufacturer, the studies cited in the report used the Lipoprint® test to determine CVA risk by measuring lipoprotein subfraction by particle size or complicated LDL scores.

Incidence or progression of CVD

No studies evaluated the association between LipoPrint® GE-measured LDL subfractions and incident CVD or progression of CVD.

Prevalent CVD

Two case-control studies[43,50] (Tables 6a & 6b) and six cross-sectional studies[44-49] (Tables 7a & 7b) reported on the association between LipoPrint® GE-measured LDL subfractions and prevalent CVD. Six studies were of fair methodological quality and two were of poor methodological quality. The number of subjects in these studies ranged from 27 to 792. Many of the studies have different definitions of small LDL subfractions (eg, one study defined pattern B as LDL <255 Å,[50] while another study defined pattern B as LDL <265 Å[45]). One study enrolled only men.[49] Some studies enrolled subjects without clinical CVD at baseline and some studies enrolled patients with established CVD. Half of the studies enrolled 40 percent or more patients older than 65 years.

Diagnosis of CVD

One fair quality case-control study found that LDL pattern B (<255 Å), compared to pattern A or I (intermediate), was associated with prevalent coronary artery disease (recent myocardial infarction or angina) after adjusting for other lipid variables (adjusted OR 4.4 (1.2-16.1), P=0.03).[50] A poor quality study on patients with type 2 diabetes found that small LDL was an independent factor in determining the average carotid IMT in a multivariate analysis that included other lipid variables. This multivariate analysis that included a total of 17 variables had only 27 patients.[43]

Three fair and one poor quality cross-sectional studies analyzed the relationships between LDL subfractions and prevalent CVD. The first study found that there was a statistically significant variation in LDL score ("the relative percentage of the area under the curve of each LDL band was multiplied by its band number, then the sum of all LDL bands present was calculated to produce a final LDL score") between the subjects who had prevalent coronary artery disease (>50% stenosis in ≥1 major epicardial arteries) and the subjects who did not (P<0.001), after adjusting for triglyceride.[49] The second study found that small dense LDL was an independent risk factor for prevalent coronary artery disease (>50% stenosis in ≥1 coronary artery branches) in a multiple logistic regression that included low and high HDL-cholesterol.[45] The third study found that LDL score was significantly different between patients who had prevalent carotid atherosclerosis and those who did not in an unadjusted analysis (1.56 vs. 1.26, P=0.04), but a stepwise logistic regression that included other lipid variables rendered the association non-significant (adjusted OR 2.20 (95% CI 0.91-5.29); the odds of higher LDL score in patients with carotid atherosclerosis; the exact units of the OR were not reported).[46] The last study found that in patients with type 2 diabetes, those with a history of coronary artery disease (myocardial infarction and/or nitrates, revascularization, or EKG changes) had statistically significantly different small LDL profile (LDL 3 and above) than the patients who did not (overall sum of LDL3 to 5: 16.7 vs. 11.1, P<0.05) in an unadjusted anal ysis.[47]

Intermediate markers of CVD

Two fair quality cross-sectional studies analyzed the relationships between LDL subfractions and intermediate markers of prevalent CVD. The first study did not find an association between LDL particle size and the measurement of coronary artery calcium (CAC) after adjustment for other lipid variables in a multiple regression.[44] The second study did not find an association between LDL particle size and carotid IMT (Pearson correlation r = -0.172, P=0.075).[48]

Summary

Three fair quality studies found an association between small LDL (as measured by LipoPrint® GE) and prevalent CVD or intermediate markers in adjusted analyses. Three fair quality studies did not find such an association. The study populations, definitions of small LDL subfraction, and outcomes evaluated were heterogeneous.

Berkeley HeartLab® gradient GE measured LDL subfractions

One fair quality study evaluated the association between gradient GE performed at the Berkeley HeartLab® and cardiovascular outcomes.[51] The investigators evaluated the "usual care" arm of a randomized trial of men under age 75 with known coronary artery disease (Table 8). The only outcome evaluated was annual progression, over 4 years, of coronary artery stenosis. They found that the percentage of LDL in the IVb category (220-233 Å) was the strongest predictor of progression among subfractions (including HDL and IDL), also adjusting for lipoprotein concentrations. When LDL IVb was above 5.2 percent, the rate of progression was about six times faster than when LDL IVb was below 2.5 percent. Notably, this association was stronger for patients with baseline stenosis below 30 percent, and the association did not hold for patients with baseline stenosis at or above 30 percent. However, a major caveat to this study is that the LDL subfraction estimates used in the regressions are an average of the baseline and the fourth year data. Thus, the study does not evaluate whether LDL subfractions are a predictor of future coronary artery disease progression, but instead evaluate a difficult to interpret association between LDL subfractions over time and coronary artery stenosis over the same period of time.

Summary

One study of men with coronary artery disease found that the average LDL IVb percentage over a 4 year period was associated with an increased rate of coronary artery stenosis over that same period, particularly in artery segments with less than 30 percent stenosis at baseline.

Table 3a. Characteristics of the nested case-control studies of incident CVD and NMR-measured LDL subfractions

Author, Year Country UI	Population	Mean Age, [A] years	>65, [A,B] %	Male, [A] %	DM, [A] %	Smoke, [A] %	Mean LDL-c, [A] mg/dL
Blake, 2002[27] US 12370215	Women's Health Study: RCT of aspirin vs. vitamin E vs. placebo. Subjects had baseline blood sample with subsequent cardiovascular event	60	~30	0	11	59	129
El Harchaoui, 2007[35] UK 17276177	European Prospective Investigation into Cancer and Nutrition (EPIC), age between 45 and 79 years	65	~50	64	6	16	164
Kuller, 2002[37] US 12117734	Cardiovascular Health Study, Age ≥65 years, noninstitutionalized, 95% White	73	100	56	nd	nd	129
Otvos, 2006[40] US 16534013	Veterans Affairs HDL Intervention Trial (VA-HIT) (gemfibrozil vs. placebo), age <74 years, established CHD, HDL-c≤40 mg/dL, LDL-c≤140 mg/dL, Tg≤300 mg/dL	64	~45	100	37	22	113
Soedamah-Muthu, 2003[42] US 12743701	Pittsburgh Epidemiology of Diabetes Complications (EDC) study, type 1 DM diagnosed before age 17 years	35	0	28	100	31	126

[A] Of cases in case-control or cross-sectional studies (when reported separately); Data at baseline
[B] ~ = estimated from mean and standard deviation, otherwise reported value (rounded to nearest 5%)

Table 3b. Nested case-control studies of incident CVD and NMR-measured LDL subfractions

Author Year Country UI	Population	Definitions	Cases	Control	P	Other results	Quality
Blake 2002[27] US 12370215	Women's Health Study	Case=death due to CAD, nonfatal MI, or stroke	n=130	n=130		Risks of event (adjusted for Tg and TC/HDL-c) 4th quartile compared to 1st quartile:	A
		Large: 213-227 Å	886 [A] (nmol/L)	1001	0.50		
		Medium: 198-212 Å	201	126	.008		
		Small: 183-197 Å	0	0	.80		
		LDL particle concentration	1597	1404	<.001	RR=2.90 (1.16, 7.30) P=.03	
		LDL particle size, Å	215	218	.046	RR=1.20 (0.51, 2.82) P=.70	
El Harchaoui, 2007[35] UK 17276177	EPIC (European prospective cancer and nutrition study)	Case=fatal or nonfatal CAD	n=1003	n=1885		Adjusted risks of event, per quartile of risk factor	A
		Large: 212-230 Å	43 (nmol/L)	36	.003		
		Medium: nd	568	572	0.60		
		Small: 180-212 Å	999	885	<.0001		
		LDL particle concentration	1640	1525	<.0001	OR=1.37 (1.04, 1.83) P=.02 adjusted for HDL-c, Tg	
		LDL particle size, Å	210	211	.002	OR= 0.86 (0.65, 1.15) P=.50 adjusted for LDL particle concentration	
Kuller, 2002[37] US 12117734	CV Health Study	Case=incident MI & no stroke before MI; incident angina & no stroke or MI	Women n=191	Women n=182		Risks of event, adjusted for age, race, LDL-c, per quartile of risk factor	B
		Large: 213-230 Å	96 (mg/dL cholesterol)	104	NS		
		Medium: 198-212 Å	8.2	6.8	NS		
		Small: 183-197 Å	7.1	0	<.05		
		LDL particle concentration	1680	1501	<.05	OR=1.11 (1.03, 1.09) per 100 nmol/L P<.05	
		LDL particle size, Å	213	216	<.05	nd	
			Men n=243	Men n=67			
		Large: 213-230 Å	57.3	58	NS		
		Medium: 198-212 Å	36	34.5	NS		
		Small: 183-197 Å	25.7	22.7	NS		
		LDL particle concentration	1676	1597	NS	nd	
		LDL particle size, Å	209	210	NS	nd	

Continued

Table 3b. Continued

Author Year Country UI	Population	Definitions	Cases	Control	P	Other results	Quality
Otvos, 2006[40] US 16534013	VA patient with CHD; LDL-c≤140 mg/dL; HDL-c≤40 mg/dL; Tg≤300 mg/dL	Case=New nonfatal MI or CHD death	n=364	n=697		Risks of event, adjusted for treatment group, age, HTN, smoking, BMI and DM; Adjustment for LDL-c, HDL-c, and Tg did not "appreciably change these relations [nd])." per 1 SD increase in parameter	B
		Large: 212-230 Å			nd	OR=1.08 (0.95-1.23) NS	
		Small: 180-212 Å			nd	OR=1.11 (0.98-1.27) NS	
		LDL particle concentration			nd	OR=1.20 (1.05-1.37) P<.05	
		LDL particle size, Å			nd	OR=0.97 (0.85-1.10) NS	
Soedamah-Muthu 2003[42] US 12743701	Prospective study of type 1 DM	Case=CAD	n=59 (nmol/L cholesterol)	n=59		Small, medium and total LDL particle concentration failed to predict CAD independently in multivariate analysis (included Tg, HDL particle number in analysis).	B
		Large: 213-230 Å	603	688	NS		
		Medium: 198-212 Å	120	111	≤.01		
		Small: 183-197 Å	800	526	≤.001		
		LDL particle size, Å	206	210	≤.01		

[A] median

Table 4. Longitudinal study of NMR-measured LDL subfractions and progression of CVD

Author Year Country UI	Population	Mean Age, [A] years	>65, [A,B] %	Male, [A] %	DM, [A] %	Smoke, [A] %	Mean LDL-c, [A] mg/dL
Rosenson 2002[41] US 12106834	Pravastatin Limitation of Atherosclerosis in the Coronary Arteries (PLAC-I) trial, completed 3 years in the RCT, frozen plasma and coronary angiogram at baseline Patients with CAD in RCT of pravastatin (n=130) vs. placebo (n=111)	58	~20	76	nd	nd	163
		Outcome	**Definitions**	**Results**			**Quality**
		Change in minimum lumen diameter (MLD) over 3 years n=241		Spearman correlations, adjusted for LDL-c, HDL-c, Tg and other factors			B
			Large: 213-230 Å	0.03 (NS)			
			Medium: 198-212 Å	nd			
			Small: 183-197 Å	-0.17 (P<0.01)			
		Progression of MLD: ↓MLD>0.07 mm/y, over 3 years n=111 (placebo arm only)		Risk of progression, adjusted for LDL-c, HDL-c, Tg and other factors			
			Large: 213-230 Å	≥84 mg/dL vs.<84 mg/dL OR=0.4 (0.1-1.4) NS			
			Small: 183-197 Å	≥30 mg/dL vs.<30 mg/dL OR=9.1 (2.1-39) P<.05			
			LDL particle concentration	≥1825 vs.<1825 OR= 1.4 (0.3-6.7) NS			
			LDL particle size	≥200 vs <200 Å OR=0.2 (0.1-0.9) P<.05			

[A] Of cases in case-control or cross-sectional studies (when reported separately); Data at baseline
[B] ~ = estimated from mean and standard deviation, otherwise reported value (rounded to nearest 5%)

Table 5a. Characteristics of patients in the cross-sectional studies of prevalent CVD and NMR-measured LDL subfractions

Author Year Country UI	Population	Mean Age, [A] years	>65, [A,B] %	Male, [A] %	DM, [A] %	Smoke, [A] %	Mean LDL-c, [A] mg/dL
Mora 2007[39] US 16765964	Multi-Ethnic Study of Atherosclerosis (MESA), age 45-84 years, no self-reported CHD, from 6 centers	61	~40	47	12	14	120
Freedman 1998[36] US 9672064	Men admitted for coronary angiogram (severe or unstable angina, myocardial ischemia after MI, recurrent chest pain of unknown origin), did not use cholesterol-lowering medications, Tg<400	63	~40	100	20	nd	129
Mackey 2002[38] US 12419483	Women 8 years postmenopause; were premenopausal when enrolled into the Healthy Women Study (HWS)	62	~5	0	nd	13	128
Barzilai 2003[34] US 14559957	Offspring and spouses of offspring of Ashkenazi Jews with exceptional longevity (mean age 98 years)	69	nd	44	nd	nd	nd

[A] Of cases in case-control or cross-sectional studies (when reported separately); Data at baseline
[B] ~ = estimated from mean and standard deviation, otherwise reported value (rounded to nearest 5%)

Table 5b. Cross-sectional studies of NMR-measured LDL subfractions and prevalent CVD outcomes

Author Year Country UI	Population	Outcome	Definitions	Results	Quality
Mora 2007[39] US 16765964	No self-reported CVD n=5538	Carotid IMT		Association, adjusted for LDL subfractions, age, sex, race, HTN, smoking, LDL-c, HDL-c, and Tg ΔIMT per one SD of parameter	B
			Large: 212-230 Å	+30.3 (11.9, 48.7) P<.05	
			Small: 180-212 Å	+34.8 (15.0, 54.6) P<.05	
Freedman 1998[36] US 9672064	Admitted for angiography n=158	Coronary lumen diameter (occlusion score)		Association, adjusted for age, LDL-c, HDL-c, Tg	B
			Large: 230-300 Å (including IDL)	Correlation with occlusion score: -0.12 (NS) (also adjusted for age in addition to lipid variables)	
			Small: 180-205 Å	<20 vs. ≥20 mg/dL OR=1.8 NS	
Mackey 2002[38] US 12419483	Postmenopausal n=286	Coronary calcification (CAC category)		Risk of a higher CAC category, adjusted for LDL-c and Tg, per 1 SD increase in parameter	B
			Large: 213-230 Å	OR=1.03 (0.77-1.39) NS	
			Medium: 198-212 Å	OR=0.78 (0.60-1.02) NS	
			Small: 183-197 Å	OR=1.36 (1.04-1.77) P<.05	
			LDL particles	OR=1.44 (1.04-1.99) P<.05	
			LDL size	OR=0.55 (0.31-0.99) P<.05	
Barzilai 2003[34] US 14559957	Offspring of Ashkenazi Jews with longevity n=229	Prevalent CVD		Unadjusted associations (Cases, n=20 vs healthy, n=209)	C
			Large: 213-230 Å	66.5% vs 43.3% P=.001	
			Medium: 198-212 Å	nd	
			Small: 183-197 Å	nd	
			Particle size, Å	214 vs 208 P=.001	

Table 6a. Characteristics of case-control studies of prevalent CVD and LipoPrint GE-measured LDL subfractions

Author Year Country UI	Population	Mean Age [A], years	>65, [A,B] %	Male, [A] %	DM, [A] %	Smoke, [A] %	Mean LDL-c [A] (mg/dL)
Yoon 2005[50] S. Korea 15899660	Consecutive patients who underwent coronary angiogram, age <80 years, not on lipid-lowering drugs, recent MI or angina, with or without type 2 DM, blood sample obtained 2 months after MI	59	~20	72	26	29	122
Inukai 2005[43] Japan 16112502	Type 2 DM, 26% on statin	63	~40	52	100	nd	118

[A] Of cases in case-control or cross-sectional studies (when reported separately); Data at baseline
[B] ~ = estimated from mean and standard deviation, otherwise reported value (rounded to nearest 5%)

Table 6b. Case-control studies of LipoPrint GE-measured LDL subfractions and prevalent CVD

Author Year Country UI	Population	Definitions	Cases		Controls	Unadjusted P Value	Other Results (Including Multivariable Analyses)	Quality
Yoon 2005[50] S. Korea 15899660	CHD ± type 2 DM		CHD only (n=100)	CHD with DM (n=35)	n=88		Association with prevalent CHD, adjusted for TC, HDL-c, LDL-c, Tg and other factors	B
		Pattern B prevalence	54%	62%	10%	<0.05	OR=4.4 (1.2, 16) P=.03 (vs. pattern A)	
		Mean±SD LDL size, Å	252±14	249±16	262±14	<0.05	nd	
Inukai 2005 [43] Japan 16112502	Type 2 DM		Increased average IMT (≥1 mm) (n=16)		Normal average IMT (<1 mm) (n=11)		Independent risk factors for determining average IMT, adjusted for Tg, HDL-c, LDL-c and other variables; unit of analysis not reported	C
		Small LDL (mg/dL): subfractions 3-7	107±28		68±21	<0.01	OR=1.61 P=.01	
		Mean±SD Small LDL / Total LDL	0.81±0.13		0.69±0.11	<0.05	OR=1.59 P=.03	

Table 7a. Characteristics of patients in the cross-sectional studies of prevalent CVD and LipoPrint GE-measured LDL subfractions

Author Year Country UI	Population	Mean Age [A], years	>65, [A,B] %	Male, [A] %	DM, [A] %	Smoke, [A] %	Mean LDL-c [A] (mg/dL)
Kullo 2004[44] US 15363830	Genetic Epidemiology Network of Arteriopathy (GENOA) study, community-based, Sibships with ≥2 full siblings with essential HTN before the age of 60; measurement of coronary artery calcium (CAC), excluded those with history of CABG or PTCA (previous MI or stroke okay)	62	~40	41	16	49	119
Rajman 1996[49] UK 8842354	Men who had coronary angiogram, Tg≤204 mg/dL, no lipid lowering drugs, no DM, no kidney disease, no MI or CABG within 6 weeks prior to angiogram	61	~30	100	0	16	160
Park 2006[48] Korea 17142132	Subjects who visited a health screening program, no CVD, no DM, not on treatments with cardiovascular medications (not defined) that might interfere with measurements	52	~5	35	0	22	119
Kwon 2006[45] S. Korea 16807992	Patients who underwent coronary angiogram, no MI, no ESRD, no liver failure, excluded those who had previous coronary angiograms	60.4	~45	nd	22	34	106
Landray 1998[46] UK 9709468	Patients with stroke, TIA, amaurosis fugax, or presyncope referred for carotid ultrasonography	62.4	~40	65	18	72	nd
Mohan 2005[47] India 15847025	Study sample randomly drawn from Chennai Urban Rural Epidemiology Study (CURES), type 2 DM with or without CAD (history of MI and/or nitrates or revascularization or ECG changes)	57	~20	50	100	nd	131

[A] Of cases in case-control or cross-sectional studies (when reported separately); Data at baseline
[B] ~ = estimated from mean and standard deviation, otherwise reported value (rounded to nearest 5%)

Table 7b. Cross-sectional studies of LipoPrint GE-measured LDL subfractions and prevalent CVD

Author Year Country UI	Population	Outcome	Definitions	Results	Quality
Kullo 2004[44] US 15363830	Sibships with ≥2 full siblings with essential HTN before age 60 years n=792	Coronary artery calcium (CAC)		Associations with CAC, unit not defined	B
			LDL particle size	Unadjusted Women: OR=0.94 (0.90, 0.99) P=.008 Men: OR=1.02 (0.97, 1.07) NS	
				Adjusted for HDL-c, Tg, and conventional risk factors Women: OR=0.98 (0.92,1.04) NS Men: OR=1.02 (0.96, 1.08) NS	
Rajman 1996[49] UK 8842354	Men who had coronary angiogram n=68	Prevalent CAD	LDL score [A]	Correlation, r = 0.49 P<0.001 Score: 1.48 (CAD) vs 0.96 (no CAD) P<.001 Adjusted for Tg, P<.001	B
		No. of diseased vessels		Correlation, r = 0.46 P<0.001	
		History of MI		Compared to no history of MI, NS (data not shown).	
Park 2006[48] S. Korea 17142132	Preclinical non-diabetic patients n=136	Carotid IMT	LDL particle size	Pearson correlation = -0.172 P=0.08	B
Kwon 2006[45] S Korea 16807992	Subjects who had angiogram n=504 (262 cases, 242 controls)	Prevalent CAD	Pattern B	49% (cases) vs 26% (controls) P<.001	B
			Small dense LDL: subtypes 3-7 divided by subtypes 1-7	18% (cases) vs 12% (controls) P<.001 Adjusted for LDL-c, HDL-c, and other risk factors, vs. less dense LDL: OR=2.3 (1.5, 3.5) P<.001	
			LDL particle size, Å	264.1 (cases) vs 267.3 (controls) P<.001	
Landray 1998[46] UK 9709468	Stroke, TIA, presyncope or amaurosis fugax referred for ultrasonography n=79	Carotid atherosclerosis	LDL Score [B]	1.56 (disease) vs 1.26 (no disease) P=.04 Adjusted for TC, Tg, OR=2.20 (0.91-5.29) NS, unit of analysis not reported	B
Mohan 2005[47] India 15847025	Type 2 DM N=60	Prevalent CAD	LDL 3	12.2 (CAD) vs 9.6 mg/dL NS	C
			LDL 4	3.7 (CAD) vs 1.5 P<.05	
			LDL 5	0.79 (CAD) vs 0.06 P<.05	
			Small LDL (LDL ≥3)	16.7 (CAD) vs 11.1 P<.05	

[A] "The relative percentage of the [area under the curve] of each LDL band was multiplied by its band number. The sum of all LDL bands present was calculated to produce a final LDL score."
[B] Area under the curve of each LDL band multiplied by its band number for bands 1-6.

Table 8. Longitudinal study of time-averaged Berkeley HeartLab GE-measured LDL subfractions and progression of CVD

Author Year Country UI	Population	Mean Age, [A] years	>65, [A] %	Male, [A] %	DM, [A] %	Smoke, [A] %	Mean LDL-c, [A] mg/dL
Williams 2003[51] US 12588777	Men with CAD <age 75, under "usual care" (n=106)	56	100	100	nd	nd	151
		Outcome	**Definitions**	**Results**			**Quality**
		Annualized rate of stenosis (%/year), 4 years	Small LDL mass (S_f0-7), mg/dL	Regression slope=0.006, P=0.09			B
			Large LDL mass (S_f7-12), mg/dL	Regression slope=0.002, NS			
			LDL peak diameter, nm	Regression slope=-0.391, NS			
			LDL IIIb (242-247 Å), %	Regression slope=0.149, P=0.06 (P=0.02 if baseline stenosis <30%; NS if ≥30%)			
			LDL IVb (220-233 Å), %	Regression slope=0.238, P=0.01 (P=0.002 if baseline stenosis <30%; NS if ≥30%) (Adjusted for other lipoproteins, P=0.06 overall; P<0.05 if stenosis <30%; NS if stenosis ≥30%; the best fitting multivariable model with lipoproteins includes only LDL IVb, among subfractions)			
			LDL IVb, quartiles (2.5%, 3.7%, 5.2%)	Trend for more rapid stenosis with increasing quartile, P=0.04 4th vs 1st quartiles: Rate ~6x greater, P=0.03; stronger association if stenosis <30%; NS if stensosis≥30%			
			LDL I, IIa, IIb, IIIa, or IVa, %	NS			

[A] Data at baseline

Other methods to measure LDL subfractions

As described above, full data extraction and study analysis were performed only on those studies that used methods available for clinical use to measure LDL subfractions. We performed only limited extraction of other studies. We did not extract detailed results, nor did we assess study quality. We extracted only data presented in Tables 9-13. Measurements of LDL subfractions were classified as "size" (measured in angstroms), "pattern" (where the measurement was of a described pattern based on subfraction distribution or of a specific subfraction such as small dense LDL). In an overall summary table described below, NMR measurements of LDL subfraction number (or concentration) are classified as "number." Within each classification, the magnitude and statistical association between the LDL subfraction and the CVD outcome are presented as symbols as described in Table 9 and following. Analyses that were unadjusted or adjusted for LDL or HDL cholesterol, triglycerides, or other commonly used lipid measurements are separated (and given different symbols). Note that analyses that are categorized here as unadjusted may have been adjusted for such factors as treatment, demographics, or past medical history, but not lipids. We ignored adjustments for other lipid subfractions.

Results

Forty-one studies evaluated the associations between LDL subfraction measurements and CVD outcomes using measurement methods not clinically available. Among these, 30 used GE, 8 used ultracentrifugation, 2 used HPLC, and 1 did not report its methodology; 32 measured the size of the LDL particles and 29 evaluated different patterns. Seven studies evaluated incident CVD events in 5 nested case control studies and 2 prospective longitudinal studies (Table 9); followup occurred at averages ranging from 3.5 to 13 years. Five studies evaluated progression of coronary artery disease (Table 10), measured by angiography, in prospective longitudinal studies; followup occurred between 2 and 5 years. Twenty studies evaluated prevalent coronary artery disease in 16 case control studies and 4 prospective cohort studies (Table 11). Eight studies evaluated prevalent carotid atherosclerotic disease, primarily measuring IMT, in 1 case control study and 7 prospective cohort studies (Table 12). Lastly, a singly study evaluated prevalent cerebrovascular disease (silent lacunar infarcts) in a prospective cohort study (Table 13).

These additional studies were generally consistent with the studies that evaluated LipoPrint® GE or NMR. They evaluated a wide range of populations, including those with and without baseline CVD, with various comorbidities, on a wide range of medications (though this was generally not explicitly described). In most studies, participants tended to be relatively young. Eighteen studies included very few or no subjects above age 65 years; 3 studies had more than half the subjects over age 65 years, none of which included only older subjects.

Tables 14-16 summarize findings across all studies (including those that used the clinically available methods). Table 14 summarizes the studies that reported unadjusted analyses of LDL subfractions and cardiovascular outcomes, Table 15 summarizes the studies that reported analyses of LDL subfractions and cardiovascular outcomes adjusted for lipid and other cardiovascular risk factors, and Table 16 summarizes the studies that reported both unadjusted and adjusted analyses of LDL subfractions and cardiovascular outcomes. For the purpose of these analyses, the many specific measurements were categorized as being measurements of LDL subfraction size, number, or pattern. The numbers of studies that reported statistically significant "positive" or "negative" associations or no significant associations are summarized.

Only those studies that reported associations not adjusted for other cardiovascular risk factors are included in Table 14; only those studies that reported associations that were adjusted for other cardiovascular risk factors are included in Table 15; and only studies that reported both unadjusted and adjusted associations are included in Table 16.

Unadjusted analyses

Looking at Table 14 alone, the majority of studies found that LDL subfraction size, number, and patterns were significantly associated with CVD outcomes. Overall, 64 percent of analyses found statistically significant associations with incident CVD or progression, and 78 percent with prevalent CVD. Interestingly, a minority of studies found that *larger* LDL subfractions were associated with prevalent disease (10 percent). There were no obvious factors among these seven studies to explain this heterodox finding, other than chance.

Lipid-adjusted analyses

Given the wide variety of participants across the studies, particularly that many studies included very narrow populations (eg, cases and controls selected among patients having coronary angiograms, patients with a history of myocardial infarction before age 45) and that many studies were case control (ie, matched retrospective), the unadjusted analyses may be misleading. Particularly, given that the major potential treatments for abnormal LDL subfractions also treat dyslipidemias, it is important to evaluate the lipid-adjusted associations to have a better understanding of the clinical value of LDL subfractions. Only half the number of lipid-adjusted analyses were performed as unadjusted analyses. The distribution of statistically significant and nonsignificant associations was more evenly split among these analyses; 50 percent of analyses found significant adjusted associations with incident CVD or progression, and 58 percent with prevalent CVD. Only 1 adjusted analysis found a significant association between *larger* subfractions and prevalent CVD. An important caveat to these analyses, though, is that studies used different statistical (or clinical) methods to determine which variables would be adjusted for, including the various lipoprotein cholesterol and triglyceride concentrations, lipid ratios, along with other CVD risk factors (such as blood pressure) and other variables (such as demographics). It is impossible to evaluate how the distribution of findings would have changed had all researchers used the same analytic technique.

Importantly, many of these adjusted analyses were reported without presenting the unadjusted analyses. To understand the impact of adjustment on the findings of significant associations, we evaluated how findings changed within those studies that reported both the unadjusted and lipid-adjusted analyses (Table 16). As displayed in the striped columns to the right of the table, possible findings within studies include similar conclusions regardless of adjustment (the grey columns) or changes between significant and nonsignificant associations after adjustment (the white columns). Among the 17 analyses that found a statistically significant unadjusted association between LDL subfractions and incident CVD or progression (▽ in the table), 8 (47 percent) became nonsignificant after adjusting for lipid and other factors; 5 analyses remained nonsignificant regardless of adjustment. Among the 27 analyses that found a statistically significant unadjusted association between smaller LDL subfractions and prevalent CVD, 10 (37 percent) became nonsignificant after adjusting for lipid and other factors; 1 analysis remained nonsignificant regardless of adjustment, 1 nonsignificant unadjusted analysis was statistically significant after adjustment, and 2 analyses that found an association between *larger* LDL subfractions and CVD both lost significance after adjustment.

Summary of specific measures

To further understand whether there are specific measures of LDL subfractions that are associated with CVD outcomes, we focused on those studies that evaluated GE or NMR for incident CVD or progression of CVD in lipid-adjusted analyses (studies had to adjust for lipoprotein cholesterol or triglycerides, they may also have adjusted for other CVD risk factors). We chose these studies for clinical reasons, as these are the measures that could be available to clinicians and the outcomes of interest to clinicians and patients, if treatment options are being considered.

None of the studies of the clinically available GE evaluated incident CVD or progression. We evaluated the other GE studies under the assumption that the specific measurements would be available using all GE methods. Eight GE studies and six NMR studies reported lipid-adjusted associations with incidence or progression of CVD.[27,35,37,40-42,51-58] GE and NMR results are analyzed separately.

Six GE studies evaluated LDL particle size,[52-56,58] four of which found no significant adjusted association with CVD. Three GE studies analyzed the percent of LDL which was defined as small; though each used a different definition for small LDL: less than 228 Å, between 220 and 233 Å (LDL IVb), or less than 255 Å.[51,56,57] The study that measured the smallest particles (less than 228 Å) found no significant association,[56] in contrast with the other two studies. Despite the generally common use of describing peoples as having LDL Pattern A or B (or intermediate), only one GE study of incident CVD performed lipid-adjusted analyses. This study found no significant association with Pattern B (defined as less than 258 Å).[58] Two GE studies evaluated both size and another measure, but both found no association with CVD with either measure evaluated.[56,58] Overall, none of the specific measures of LDL subfractions determined by GE consistently was associated with incidence or progression of CVD after adjustment for lipid concentrations.

The most common measurement by NMR was concentration of LDL particles. The four studies that evaluated incident disease all found a significant lipid-adjusted association between LDL particle concentration and CVD,[27,35,37,40] in contrast with the one evaluation of CVD progression, which found no association.[41] Three studies each evaluated LDL particle size[27,40,41] and small LDL particles (defined as either 183 to 197 Å in two studies or 180 to 212 Å in one).[40-42] For both measures, only one study[41] found a statistically significant association (with progression of CVD); the other studies found no significant associations with incident CVD with either measure. Three NMR studies evaluated both LDL particle size and concentration; two of which also evaluated small LDL (defined differently).[27,40,41] The two studies of CVD incidence were consistent in finding that concentration was significantly associated with CVD, but not particle size (or small LDL in one study). The study of CVD progression had the opposite finding, that size and small LDL, but not concentration, were associated with CVD.

In summary, only LDL particle concentration, as measured by NMR, was consistently found to be associated with incident CVD after adjustment for lipids (and other risk factors). Other specific measures have been found to be associated with incidence or progression of CVD by only a minority of studies.

Table 9. Association between LDL Subfraction and incident CVD events (not full extraction)

▽/▼ *Smaller* particles associated with more CAD outcome: Statistically significant association in unadjusted (unadj)/**adjusted (adj)** analysis
○/**O** No statistically significant association in unadjusted (unadj)/**adjusted (adj)** analysis
△/▲ *Larger* particles associated with more CAD outcome: Statistically significant association in unadjusted (unadj)/**adjusted (adj)** analysis
▼/▲ (grey) An association was reported, but no statistical analysis was performed

Author Year Country UI	Test Category [A] / Study Design	Mean Age [B]	%>65 [C]	%Male [B]	%DM [B]	%Smoke [D]	LDL-c [B] (mg/dL) / Subfraction Data		Group (Arm)	N	Follow-up Time	Outcome (Definition)		Predictor	Results Unadj	Results Adj
Howard 2000[59] US 10712410	nd	56	~15	nd	47	35c	LDL-c	113	American Indian	3668? or 4378? [E]	mean 4.8 yr	Fatal and nonfatal CVD event	Women	Size	▽	
	Prospective longitudinal						mean 259.1 Å						Men		○	
													DM			**O**
St. Pierre 2005[57] Canada 15618542	GE	57	0	100	5	23c	LDL-c	148	Healthy	2072	13 yr unadj 5 yr adj [F]	Ischemic CAD event		Size	○	
	Prospective longitudinal						mean 256.9 Å B (<255 Å): 40%							Pattern	▽	▼
Stampfer 1996[58] US 8782637	GE	59	~25	100	6	56e	Total:HDL-c ratio	5.2	CAD event	266	7 yr	Incident MI or CAD death		Size	▽	**O**
	Nested case control						mean 256 Å B: 47% I: 20%		Control	308				Pattern	▽	**O**
Austin 2000[52] US (HI [G]) 10946034	GE	68	~70	100	17	63e	LDL-c:	142	Incident CHD	145	12 yr	Incident CAD: MI or coronary intervention		Size	▽	**O**
	Nested case control						mean 260.0 Å		Control	296				Pattern	▽	
Gardner 1996[54] US 8782636	GE	59	33	73	nd	42n	Non-HDL-c:	176	CAD event	124	mean 5 yr to CAD event	Incident MI or CAD death		Size	▽	▼
	Nested case control						mean 261.7 Å <260 Å: 40% >274.2 Å: 10%		No event	124				Pattern	▼ (grey)	
Mykkanen 1999[55] Finland 10559020	GE	69	100	50	33	20c	Total:HDL-c ratio	6.09	CAD event	86	mean 3.5 yr	Incident MI or CAD death		Size	○	**O**
	Nested case control						mean 268.2 Å B or I: 21%		Control	172				Pattern	○	
Campos 2001[53] US 11572739 [H]	GE	60	~30	87	16	17c	LDL-c	139	CAD event	242	median 5 yr	Confirmed MI or CAD death (on placebo)		Size	▽	▼
	Nested case control						mean 256 Å		Control	218						

A, Pattern A (if no definition included, the article did not define); I, Indeterminate pattern (not A or B); B, Pattern B;
Pattern: analysis based on distribution across categories of LDL subfractions (eg, small, medium, large)

Size: analysis based on actual particle size (eg, regression or comparisons of mean sizes)

[A] CITP; GE; HPLC; NMR; UC; Other
[B] Of cases in case-control or cross-sectional studies (when reported separately); Data at baseline
[C] ~ = estimated from mean and standard deviation, otherwise reported value (rounded to nearest 5%)
[D] c = current smokers; e = ever smoked; n = not defined.
[E] Unclear how many of the subjects were analyzed.
[F] Lamarche 2001[60] UI 11521128.
[G] Japanese Americans in the Honolulu Heart Program.
[H] Also in section 4.4.

Table 10. Association between LDL Subfraction and progression of coronary artery disease (not full extraction)

▽/▼ *Smaller* particles associated with more CAD outcome: Statistically significant association in unadjusted (unadj)/**adjusted (adj)** analysis
○/**O** No statistically significant association in unadjusted (unadj)/**adjusted (adj)** analysis
△/▲ *Larger* particles associated with more CAD outcome: Statistically significant association in unadjusted (unadj)/**adjusted (adj)** analysis
▼/▲ An association was reported, but no statistical analysis was performed

Author Year Country UI	Test Category [A] Study Design	Mean Age [B]	%>65 [C]	%Male [B]	%DM [B]	%Smoke [D]	LDL-c [B] (mg/dL) / Subfraction Data	Group (Arm)	N	Follow-up Time	Outcome (Definition)	Predictor	Results Unadj	Results Adj
Zhao 2003[61] US 12601286	GE	62	40	74	23	64e	LDL-c 141	CAD requiring PTCA or CABG	278	3 yr	Native CAD progression (angiography)	Size	▽	
	Prospective longitudinal (RCT)						mean 266.8 Å B: 26% A: 64% Small (<256 Å): 26%					Pattern	▽	[E]
Watts 1993[62] UK 8231842	UC	nd	nd	100	nd	nd	TC 280	Angina pectoris not requiring revascularization	74	38 mo	Change coronary atherosclerosis (angiography)	Pattern	▽	
	Prospective longitudinal (RCT)						LDL_2 (d=1.019-1.040 Kg/L): 36 mg/dL LDL_3 (d=1.040-1.063 kg/L): 92 mg/dL							
Ruotolo 1998[56] Sweden 9822092 [F]	GE	42	0	100	nd	24c	LDL-c 180	MI<45 yo	92	5 yr	Change coronary atherosclerosis (angiography)	Size	○	**O**
	Prospective longitudinal (RCT)						mean 230 Å Small (<228 Å): 39%					Pattern	○	**O**
Miller 1996[63] US 8901665 [F]	UC	57	~20	100	nd	nd	LDL-c 156	Coronary stenosis (usual care arm)	116	4 yr	Change coronary atherosclerosis (angiography)	Pattern	○	
	Prospective longitudinal (RCT)						sdLDL (S_f° 0-5): 44% B: 41% I: 31% A: 28%							
Mack 1996[64] US 8963728 [F]	UC	58	~15	92	nd	79e	LDL-c 156	Coronary stenosis	220	2 yr	Change coronary atherosclerosis (angiography)	Size	○	
	Prospective longitudinal (RCT)						Peak flotation rate, S_f: 5.4 IV (S_f 0-3): 14.7 mg/dL					Pattern	▽	

A, Pattern A (if no definition included, the article did not define); I, Indeterminate pattern (not A or B); B, Pattern B;
Pattern: analysis based on distribution across categories of LDL subfractions (eg, small, medium, large)
Size: analysis based on actual particle size (eg, regression or comparisons of mean sizes)

[A] CITP; GE; HPLC; NMR; UC; Other
[B] Of cases in case-control or cross-sectional studies (when reported separately); Data at baseline
[C] ~ = estimated from mean and standard deviation, otherwise reported value (rounded to nearest 5%)
[D] c = current smokers; e = ever smoked; n = not defined.
[E] Significant association adjusted for history of hypertension, ST depression   1 mm at baseline exercise tolerance test. Lipids not included in model.
[F] Also in section 4.4.

Table 11. Association between LDL Subfraction and prevalent coronary artery disease (not full extraction)

▽/▼ *Smaller* particles associated with more CAD outcome: Statistically significant association in unadjusted (unadj)/**adjusted (adj)** analysis
○/**O** No statistically significant association in unadjusted (unadj)/**adjusted (adj)** analysis △/▲ *Larger* particles associated with more CAD outcome: Statistically significant association in unadjusted (unadj)/**adjusted (adj)** analysis ▼/▲ An association was reported, but no statistical analysis was performed

Author Year Country UI	Test Category [A] Study Design	Mean Age [B]	%>65 [C]	%Male [B]	%DM [B]	%Smoke [D]	LDL-c [B] (mg/dL)		Group (Arm)	N	Follow-up Time	Outcome (Definition)	Predictor	Results Unadj	Results Adj
							Subfraction Data								
Jang 2006[65] S Korea 16787988	UC	55	~0	100	0	71c	LDL-c	106	CAD	532	XS	CAD (angiography)	Size	▽	
	Case control						mean 255 Å		Control	670					
Campos 1992[66] US 1543692	GE	50	0	100	nd	nd	LDL-c	143	CAD	275	XS	CAD (angiography)	Size	▽	**O**
	Case control						LDL particle score [E]: 4.32		Control	822			Pattern	▽	**O**
Koba 2002[67] Japan 12486427 [F]	GE	63	~40	79	34	35c	LDL-c	123	CAD	571	XS	CAD (angiography)	Size	▽	
	Case control						mean 255 Å B (≤255 Å): 53%		Control	263			Pattern	▽	▼
Alabakovska 2002[68] Macedonia 12035134	GE	49	0	73	0	nd	LDL-c	135	CAD	132	XS	CAD (previous MI)	Size	▽	
	Case control						mean 244 Å B: 81%		Control	334			Pattern	▽	
Koba 2006[69] Japan 16414053	GE	60	~30	77	24	66e	LDL-c	127	Coronary angiography performed	367	XS	CAD (angiography)	Size	▽	
													Pattern	▽	
	Prospective cohort						mean 257.1 Å B (<255 Å): 37%					CAD severity (affected vessels, n=225)	Size	○	
Koba 2002[70] Japan 11755944 [F]	GE	63	~40	100	100	51c	LDL-c	108	CAD+DM+	45	XS	CAD (clinical diagnosis)	Size	▽	
							mean 250 Å B (≤255 Å): 76%		CAD-DM+	76			Pattern	▽	▼
	Case control	60	~30		0	49c	LDL-c	116	CAD+DM-	85			Size	▽	
							mean 252 Å B (≤255 Å): 71%		CAD-DM-	142			Pattern	▽	▼
Erbey 1999[71] US 10206450	GE	42	0	52	100	32c	LDL-c	125	CAD	44	XS	CAD (clinical diagnosis)	Size	▽	**O**
	Prospective cohort						mean 261.3 nmol/L B (<235.5 nmol/L): 3% A (>257 nmol/L): 9%		Control	297					

Continued

Table 11. Continued

▽/▼ *Smaller* particles associated with more CAD outcome: Statistically significant association in unadjusted (unadj)/**adjusted (adj)** analysis
○/**O** No statistically significant association in unadjusted (unadj)/**adjusted (adj)** analysis △/▲ *Larger* particles associated with more CAD outcome: Statistically significant association in unadjusted (unadj)/**adjusted (adj)** analysis ▼/▲ (grey) An association was reported, but no statistical analysis was performed

Author Year Country UI	Test Category [A] Study Design	Mean Age [B]	%>65 [C]	%Male [B]	%DM [B]	%Smoke [D]	LDL-c [B] (mg/dL) Subfraction Data		Group (Arm)	N	Follow-up Time	Outcome (Definition)	Predictor	Results Unadj	Results Adj
Kamigaki 2001[72] US 11384949	GE	40	0	0	14	86e	LDL-c	139	Premature CAD	72	XS	CAD (previous MI)	Size	▽	▼
	Case control						mean 264 Å B (<255 Å): 36%		Control	159			Pattern	▽	
Austin 1988[73] US 3418853	GE	52	0	84	nd	nd	LDL-c	144	CAD	109	XS	CAD (previous MI)	Pattern	▽	**O**
	Case control						B (<255 Å): 50%		Control	121					
Sherrard 1996[74] Australia 8902153	UC	nd	nd	64	nd	nd	LDL-c	~138	CAD	53	XS	CAD (angiography)	Size	○	
	Case control						mean 250 Å		Control	167			Pattern	▲ (grey)	
Coresh 1993[75] US 8245719	GE	48	0	54	22	85e	LDL-c	127	CAD	107	XS	CAD (angiography)	Size	▽	**O**
	Prospective cohort						mean 251.6 Å		Control	91					
Campos 1995[76] US 7627694	GE	58	~22	100	0	nd	LDL-c	126	CAD	92	XS	CAD (angiography)	Size	△	▲
	Case control						mean 268 Å III (<260 Å): 43% I (>268 Å): 14%		Control	92			Pattern	△	
Griffin 1994[77] UK 8060384	UC	53	0	100	?0	70e	LDL-c	154	CAD+/MI-	46	XS	CAD (angiography or recent MI)	Pattern	○	▼
									CAD-	24					
	Case control						%LDL: I (≤1.034 mg/dL): 15% III (≥1.044 mg/dL): 38%		MI+	40			Pattern	▽	▼
									Healthy	58					
Miwa 2003[78] Japan 12559540	GE (Lipophor)	61	~35	80	31	74n	LDL-c	124	Spastic angina	49	XS	CAD (angiography)	Size	▽	
	Case control						Relative migratory distance [G]:0.370 Small (>0.36): 48%		Stable angina	56			Pattern	▽	
									Control	40					

Continued

Table 11. Continued

▽/▼ *Smaller* particles associated with more CAD outcome: Statistically significant association in unadjusted (unadj)/**adjusted (adj)** analysis
○/**O** No statistically significant association in unadjusted (unadj)/**adjusted (adj)** analysis △/▲ *Larger* particles associated with more CAD outcome: Statistically significant association in unadjusted (unadj)/**adjusted (adj)** analysis ▼/▲ An association was reported, but no statistical analysis was performed

Author Year Country UI	Test Category [A] Study Design	Mean Age [B]	%>65 [C]	%Male [B]	%DM [B]	%Smoke [D]	LDL-c [B] (mg/dL) / Subfraction Data	Group (Arm)	N	Follow-up Time	Outcome (Definition)	Predictor	Results Unadj	Results Adj
Crouse 1985[79] US 4020295	Ultracentrifugation	57	nd	100	13	76e	LDL-c 140	CAD	46	XS	CAD (angiography)	Size	▽	
	Case control						mean 279 Å	Control	47					
Okazaki 2006[80] Japan 16990425	HPLC	64	~45	100	0	51c	LDL-c 122	CAD	45	XS	CAD (angiography)	Pattern	▽	
	Prospective cohort						Large (Peak 286 Å): ~35 mg/dL Medium (255 Å): ~50 mg/dL Small (230 Å): ~30 mg/dL Very small (207, 186, & 167 Å): ~10 mg/dL	Control	17					
Hitsumoto 2002[81] Japan 12226547	GE	61	~35	100	3	68n	LDL-c 124	Recent MI	44	XS	Recent MI	Pattern	▽	**O**
	Case control						Small (Relative migratory distance>0.35): 68%	Control	16					
Barbagallo 2006[82] Italy 16631444	GE	43	0	100	0	0c	LDL-c 111	CAD	29	XS	CAD (angiography)	Size	▽	
	Case control						mean 262.7 Å	Control	29					
Karpe 1993[83] Sweden 8457249	UC	49	0	100	25	91e	LDL-c 154; Dense (1.040<d<1.063 kg/L): 56%	CAD, elevated Tg	15	XS	History of early MI (<age 45 yr)	Pattern	▽	
	Case control						LDL-c 171	CAD, normal Tg	17			Pattern	○	
							Dense (1.040<d<1.063 kg/L): 43%	Control	10					

Continued

Table 11. Continued

▽/▼ *Smaller* particles associated with more CAD outcome: Statistically significant association in unadjusted (unadj)/**adjusted (adj)** analysis
○/**O** No statistically significant association in unadjusted (unadj)/**adjusted (adj)** analysis △/▲ *Larger* particles associated with more CAD outcome: Statistically significant association in unadjusted (unadj)/**adjusted (adj)** analysis ▼/▲ An association was reported, but no statistical analysis was performed

Author Year Country UI	Test Category [A] Study Design	Mean Age [B]	%>65 [C]	%Male [B]	%DM [B]	%Smoke [D]	LDL-c [B] (mg/dL) / Subfraction Data		Group (Arm)	N	Follow-up Time	Outcome (Definition)	Predictor	Results Unadj	Results Adj
Tilly-Kiesi 1992[84] Finland 1569383	GE	57	~0	100	100	nd	LDL-c	117	CAD+DM+	10	XS	CAD (angiography)	Size	○/△ [H]	
							mean 259 Å 2nd peak (>255 Å): 80%		CAD-DM+	10			Pattern	▲	
	Case control				0		LDL-c	133	CAD+DM-	10			Size	○/△ [H]	
							mean 259 Å 2nd peak (>255 Å): 60%		CAD-DM-	10			Pattern	○	

A, Pattern A (if no definition included, the article did not define); I, Indeterminate pattern (not A or B); B, Pattern B;

A CITP; GE; HPLC; NMR; UC; Other
B Of cases in case-control or cross-sectional studies (when reported separately); Data at baseline
C ~ = estimated from mean and standard deviation, otherwise reported value (rounded to nearest 5%)
D c = current smokers; e = ever smoked; n = not defined.
E Area under curve for each LDL band (1-5, 5 densest) multiplied by the band number, summed across bands.
F Probably some overlap in subjects.
G Measure of LDL particle size: “relative migratory distance of LDL [compared] to that of HDL from VLDL”. Relative migratory distance LDL >0.36 corresponded to the particle diameter <255 Å.
H NS when subjects with and without diabetes analyzed separately, but statistically significant when combined.

Table 12. Summary: Association between LDL Subfraction and CVD outcomes (not full extraction)

▽/▼ *Smaller* particles associated with more CAD outcome: Statistically significant association in unadjusted (unadj)/**adjusted (adj)** analysis
○/**O** No statistically significant association in unadjusted (unadj)/**adjusted (adj)** analysis
△/▲ *Larger* particles associated with more CAD outcome: Statistically significant association in unadjusted (unadj)/**adjusted (adj)** analysis
▼/▲ An association was reported, but no statistical analysis was performed

Author Year Country UI	**Test Category [A] Study Design**	**Mean Age [B]**	**%>65 [C]**	**%Male [B]**	**%DM [B]**	**%Smoke [D]**	**LDL-c [B] (mg/dL) / Subfraction Data**		**Group (Arm)**	**N**	**Follow-up Time**	**Outcome (Definition)**	**Predictor**	**Results**	
														Unadj	**Adj**
Hallman 2004[85] US 15370875	GE	57	0	68	nd	84e	LDL-c	146	Carotid disease, White	151	XS	Carotid atherosclerosis (ultrasonography)	Pattern	▽	▼ [E]
							B: 29%; A: 40%		Control	237					
	Case control	54	0	40	nd	51e	LDL-c	135	Carotid disease, Black	47			Pattern	○	
							B: 19 A: 49		Control	81					
Hulthe 2000[86] Sweden 10978261	GE	58	0	100	0	63e	LDL-c	157	Healthy [F]	380	XS	Carotid & Femoral IMT [G] (mm)	Size	▽	
	Prospective cohort						Peak size: 263 Å B (<255 Å): 16%						Pattern	○	
Hulthe 2000[87] Sweden 10947880	GE	60	~30	51	nd	57e	LDL-c	267	Hyper-cholesterolemia, Carotid IMT ≥1 mm	102	XS	Carotid & Femoral IMT [G] (mm)	Size	○ [H]	
							Peak size: 266.9 Å B (<255 Å): 9%								
	Prospective cohort						LDL-c	142	Healthy	102			Size	○ [J]	
							Peak size: 271.4 Å B (<255 Å): 7%								
Hayashi 2007[88] Japan 17445534	GE	67	~55	65	100	nd	LDL-c	109	Diabetes	172	XS	Carotid IMT (mm, maximum value)	Size	▽	▼
	Prospective cohort						mean 251 Å								
Liu 2002[89] Finland 11988600	GE	40	0	36	0	57e	LDL-c	136	Dyslipidemia or family history	148	XS	Carotid IMT (mm, mean)	Size	▽	▼
	Prospective cohort						mean 267 Å								

Continued

Table 12. Continued

▽/▼ *Smaller* particles associated with more CAD outcome: Statistically significant association in unadjusted (unadj)/**adjusted (adj)** analysis
○/**O** No statistically significant association in unadjusted (unadj)/**adjusted (adj)** analysis
△/▲ *Larger* particles associated with more CAD outcome: Statistically significant association in unadjusted (unadj)/**adjusted (adj)** analysis
▼/▲ (grey) An association was reported, but no statistical analysis was performed

Author Year Country UI	**Test Category [A] Study Design**	**Mean Age [B]**	**%>65 [C]**	**%Male [B]**	**%DM [B]**	**%Smoke [D]**	**LDL-c [B] (mg/dL) / Subfraction Data**		**Group (Arm)**	**N**	**Follow-up Time**	**Outcome (Definition)**	**Predictor**	**Results Unadj**	**Results Adj**
Watanabe 2004[90] Japan 15045695	GE / Prospective cohort	74	~85	100	0	22c	LDL-c	99	Dementia	134	XS	Carotid IMT (mm, mean)	Size	▽	
							B (<255 Å): 26%								
Skoglund 1999[91] Sweden 10521372	GE / Prospective cohort	50	0	100	0	nd	LDL-c	139	Healthy	94	XS	Carotid IMT (mm, common carotid)	Size	▽	
							Peak size 236 Å IV (<225 Å): 5% I (>250 Å): 20%						Pattern	▽	▼
Raal 1999[92] S Africa 10235090	GE / Prospective cohort	29	0	52	0	0c	LDL-c		With or without FH	62	XS	Carotid IMT (mm, common carotid)	Size	△	**O**
							homozygous	526							
							heterozygous	243							
							control	124							
							mean 261 Å								

A, Pattern A (if no definition included, the article did not define); I, Indeterminate pattern (not A or B); B, Pattern B;
Pattern: analysis based on distribution across categories of LDL subfractions (eg, small, medium, large)
Size: analysis based on actual particle size (eg, regression or comparisons of mean sizes)

A CITP; GE; HPLC; NMR; UC; Other
B Of cases in case-control or cross-sectional studies (when reported separately); Data at baseline
C ~ = estimated from mean and standard deviation, otherwise reported value (rounded to nearest 5%)
D c = current smokers; e = ever smoked; n = not defined.
E Not statistically significant if all variables included (age, smoking, body mass index, triglycerides, and HDL cholesterol), but statistically significant if one or more of the variables are omitted).
F Uneven selection of participants based on insulin sensitivity, overrepresenting highest and lowest sensitivity quintiles.
G Separate analyses for common carotid, carotid bulb, and common femoral. Same results for all.
H Adjusted for age, common carotid IMT was positively associated (△) with LDL peak particle size in women, but not men. No association in other arteries.
J Adjusted for age, carotid bulb IMT was positively associated (△) with LDL peak particle size in men, but not women. No association in other arteries.

Table 13. Association between LDL Subfraction and cerebrovascular outcomes (not full extraction)

▽/▼ *Smaller* particles associated with more CAD outcome: Statistically significant association in unadjusted (unadj)/**adjusted (adj)** analysis
○/**O** No statistically significant association in unadjusted (unadj)/**adjusted (adj)** analysis
△/▲ *Larger* particles associated with more CAD outcome: Statistically significant association in unadjusted (unadj)/**adjusted (adj)** analysis
▼/▲ An association was reported, but no statistical analysis was performed

Author Year Country UI	Test Category [A] Study Design	Mean Age [B]	%>65 [C]	%Male [B]	%DM [B]	%Smoke [D]	LDL-c [B] (mg/dL)		Group (Arm)	N	Follow-up Time	Outcome (Definition)	Predictor	Results	
							Subfraction Data							Unadj	Adj
Kato 2006[93] Japan 16832149	HPLC Prospective cohort	61	~35	66	0	0c	LDL-c	123	Essential hypertension	100	XS	Silent lacunar infarct (brain MRI)	Pattern	▽	▼
							LDL-3 (fastest): 8.3 mg/dL								

Pattern: analysis based on distribution across categories of LDL subfractions (eg, small, medium, large)
Size: analysis based on actual particle size (eg, regression or comparisons of mean sizes)

[A] CITP; GE; HPLC; NMR; UC; Other
[B] Of cases in case-control or cross-sectional studies (when reported separately); Data at baseline
[C] ~ = estimated from mean and standard deviation, otherwise reported value (rounded to nearest 5%)
[D] c = current smokers; e = ever smoked; n = not defined.

Table 14. Overall summary of unadjusted analyses of LDL subfractions and cardiovascular outcomes

Outcome	Predictor	Study Design	No. Studies	N	Unadjusted [A] Analyses		
					▽ [B]	○	△ [B]
CAD, Incident	Size	P Long	2	5740 [C]	10	5	
		nCC	11	7382			
	Number	nCC	7	5401	5	2	
	Pattern	P Long	1	2072	6	4	
		nCC	8	5996			
CAD, Progression	Size	P Long	7	6430	4	3	
	Number	P Long	1	111		1	
	Pattern	P Long	7	6472	5	2	
CAD, Prevalent	Size	P Cohort	6	2488	19	3	3
		CC	14	5414			
	Number	P Cohort	1	286	1		
	Pattern	P Cohort	5	1377	24		3
		CC	17	4688			
CerebroVD, Prevalent	Pattern	P Cohort	2	179	2		
IMT	Size	P Cohort	8	1330	5	3	1
	Pattern	P Cohort	3	513	3	2	
		CC	1	516			
Summary by disease type							
Incident Disease or Progression	Size		20	19,522 [D]	14	8	
	Number		8	5512	5	3	
	Pattern		16	14,540	11	6	
Prevalent Disease	Size			9232	24	6	4
	Number		1	286	1		
	Pattern		36	7273	29	2	3

CC, case control; nCC, nested case control; P Cohort, prospective cohort (cross-sectional);P Long, prospective longitudinal.

[A] ▽/▼ *Smaller* particles associated with more CAD outcome: Significant association in unadjusted (unadj)/**adjusted (adj)** analysis.
○/**O** No statistically significant association in unadjusted (unadj)/**adjusted (adj)** analysis.
△/▲ *Larger* particles associated with more CAD outcome: Significant association in unadjusted (unadj)/**adjusted (adj)** analysis.
[B] or an association was reported, but no statistical analysis was performed.
[C] or 6450 (number of analyzed subjects unclear in one study).
[D] or 8366 (number of analyzed subjects unclear in one study).

Table 15. Overall summary of lipid-adjusted analyses of LDL subfractions and cardiovascular outcomes

Outcome	Predictor	Study Design	No. Studies	N	Adjusted [A,B] Analyses ▼	○	▲
CAD, Incident	Size	P Long	1	2072	3	6	
		nCC	8	2507			
	Number	nCC	2	3148	2		
	Pattern	P Long	1	2072	1	3	
		nCC	3	840			
CAD, Progression	Size	P Long	3	5741	1	2	
	Number	P Long	1	111		1	
	Pattern	P Long	4	5858	3	1	
CAD, Prevalent	Size	P Cohort	4	1617	2	5	1
		CC	3	1512			
	Number	P Cohort	1	286	1		
	Pattern	P Cohort	3	948	9	5	
		CC	8	3071			
CerebroVD, Prevalent	Pattern	P Cohort	2	179	1	1	
IMT	Size	P Cohort	3	382	2	1	
	Pattern	P Cohort		133	3		
		CC	1	516			
Summary by disease type							
Incident Disease [C]	Size		12	9879	4	8	
	Number		3	3259	2	1	
	Pattern		9	9211	4	5	
Prevalent Disease	Size		10	3511	4	6	1
	Number		1	286	1		
	Pattern		16	4847	13	6	

[A] ▽/▼ *Smaller* particles associated with more CAD outcome: Significant association in unadjusted (unadj)/**adjusted (adj)** analysis.
○/**O** No statistically significant association in unadjusted (unadj)/**adjusted (adj)** analysis.
△/▲ *Larger* particles associated with more CAD outcome: Significant association in unadjusted (unadj)/**adjusted (adj)** analysis.

[B] Adjusted for cardiovascular risk factors, including LDL and/or HDL cholesterol and/or total:HDL cholesterol ratio, and possibly other items such as age, weight, and diagnosis of diabetes.

[C] Including progression of coronary atherosclerosis, by angiography.

Table 16. Overall summary of studies that reported both unadjusted and lipid-adjusted analyses of LDL subfractions and cardiovascular outcomes

Outcome	Predictor	Study Design	No. Studies	N	Unadjusted [A] → Adjusted [A, B] Analyses					
					▽→▼	▽→O	○→O	○→▼	△→▲	△→O
CAD, Incident	Size	nCC	8	2507	2	5	1			
	Number	nCC	2	3148	2					
	Pattern	P Long	1	2072	1	2	1			
		nCC	3	840						
CAD, Progression	Size	P Long	3	5741	1	1	1			
	Number	P Long	1	111			1			
	Pattern	P Long	4	5858	3		1			
CAD, Prevalent	Size	P Cohort	4	1617	2	4	1			1
		CC	2	1512						
	Number	P Cohort	1	286	1					
	Pattern	P Cohort	3	948	8	5		1		
		CC	8	3071						
CerebroVD, Prevalent	Pattern	P Cohort	2	179	1	1				
IMT	Size	P Cohort	3	382	2					1
	Pattern	P Cohort	2	133	3					
		CC	1	516						
Summary by disease type										
Incident Disease or Progression	Size		11	8248	3	6	2			
	Number		3	3259	2		1			
	Pattern		8	8770	4	2	2			
Prevalent Disease	Size		9	3511	4	4	1			2
	Number		1	286	1					
	Pattern		16	4847	12	6		1		

[A] ▽/▼ *Smaller* particles associated with more CAD outcome: Significant association in unadjusted (unadj)/**adjusted (adj)** analysis.
○/**O** No statistically significant association in unadjusted (unadj)/**adjusted (adj)** analysis.
△/▲ *Larger* particles associated with more CAD outcome: Significant association in unadjusted (unadj)/**adjusted (adj)** analysis.

[B] Adjusted for cardiovascular risk factors, including LDL and/or HDL cholesterol and/or total:HDL cholesterol ratio, and possibly other items such as age, weight, diabetes.

Question 4.2
If these tests are used in combination with other cardiovascular risk assessment technologies, what is the incremental increase of diagnostic performance?

Among the studies that used the clinically available GE test (LipoPrint®) or NMR, none revised a cardiovascular risk assessment technology (such as the Framingham Risk Score) by adding data on LDL subfractions or compared predictive models with and without LDL subfractions. Thus, there are no data on how cardiovascular risk assessment technologies are affected by the addition of information from clinically available LDL subfraction tests.

Question 4.3
If there is a relationship between LDL subfractions and CVD how strong is it relative to other risk factors?

Seven studies that used the LipoPrint® GE and all 12 studies that used NMR directly or indirectly compared the relative strengths of various risk factors, including LDL subfraction, for cardiovascular outcomes (Tables 17-23).[27,34-47,49,50,94] The studies have been described in the first section of the results for Question 4.1. In Tables 17-23, we did not include the increments implied by the associations reported (eg, whether OR was per 1 SD increment of the predictor or per 1 mg/dL). The goal of these tables is to evaluate the relative strengths of the risk factors (as per Question 4.3) within studies. We thus decided that the details would add complexity to the tables without adding value to answer the question at hand. Readers are referred to the primary studies for more details.

To address this question across studies, it would be ideal if all studies performed multivariable analyses using a standard set of risk factors for CVD. As can be seen in Tables 17-23, different studies evaluated different risk factors. None evaluated all the risk factors used by the ATP III or JNC 7 guidelines to determine treatment goals for dyslipidemia or hypertension.[2,13] Notably, history of atherosclerotic CVD, family history of CVD, and chronic kidney disease were rarely evaluated; though, this may be a study applicability issue, since eligibility criteria were based on these factors.

As discussed above (Question 4.1), the LipoPrint® studies predominantly evaluated prevalent disease while the NMR studies mostly evaluated incident disease or progression of CVD. The clinical utility of using LDL subfraction (or other risk factors) as a predictor for prevalent disease is unclear.

Results

Only three studies, two using NMR (Table 23) and one using GE (Table 21), reported on the association between cardiovascular risk factors and incident disease in a multivariable model together with LDL subfraction data.[35,42,45] All reported that other CVD risk factors had stronger associations with incident coronary disease than LDL subfraction.

Eight NMR studies and one GE study reported univariable associations of LDL subfraction together with other CVD risk factors (Tables 21 & 22). [27,35,37,38,40-42,45,94] The studies evaluated different incident CVD outcomes, including coronary disease, acute myocardial infarction (or death), acute coronary syndrome (including angina), stroke, and progression of coronary calcification or coronary minimum lumen diameter. Seven of the nine studies found that one or more measures of smaller LDL subfractions were among the most strongly associated risk factors for incident CVD. Three of these studies found that LDL subfractions were more strongly associated with CVD than other risk factors, while the other four found that other risk factors, including lipoprotein cholesterol, smoking, diabetes, weight, blood pressure or hypertension, and high sensitivity C reactive protein, were similarly associated with incident CVD. One study (Campbell 2007) found that LDL particles were larger among patients who developed an intracranial hemorrhage. The remaining study (Kuller 2002) did not clearly report which risk factors were most strongly associated with acute myocardial infarction.

Six studies evaluated LDL subfraction by GE and two evaluated NMR in multivariable models for prevalent CVD – coronary calcification, existing coronary or carotid atherosclerosis (Tables 19 & 20). The studies did not have consistent findings regarding the relative strength of LDL subfraction and other risk factors and their association with prevalent CVD. Three studies found no association between LDL subfraction and prevalent disease (coronary calcification or atherosclerosis, or carotid disease) (Kullo 2004, Landry 1998, Freeman 1998). Two studies found that LDL subfraction (pattern B) had broadly similar strengths of association with prevalent disease (coronary atherosclerosis) as other risk factors (age and diabetes, or HDL and smoking) (Kwon 2006, Yoon 2005). Three studies found that LDL subfraction (LDL score or small dense LDL) was most strongly associated with prevalent CVD (coronary or carotid atherosclerosis) (Rajman 1996, Inukai 2005, Mora 2007).

Seven GE studies and three NMR studies reported univariable associations of LDL subfraction and other risk factors versus prevalent CVD (coronary atherosclerotic disease, carotid atherosclerosis, and coronary calcification).[34,36,39,43-47,49,50] The studies were again inconsistent. Four of ten studies found that other risk factors were stronger predictors of prevalent disease; including a subgroup analysis of patients without diabetes (Yoon 2005). Three found that LDL subfractions were similarly predictive of CVD as other risk factors (lipoprotein cholesterol, hypertension, diabetes, and overall Framingham risk score); including the other subgroup analysis of patients with diabetes (Yoon 2005). The remaining four found that various measures of smaller LDL subfractions were most strongly associated with prevalent disease.

Summary

Among the four groups of analyses (univariable and multivariable, incident and prevalent CVD), the multivariable analyses of incident disease are the most clinically and methodologically relevant. The clinical utility of LDL subfraction as a predictor of prevalent disease is limited. The methodological value of univariable analyses, particularly among nonrandomized studies, is questionable. Also, since both ATP III and JNC 7 use multivariable approaches to determine thresholds for lipoproteins or blood pressure, multivariable analyses are clinically pertinent. All such analyses found that other risk factors were more strongly associated with CVD than LDL subfractions; though only three of 18 studies evaluated this association. The univariable analyses were inconsistent regarding how strongly (relatively) LDL subfractions were associated with incident disease. Similar to both univariable and multivariable analyses of prevalent disease, about equal numbers of studies found that LDL subfraction was most strongly associated with CVD, was similarly associated as more traditional risk factors, or were less strongly (or not) associated with CVD, regardless of the specific CVD outcome. Overall, the data do not adequately answer the question of how strongly LDL subfraction information is associated with CVD, in relation to other known and putative risk factors.

Table 17. LipoPrint: Prevalent Disease: Univariable

Predictor	Kullo 2004[44]				Kwon 2006[45]		Yoon 2005[50]				Landry 1998[46]		Rajman 1996[49]		Mohan 2005[47]		Inukai 2005[43]	
	OR 95% CI	P	OR 95% CI	P	Diff	P	Diff	P	Diff	P	Diff	P	Diff	P	Diff	P	Diff	P
Outcome:	CAC Women		CAC Men		CAD		CAD & DM		CAD, No DM		Carotid Dz		CAD		CAD		IMT	
N:	470		322		504		79		188		79		68		60		27	
Pattern B					+23.3%	<.001	~-25%	<.05	~-40%	<.05								
LDL size	0.94 0.90-0.99	.008	1.02 0.97-1.08	NS	-3.2	<.001	-8	<.05	-10	<.05								
sdLDL					+6.0%	<.001									+5.6 mg/dL	<.05	+39 mg/dL	<.01
LDL score											+0.30	.04	+0.52	<.001				
LDL-c	1.001 1.00-1.01	.05	0.998 0.99-1.01	NS	+7.1	.02	+10	NS	+9	NS			+24	<.05	+16	NS	+34	<.05
HDL-c [A]	0.98 0.97-0.99	.003	0.99 0.98-1.01	NS	-3.8	<.001	-10	<.05	-20	<.0001			-3	.08	0	NS	+7	NS
Tg	1.8 1.2-2.6	.004	0.9 0.6-1.4	NS	+12.5	NS	-2	NS	+28	<.05	+24	NS	+4	NS	+18	NS	-12	NS
TC	1.005 0.99-1.01	.10	0.997 0.99-1.01	NS	+5.6	NS	-7	NS	-5	<.05	+23	NS	+21	.07	+15	NS	+21	NS
Age [A]	1.15 1.12-1.18	<.001	1.15 1.12-1.18	<.001	+5.7	<.001	+1.7	NS	+1.4	NS	+9.9	<.001	+2	NS	0	NS		
HTN [A]	2.1 1.3-3.5	.003	1.3 0.8-2.4	NS	+16.4%	<.001	+29.8%	<.05	+49%	<.05	+16%	NS						
Smoker [A]	2.4 1.6-3.6	<.001	1.7 1.0-2.7	.03	+12.1%	.005	+8.3%	NS	+13.8%	<.05	+24%	<.005						
CVD [A]																		
FHx CVD [A]																		
DM [A,B]	3.4 1.9-6.0	<.001	2.1 0.8-2.4	.02	+20.8%	<.001					+12%	NS						
FPG [C]															+4	NS	-51	NS
Hb A1c [C]															+0.9	NS	-0.8	NS
CKD [B]																		
Fram Sc					+2.9	<.001												
BMI					-0.7	.04	+0.8	NS	+1.3	<.05	-0.2	NS	+0.9	NS	-0.9	NS	+2.7	<.05
SBP											+16	<.005			+2	NS	-7	NS
DBP											-2	NS			+3	NS	-4	NS
Male							+11.1%	NS	+16.6%	<.05	+39%	<.001			0%	NS		
hsCRP					+2.9	NS												
Strongest Assns:	**Age, Smoking, Diabetes**		**Age**		**Subfraction & Others**		**Subfraction & Others**		**HDL-c**		**Age, Sex**		**Subfraction**		**Subfraction**		**Subfraction**	

[A] Cardiovascular risk factors used in consideration of LDL-c treatment. www.nhlbi.nih.gov/guidelines/cholesterol/atglance.htm
[B] Cardiovascular risk factors used in consideration of hypertension treatment. www.nhlbi.nih.gov/guidelines/hypertension/express.pdf
[C] Cardiovascular risk factors associated with risk factors in footnotes A and B.

Table 18. NMR: Prevalent Disease: Univariable

Predictor	Mora 2007[39]		Barzilai 2003[34]		Freedman 1998[36]	
	Change	P	Diff	P	Correlation	P
Outcome:	IMT		CVD		CAD score	
N:	5538		229		158	
Particle No.	40.2	<.001				
LDL size	-20.9 [D]	<.001 [D]	-6	.001		
sdLDL	31.7 [D]	<.001 [D]	+23.2%	.001		
LDL score					-0.17	<.05
LDL-c	37.4 [D]	<.001 [D]	-16.2	.03	0.26	<.001
HDL-c [A]	-22.4 [D]	<.001 [D]			0.27	<.001
Tg	13.1 [D]	.002 [D]			0.20	<.05
TC					0.25	<.05
Age [A]					0.33	<.001
HTN [A]						
Smoker [A]						
CVD [A]						
FHx CVD [A]						
DM [A,B]						
FPG [C]						
Hb A1c [C]						
CKD [B]						
Fram Sc						
BMI					0.08	NS
SBP						
DBP						
Male						
hsCRP						
Strongest Assns:	**Subfraction Lipoproteins**		**Subfraction**		**Lipoproteins, Age**	

[A] Cardiovascular risk factors used in consideration of LDL-c treatment. www.nhlbi.nih.gov/guidelines/cholesterol/atglance.htm
[B] Cardiovascular risk factors used in consideration of hypertension treatment. www.nhlbi.nih.gov/guidelines/hypertension/express.pdf
[C] Cardiovascular risk factors associated with risk factors in footnotes A and B.
[D] Adjusted for age, sex, race, hypertension, and smoking, but not other risk factors in table.

Table 19. LipoPrint: Prevalent Disease: Multivariable

Predictor	Kullo 2004[44]				Kwon 2006[45]		Yoon 2005[50]		Landry 1998[46]		Rajman 1996[49]		Inukai 2005[43]	
	OR 95% CI	**P**	**OR 95% CI**	**P**	**OR 95% CI**	**Diff**	**OR 95% CI**	**P**	**OR 95% CI**	**P**	**F statistic**	**P**	**OR**	**P**
Outcome:	CAC Women		CAC Men		CAD		CAD		Carotid Dz		CAD		IMT	
N:	470		322		504		267		79		68		27	
Pattern B					2.3 1.5-3.5	<.001	4.4 1.2-16	.03						
LDL size	0.98 0.92-1.04	NS	1.02 0.96-1.08	NS										
sdLDL													1.6	.01
LDL score									2.2 0.9-5.3	NS	22.3	<.001		
LDL-c					2.2 0.8-2.0	NS					4.21	NS	1.5	.04
HDL-c [A]	0.98 0.96-1.00	.04	0.99 0.97-1.01	NS	1.2 0.7-2.0	NS	0.9 0.8-0.97	.01			1.72	NS	0.8	NS
Tg	0.9 0.5-1.5	NS	0.8 0.4-1.6	NS			adjusted	nd		NS	0.33	NS	1.1	NS
TC	1.01 1.01-1.01	.04	1.00 1.00-1.00	NS			adjusted	nd		NS	0.98	NS	1.3	.07
Age [A]	1.14 1.12-1.16	<.001	1.14 1.12-1.16	<.001	3.7 2.1-6.8	<.001	adjusted	nd	1.09 1.03-1.15	<.05	nd	NS		
HTN [A]	1.8 1.1-2.9	.02	1.2 0.7-2.2	NS	1.5 1.0-2.3	.05	adjusted	nd		NS				
Smoker [A]	2.5 1.7-3.8	<.001	1.6 1.0-2.7	.05	1.8 1.2-2.8	.006	4.8 1.1-22	.04	2.1 1.1-4.0	<.05				
CVD [A]														
FHx CVD [A]														
DM [A,B]	2.9 1.7-4.9	<.001	1.9 1.0-3.6	.04	3.3 2.0-5.5	<.001				NS				
FPG [C]													1.1	NS
Hb A1c [C]													1.3	NS
CKD [B]														
Fram Sc														
BMI					0.8 0.5-1.2	NS	adjusted	nd			nd	NS	1.4	.04
SBP										NS			1.2	NS
DBP										NS			1.3	NS
Male							adjusted	nd		NS				
hsCRP							adjusted	nd						
Strongest Assns:	**Age, Smoking, Diabetes**		**Age**		**Subfraction, Age, Diabetes**		**Subfraction, HDL-c, Smoking**		**Age, Smoking**		**Subfraction**		**Subfraction**	

[A] Cardiovascular risk factors used in consideration of LDL-c treatment. www.nhlbi.nih.gov/guidelines/cholesterol/atglance.htm
[B] Cardiovascular risk factors used in consideration of hypertension treatment. www.nhlbi.nih.gov/guidelines/hypertension/express.pdf
[C] Cardiovascular risk factors associated with risk factors in footnotes A and B.

Table 20. NMR: Prevalent Disease: Multivariable

Predictor	Mora 2007[39]		Freedman 1998[36]	
	Change	P	Predicted Change	P
Outcome:	IMT		CAD score	
N:	5538		158	
Particle No./Conc.				
LDL size				
sdLDL	34.8	.001	--	NS
LDL score				
LDL-c	11.8	NS	28	<.05
HDL-c [A]	-17.3	.003	-25	<.05
Tg	-1.6	NS	11	NS
TC				
Age [A]	Adjusted	nd	43	<.05
HTN [A]	Adjusted	nd		
Smoker [A]	Adjusted	nd		
CVD [A]				
FHx CVD [A]				
DM [A,B]				
FPG [C]				
Hb A1c [C]				
CKD [B]				
Fram Sc				
BMI				
SBP				
DBP				
Male	Adjusted	nd		
hsCRP				
Strongest Assns:	**Subfraction**		**Lipoproteins, Age**	

[A] Cardiovascular risk factors used in consideration of LDL-c treatment. www.nhlbi.nih.gov/guidelines/cholesterol/atglance.htm
[B] Cardiovascular risk factors used in consideration of hypertension treatment. www.nhlbi.nih.gov/guidelines/hypertension/express.pdf
[C] Cardiovascular risk factors associated with risk factors in footnotes A and B.

Table 21. LipoPrint: Incident Disease: Univariable and Multivariable

Predictor	Kwon 2006[45]			
	Diff	**P**	**OR (95% CI)**	**P**
Outcome:	ACS, Univariable		ACS, Multivariable	
N:	262			
Pattern B	+7.3%	NS	1.4 (0.8-2.5)	NS
LDL size	-4.5	.01		
sdLDL	+6.5%	.03		
LDL score				
LDL-c	+0.2	NS	1.0 (0.3-3.5)	NS
HDL-c [A]	-0.1	NS	1.0 (0.5-1.9)	NS
Tg	-2.2	NS		
TC	-1.5	NS		
Age [A]	-1.8	NS	1.0 (0.3-2.7)	NS
HTN [A]	-17.6%	.01	0.6 (0.3-1.1)	NS
Smoker [A]	+13.7%	.05	2.1 (1.2-3.9)	.01
CVD [A]				
FHx CVD [A]				
DM [A,B]	-1.9%	NS	0.9 (0.5-1.7)	NS
FPG [C]				
Hb A1c [C]				
CKD [B]				
Fram Sc	-0.4	NS		
BMI	-0.7	NS	0.5 (0.2-0.8)	.02
SBP				
DBP				
Male				
hsCRP	+11.2	.02	1.01 (1.00-1.02)	NS
Strongest Assns:	**Subfraction, HTN**		**Smoking**	

[A] Cardiovascular risk factors used in consideration of LDL-c treatment. www.nhlbi.nih.gov/guidelines/cholesterol/atglance.htm

[B] Cardiovascular risk factors used in consideration of hypertension treatment. www.nhlbi.nih.gov/guidelines/hypertension/express.pdf

[C] Cardiovascular risk factors associated with risk factors in footnotes A and B.

Table 22. NMR: Incident Disease: Univariable (part 1)

Predictor	El Harchaoui 2007[35]		Kuller 2002[37]				Otvos 2006[40]		Mackey 2002[38]		Blake 2002[27]		Campbell 2007[94]	
	Diff	**P**	**Diff**	**P**	**Diff**	**P**	**OR**	**P**	**Diff**	**P**	**Diff**	**P**	**Diff**	**P**
Outcome:	CAD		MI Women		MI Men		MI or Death		Coronary Calcification		CVD event		ICH	
N:	2888		373		310		1061		268		260		148	
Particle No./Conc.	+115	<.0001	+11	nd	+101	nd	1.2 [D]	.006 [D]	+339	.001	+193	<.001	-95	NS
LDL size	-1	.002	-3	nd	0	NS	1.0 [D]	NS [D]	-3.3	.004	-3	.05	+3	.04
sdLDL	+114	<.0001	+7.1	<.05	+3.0	nd	1.1 [D]	NS [D]	+26.2	.001	0	NS		
LDL score														
LDL-c	+8	<.0001	+8	nd	+2	nd	1.1 [D]	NS [D]	+16	.006	+11	.01	+1	NS
HDL-c [A]	-4	<.0001					0.9 [D]	NS [D]	-7.1	.02	-5.9	.004	+5	.05
Tg	+18	<.0001	+19	nd	+15	NS	1.1 [D]	NS [D]	+37.4	.005	+23	.006		
TC	+8	<.0001	+8	nd	0	NS			+16.7	.007			+4	NS
Age [A]													0	NS
HTN [A]											+22%	.001	+15%	NS
Smoker [A]	+7.4%	<.0001											-3%	NS
CVD [A]													-4%	NS
FHx CVD [A]											+12%	.01		
DM [A,B]	+4.5%	<.0001									+7.7%	.02	-3%	NS
FPG [C]									+10.1	.002				
Hb A1c [C]														
CKD [B]														
Fram Sc														
BMI	+1.1	<.0001							+2.1	NS	+1.9	.003	-1.0	NS
SBP	+5	<.0001							+4.6	NS			7	.06
DBP	+2	<.0001											+2	NS
Male													-3%	NS
hsCRP											+.23	<.001	-.07	NS
Strongest Assns:	**Subfraction & Others**		**Unclear**				**Subfraction**		**Subfraction**		**Subfraction, hsCRP**		**Subfraction (-) HDL-c**	

[A] Cardiovascular risk factors used in consideration of LDL-c treatment. www.nhlbi.nih.gov/guidelines/cholesterol/atglance.htm
[B] Cardiovascular risk factors used in consideration of hypertension treatment. www.nhlbi.nih.gov/guidelines/hypertension/express.pdf
[C] Cardiovascular risk factors associated with risk factors in footnotes A and B.
[D] Adjusted for treatment (gemfibrozil vs placebo), age, hypertension, smoking, BMI, and diabetes, but not other risk factors in table.

Table 22. NMR: Incident Disease: Univariable (part 2)

Predictor	**Soedamah-Muthu 2003[42]**		**Rosenson 2002[41]**	
	Diff	**P**	**OR**	**P**
Outcome:	CAD		MLD Progression	
N:	118		111	
Particle No./Conc.	+274	<.001	2.1	NS
LDL size	-4	<.01	0.2	<.05
sdLDL	+0.47	<.001	7.5	<.05
LDL score				
LDL-c	+12	.07	1.4	NS
HDL-c [A]	-10	<.001	1.3	NS
Tg	+38	<.001	1.9	NS
TC	+12	.09		
Age [A]			Adjusted	
HTN [A]	+5%	NS		
Smoker [A]	+16%	<.001		
CVD [A]				
FHx CVD [A]				
DM [A,B]				
FPG [C]				
Hb A1c [C]	+0.2	NS		
CKD [B]	+14%	.01		
Fram Sc				
BMI	-0.5	NS		
SBP	+1.5	NS		
DBP	+2.3	NS		
Male			Adjusted	
hsCRP				
Strongest Assns:	**Subfraction Lipoproteins, Smoking**		**Subfraction**	

Table 23. NMR: Incident Disease: Multivariable

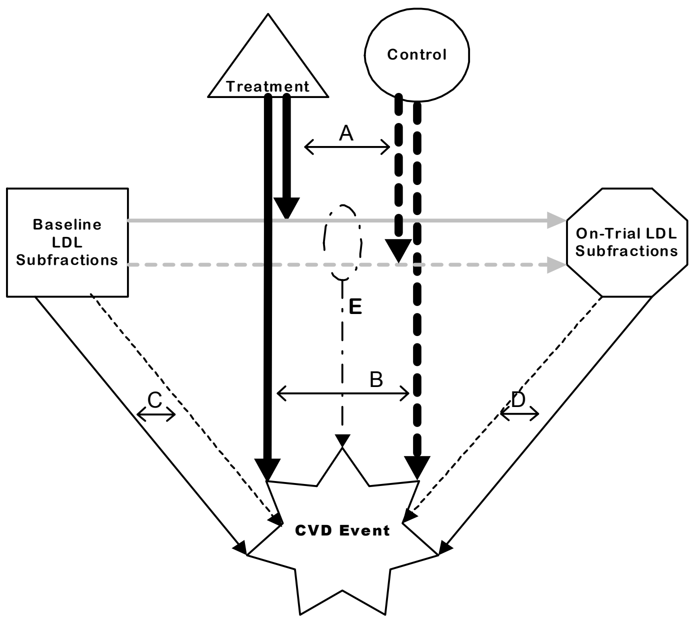
Predictor	El Harchaoui 2007[35]		Soedamah-Muthu 2003[42]	
	OR	**P**	**OR**	**P**
Outcome:	CAD		CAD	
N:	2888		118	
Particle No./Conc.	1.4 1.0-1.9	.02	--	NS
LDL size			--	NS
sdLDL			--	NS
LDL score				
LDL-c	1.6 1.2-2.0	.001		
HDL-c [A]	0.7 0.5-0.9	.001	--	NS
Tg	1.5 1.2-2.0	.001	3.1 [D]	.0004
TC				
Age [A]	Adjusted	nd		
HTN [A]				
Smoker [A]	Adjusted	nd	--	NS
CVD [A]				
FHx CVD [A]				
DM [A,B]				
FPG [C]				
Hb A1c [C]				
CKD [B]			11	.02
Fram Sc				
BMI				
SBP	Adjusted	nd		
DBP				
Male	Adjusted	nd		
hsCRP				
Strongest Assns:	**Lipoproteins**		**Overt nephropathy**	

[A] Cardiovascular risk factors used in consideration of LDL-c treatment. www.nhlbi.nih.gov/guidelines/cholesterol/atglance.htm
[B] Cardiovascular risk factors used in consideration of hypertension treatment. www.nhlbi.nih.gov/guidelines/hypertension/express.pdf
[C] Cardiovascular risk factors associated with risk factors in footnotes A and B.
[D] In one of several models.

Question 4.4
What do studies report regarding the link between therapies to alter LDL subfractions and CVD outcomes?

The aim of this question was to evaluate the link within trials between treatment effects on LDL subfractions and subsequent CVD outcomes. Upon reviewing the studies it became clear that this is a complex question that can be addressed a number of different ways. To structure our analysis (and to help determine which studies provide analyses relevant to this question) we developed an analytic framework of interventions, LDL subfractions, and CVD outcomes (Figure 1). The different possible analyses to address the question (arrows C, D, E) are described in the legend.

Figure 1. Analytic framework for association between interventions, LDL subfractions, and CVD events



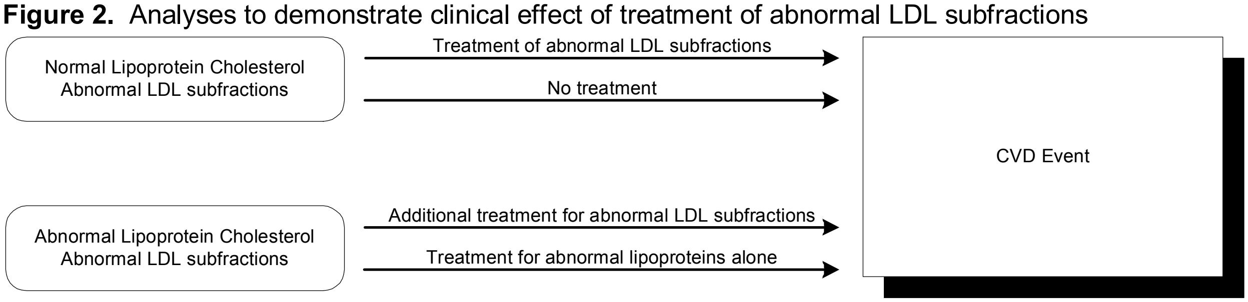
Triangle "Treatment" and circle "Control" represent the interventions in a trial.
Solid lines represent associations related to treatment; dashed lines represent associations related to control.
The interventions (treatment and control) have putative effects on LDL subfractions, displayed as the grey horizontal arrows representing the change from baseline (square) to on-trial (octagon).
The horizontal double-headed arrow A represents the comparison between the effect of treatment and control on change in LDL subfractions.
The interventions also have a putative effect on the clinical outcome incident CVD events (star), as displayed by the longer vertical arrows.
The net treatment effect on incident disease is represented by the horizontal double-headed arrow B.
The diagonal arrows from Baseline LDL Subfractions to CVD Event represent the association addressed in Question 4.1, LDL subfractions as a predictor of CVD outcomes. The difference between the associations of baseline LDL subfractions and CVD events found in the intervention arm and the control arm (arrow C) would provide evidence that treatment alters the strength of the association between the risk factor and outcome.
The other pair of diagonal arrows emanating from On-Trial LDL Subfractions (at arrow D) represent how the associations between LDL subfractions and CVD may be altered after patients have begun treatment.
The other type of analysis found is an association between the change in LDL subfractions and later CVD outcomes (E).

Briefly, associations between baseline LDL subfractions and CVD events could be analyzed separately for treatment and control arms, and then compared. In theory, if patients on treatment have a lessened association between their baseline LDL subfractions and incident CVD than patients in the control arm, then the treatment may be beneficial for those patients at increased risk of CVD based on their LDL subfractions. Associations between LDL subfractions while study subjects are being treated and CVD events can be evaluated. Interpretation of the possible meaning of differences in associations on and off treatment is complex, and may not convincingly demonstrate the connections among treatment for LDL particle size, particle size, and outcomes. Or associations between the change in LDL subfractions while on treatment (or control) and CVD events can be analyzed. In theory, the association could be analyzed separately for treated and control patients. As will be discussed below, however, studies analyzed all patients together; it is implied that changed LDL subfractions were related to treatment. These analyses address whether altering LDL subfractions may be effective at altering CVD risk; however, by lumping treatment and control, it would be unclear whether the changes related to treatment, as opposed to changes correlated to other factors, were what altered CVD event rates.

The necessary analyses (or subanalyses) in clinical trials to determine whether treatment of LDL subfractions may be effective at reducing CVD events are depicted in Figure 2. These approaches follow the reasoning that would be used by clinicians and patients to decide whether active treatment of abnormal LDL subfractions is worthwhile for reducing the risk of CVD events (this simple model does not account for other factors such as adverse events, effects on other diseases, or cost). Patients with abnormal LDL subfractions fall into two broad categories, those with normal lipoprotein (LDL and HDL) cholesterol and triglyceride concentrations and those with abnormal LDL and/or HDL cholesterol and/or triglyceride concentrations. We focus on these cholesterol risk factors, as opposed to others such as blood pressure and diabetes control, because the treatments that have been evaluated in studies addressing this question all are primarily treatments for lipoprotein cholesterol or triglyceride concentrations.

The primary question addressed by these two study designs is whether treating patients specifically for their abnormal LDL subfractions, in the setting of either normal or abnormal lipoprotein cholesterol or triglyceride concentrations, would reduce their risk of CVD.

Figure 2. Analyses to demonstrate clinical effect of treatment of abnormal LDL subfractions



See text for description of figure.

Results

Seven studies performed analyses regarding the associations among putative treatments, LDL subfractions, and CVD outcomes. [40,41,53,56,63,64,95] Two studies used NMR and are also reviewed in detail in Question 4.1.[40,41] Two studies used GE methods that are not clinically available,[53,56]; three used ultracentrifugation.[63,64,95]

All studies were secondary analyses of randomized trials of lipid reduction interventions or (in one trial) a multifactorial cardiovascular risk reduction regimen in patients who had already had a cardiovascular event (secondary prevention). Each trial evaluated a specific group of patients at risk for another cardiovascular event, including groups such as men with normal LDL cholesterol but low HDL cholesterol concentrations (VA-HIT),[40] men with a myocardial infarction before age 45 years, and other groups of patients whose lipoproteins were within constrained ranges. All trials had at least three-quarters men. No trial focused on people over age 65 years. Only one trial had almost half the subjects over age 65 years; [40] two excluded patients over 65 years.[56,95] The percentage of patients with diabetes was not reported in most trials; the percentage of people who smoked (ever or current) ranged widely. Except for VA-HIT (which excluded patients with elevated LDL cholesterol), the mean LDL cholesterol concentrations was generally elevated (for patients with a history of cardiovascular events), ranging from 139 to 194 mg/dL.

Baseline LDL subfractions

Three fair quality trials reported on the potential association between baseline LDL subfractions and CVD outcomes, stratified by treatment (analysis C in Figure 1, Table 24). Rosenson 2002 and Miller 1996 performed secondary analyses of RCTs, one of pravastatin and one of a "risk reduction" protocol. Both evaluated changes in coronary minimum lumen diameter (MLD). Campos 2001 performed a nested case control study of a pravastatin RCT, where the cases were patients with confirmed myocardial infarction or cardiac death. All were secondary prevention trials, where patients had coronary artery disease. All three studies used different definitions of LDL subfractions.

Only Miller 1996 directly compared the associations between LDL subfraction and outcome across treatments. They measured change in MLD in subgroups of patients in multiple categories of LDL subfractions. Comparing patients receiving active risk reduction and those receiving usual care, patients in the risk reduction arm with small dense LDL had significantly smaller changes in their MLD than their counterparts in the usual care arm. Other LDL subfraction subgroups of patients did not have significantly different changes in their MLD based on treatment; though P values were less than 0.10 (favoring risk reduction) for patients in the middle or high tertiles of LDL density.

Both Rosenson 2002 and Campos 2001 found that statistically significant associations between LDL subfractions and CVD outcomes in the placebo arm were smaller and not statistically significant in the pravastatin arms. This may imply that pravastatin mitigated the effect that small LDL subfractions had on the risk of CVD, perhaps by reducing the absolute number of particles.

On-treatment LDL subfractions

Two fair quality secondary prevention trials reported on the potential association between on-treatment LDL subfractions and CVD outcomes, stratified by treatment (analysis D in Figure 1, Table 25). Both Rosenson 2002 and Mack 1996 performed secondary analyses of RCTs, one of pravastatin and one of lovastatin. Both evaluated progression of coronary artery disease. The studies used different methods for measuring LDL subfractions and definitions of the subfractions.

Both studies found that on-treatment (whether with a statin or placebo) LDL size (in angstroms or concentration of small particles) were not associated with progression of CVD. For Rosenson 2002, where baseline small LDL particles were associated with MLD progression in the placebo arm but not the statin arm, in contrast, this analysis of on-treatment associations may call into question the interpretation that pravastatin mitigated the effect that small LDL particles had on the risk of CVD. Mack 1996 does not provide evidence that the effect of lovastatin on progression of coronary stenosis is related to LDL subfractions. Rosenson 2002 did, however, find that 6 month particle concentration in the placebo arm, but not the statin arm, was associated with progression. However, in the absence of data on the baseline association between particle concentration and progression for each intervention, this information is difficult to interpret.

Change in LDL subfractions

Three studies reported on the potential association between the change in LDL subfractions during the trials and incident CVD (analysis E in Figure 1, Table 26). All were of poor quality regarding this analysis. Ruotolo 1998 and Zambon 1999 were subanalyses of RCTs of either bezafibrate or different treatments including lovastatin, colestipol, and niacin. Otvos 2006 was a nested case control study of an RCT of gemfibrozil. Outcomes included nonfatal myocardial infarction or cardiac death, MLD, and percent coronary stenosis. All were secondary prevention trials. Each used a different method for measuring and defining LDL subfractions.

Otvos 2006 and Ruotolo 1998 reported only that changes in LDL subfractions during the trials were not significantly associated with CVD outcomes; however, data were not provided. Zambon 1999 reported a statistically significant negative correlation between change in LDL particle buoyancy and change in percent coronary stenosis for all patients analyzed together. None of the studies reported on associations adjusted for other CVD risk factors. None stratified their analyses based on intervention.

Summary

Only one trial addressed the question of whether treatment (with lipid lowering agents or other cardiovascular risk factor modification) based on LDL subfractions may be associated with improved cardiovascular outcomes. In the SCRIP trial of diet, exercise, counseling, and drugs, the intervention was of greatest value in slowing progression of MLD in those people with small dense LDL However, no study evaluated whether treatment based on LDL subfractions is associated with improvement in true CVD outcomes (ie, either events or clinical severity). The three studies that evaluated treatment-stratified associations between baseline LDL subfractions and CVD weakly suggest that the risk of CVD that is associated with abnormal LDL subfractions may be mitigated by pravastatin or more general risk reduction, but this conclusion is partly offset by the lack of difference (between treatment and control) in associations in analyses of on-trial LDL subfractions. Three studies were inconsistent regarding whether changes in LDL subfractions are associated with improved CVD outcomes and are hampered by their failure to stratify their analyses. The applicability of these trials to the Medicare population is somewhat limited as these are all secondary prevention trials in predominantly young men.

Table 24. Association between baseline LDL subfraction and CVD outcome, stratified by treatment vs control

Author Year Country UI Quality	Test Category [A]	Population [Design]	Intervention (Duration)	N	Mean Age (>65[C]) {% Male} <% DM> [%Smoke [D]]	LDL-c (mg/dL)	Subfraction Data		Outcome			Association	P value
							Definition	Base	Definition	Base	Rate/ Change		
Rosenson 2002[41] US 12106834 B	NMR	CAD LDL-c 130-189 Tg≤350 (PLAC-I) [RCT]	Pravastatin 40 mg/d (3 yr)	130	58 (~20%) {76%} <nd> [nd]	165 [117 [A]]			MLD	1.99	-0.018	Unadjusted Correlation [B]	
							Size, Å	207				0.11	NS
							Large, mg/dL	84				0.04	NS
							Small, mg/dL	43				-0.12	NS
			Placebo	111		161 [164 [A]]	Size, Å	207		2.00	-0.053	0.14	NS
							Large, mg/dL	79				0.05	NS
							Small, mg/dL	40				-0.21	<.01
Miller 1996[63] US 8901665 B	UC	CAD Lumen narrowing 5-69% (SCRIP) [RCT]	Diet, Exercise, Counseling, Drugs [C] (4 yr)	97	57 (~20%) {100%} <nd> [nd]	156			MLD	2.36	nd	△MLD/yr	btw
							σ<1.03007 g/mL	38%				-0.049	NS
							1.03007-1.0355	28%				-0.019	.09
							>1.0355	34%				-0.006	.06
							sdLDL (S_f° 0-5)	42%				-0.008	.007 [D]
							Buoyant (S_f° 5-12)	58%				-0.039	NS [E]
							Pattern B	39%				-0.017	NS
							Pattern I	22%				-0.017	NS
			Usual Care	116			σ<1.03007 g/mL	29%				-0.045	
							1.03007-1.0355	38%				-0.049	
							>1.0355	33%				-0.040	
							sdLDL (S_f° 0-5)	44%				-0.054	
							Buoyant (S_f° 5-12)	58%				-0.038	
							Pattern B	41%				-0.046	
							Pattern I	31%				-0.046	
Campos 2001[53] US 11572739 B	GE	Recent MI TC<240 LDL-c 115-174 Tg<350 (CARE) [Nested case control]	Pravastatin 40 mg (5 yr)	377	60 (~30) {87%} <16%> [17%c]	139			Confirmed MI or CHD death	--	46% (10.2% in RCT)	Adjusted RR [F]	
							Size, Å	256				II: ~1	NS
							I (237-247 Å)	20%				III: ~0.9	NS
												IV: ~1.2	NS
							II (248-254 Å)	20%				V: 1.33	NS
			Placebo	460			III (255-259 Å)	20%		--	53% (13.2% in RCT)	II: 2.08	.03
							IV (260-262 Å)	20%				III: 2.42	.01
							V (263-277 Å)	20%				IV: 2.72	.008
												V: 4.00	.001

[A] 6 months.
[B] Spearman (rank) correlation, adjusted for baseline MLD, race, sex, and age. An inverse association indicates that high baseline levels are associated with a reduction in lumen diameter (adverse outcome).

[C] Goal: LDL<110 mg/dL, triglycerides<100 mg/dL, HDL>55 mg/dL.
[D] For change in mean diameter and % stenosis, there was no significant difference between interventions for patients with small dense LDL (P=.09 and .08, respectively).
[E] For change in mean diameter and % stenosis, there was no significant difference between interventions for patients with buoyant LDL (P=.93 and .67, respectively).
[F] Relative risk of outcome compared to Quintile I.

Table 25. Association between on-treatment LDL subfraction and CVD outcome, stratified by treatment vs control

Author Year Country UI Quality	Test Category [A]	Population [Design]	Intervention (Duration)	N	Mean Age (>65[C]) {% Male} <% DM> [%Smoke [D]]	LDL-c (mg/dL)	Subfraction Data		Outcome			Association	P value
							Definition	On Trial	Definition	Base	Rate/ Change		
Rosenson 2002[41] US 12106834 B	NMR	CAD LDL-c 130-189 Tg≤350 (PLAC-I) [RCT]			58 (~20%) {76%} <nd> [nd]		6 mo data		MLD			Adjusted Correlation [B]	
			Pravastatin 40 mg/d (3 yr)	130		165 [117 [A]]	Particle concentration, nmol/L	1858		1.99	-0.018	-0.10	NS
							Size, Å	208				0.14	NS
							Large, mg/dL	69				0.03	NS
							Small, mg/dL	30				-0.14	NS
			Placebo	111		161 [164 [A]]	Particle concentration, nmol/L	1918		2.00	-0.053	-0.24	<.05
							Size, Å	207				0.10	NS
							Large, mg/dL	79				0.01	NS
							Small, mg/dL	43				-0.18	NS
Mack 1996[64] US 8963728 B	UC	CAD TC 190-295 37-67 yo (MARS) [RCT]			58 (~15%) {92%} <nd> [79%e]	156	IV (S_f 0-3), mg/dL	14.7	Coronary stenosis (progression)	37%	Progression in 51% of lesions	Unadjusted OR (per 10 mg/dL)	
			Lovastatin 80 mg (2 yr)	114								1.6	NS
			Placebo	106								1.6	NS

[A] 6 months.

[B] Spearman (rank) correlation, adjusted for baseline MLD, race, sex, and age; and triglycerides, LDL and HDL cholesterol. An inverse association indicates that high baseline levels are associated with a reduction in lumen diameter (adverse outcome).

Table 26. Association between change in LDL subfraction, on intervention (control), and CVD outcome

Author Year Country UI Quality	Test Category[A]	Population [Design]	Intervention (Duration)	N	Mean Age (>65[C]) {% Male} <% DM> [%Smoke[D]]	LDL-c (mg/dL), Base [on trial]	Subfraction Data: Definition	Subfraction Data: Base	Subfraction Data: Change	Outcome: Definition	Outcome: Base	Outcome: Rate/ Change	Association		P value
Otvos 2006[40] US 16534013 C	NMR	CAD; LDL-c≤140 Tg;≤300 HDL-c≤40 (VA-HIT) [Nested case control]	Gemfibrozil 1200 mg/d (5.1 yr, median)	515	64 yr (~45%) {100%} <30%> [20%c]	112 [115[B]]	Size, Å	204	+5	Nonfatal MI or CHD death	--	17%[C]	Unadj:	NR[D]	NS
							Large, nmol/L	354	+126						
							Small, nmol/L	967	-190						
			Placebo	546		112 [112[B]]	Size, nm	204	-1		--	22%[C]	Adj:	not analyzed	
							Large, nmol/L	346	-1						
							Small, nmol/L	984	+99						
Ruotolo 1998[56] Sweden 9822092 C	GE	MI <45 yo; TC≥200 Tg≥140 Coronary stenosis (BECAIT) [RCT]	Bezafibrate 600 mg/d (5 yr)	47	42 yr (0%) {100%} <nd> [24%c]	180 [159]	Size, Å	230	+3.2	MLD	1.82	-0.06	Unadj:	NR[D]	NS
							% Small	41.4	-9.7		1.91	-0.17	Adj:	not analyzed	
			Placebo	45		179 [171]	Size, Å	231	+0.2	% Stenosis	36.5	+1.70	Unadj:	NR[E]	NS
							% Small	35.9	-0.3		35.2	+4.25	Adj:	not analyzed	
Zambon 1999[95] US 10208998 C	UC	CAD Family Hx ≤62 yo Apo B≥125 (FATS) [RCT]	Lovastatin 40 mg/d & Colestipol 30 g/d (2.5 yr)	31	47 yr (0%) {100%} <nd> [24%c]	194 [102]	Buoyancy (Rf)	0.261	+0.020	% Stenosis	36%	-1.25%	Unadj:	r=-0.61	<.001
			Niacin 4 g/d & Colestipol 30 g/d	26		191 [131]		0.252	+0.026		36%	-0.7%	Adj:	not analyzed	
			Colestipol if LDL-c≥90th percentile	13		177 [160]		0.267	-0.018		30%	+1.87%			
			Placebo if LDL-c<90th percentile	18				0.250	-0.008						

A CITP; GE; HPLC; NMR; UC; Other
B 7 months
C Within complete trial (219/1264 on gemfibrozil; 275/1267 on placebo). Data within nested case control study not reported.
D Not reported. "Additional analyses (results not shown) indicated that no change (by concentration or percentage) in any of the … lipoprotein particle variables was a significant predictor of CHD risk."
E Not reported. "Percentage change in … lipoprotein … concentrations from baseline to mean on-trial levels did not correlate significantly with any of the angiographic outcome variables (… with control for treatment assignment, baseline angiographic score, age, [body mass index], smoking and alcohol consumption)."

Chapter 4. Discussion

Measurement of LDL subfractions is an increasingly studied tool for cardiovascular risk status. Although the clinical value of the tool relative to other known cardiovascular risk factors has yet to be ascertained, it is available as part of the panel of risk factors being tracked by clinicians and patients. While the ATP III guidelines do not recommend measurement of small LDL particles in routine practice, they do provide guidance on how to consider altering treatment based on elevated levels (www.nhlbi.nih.gov/guidelines/cholesterol/atp3full.pdf, accessed Feb 19, 2008; page II-21-2).

The large majority of research on LDL subfractions as a potential cardiovascular risk factor has been performed with measurement methods that are either expensive, time-consuming, or resource-intensive. In addition, the methods do not use FDA cleared medical devices. Relatively few studies have been performed using tests that are available for clinical use. As described in the results sections for Questions 1, 2 and 3, there is not yet a standard method of subfraction measurement that can be used as a reference standard, has been demonstrated to be superior to other methods, or has been demonstrated to be accurate and reliable. Each of the three major methods for measuring LDL subfractions – GE, NMR, and ultracentrifugation – describes and measures the subfractions differently. Even within a specific general type of measurement tool (eg, GE) or even within a specific test (LipoPrint® GE or NMR, all performed by LipoScience®) there is not standardization for defining or describing the LDL subfractions. A variety of outcomes are used including size (which correlate but do not agree among methods), LDL subfraction concentrations or proportions, and different patterns, among others. In addition, different researchers use different thresholds to differentiate a wide range of different numbers of LDL subfractions.

LDL subfraction methodology

The studies comparing different methods of measuring LDL subfractions are incomplete in terms of adequately comparing each of the methods. In part, this is due to the research goals of the study authors. Only a single study (Ensign 2006[17]) compared all major test methods (NMR, LipoPrint® GE, other GE, and ultracentrifugation). It was common that studies performed their analyses for the purpose of establishing that a given (often unique or new) method of measuring LDL subfractions provides similar results to other methods. Overall, the studies support fair to good correlation among the different methods; however, some studies found only low levels of agreement between LipoPrint® GE compared to other GE to classify LDL subfractions and ultracentrifugation compared to GE (LipoPrint® or other). One study found that NMR measurement of LDL sizes are on average about 54 Å smaller than measurements based on GE, with wide limits of agreement.[31] This is consistent with a widely quoted review paper in which the authors stated the fact that NMR LDL particle sizes are referenced to diameters measured by electron microscopy, which are consistently smaller by approximately 50 to 60 Å than those estimated by the gradient gel electrophoresis referencing method.[19]

It is important to note, though, that comparisons of methods based on agreement in size or phenotypes are necessary, but not sufficient, to evaluate whether the different methods are measuring the same LDL subfraction analytes. Since different combinations of physicochemical properties are used to separate lipoproteins with different methods (eg, density, size, electrophoretic mobility) the correlation between methods will inevitably be imperfect.

Development of reference materials are necessary to allow for descriptions of the similarities and differences of the various measurements produced by the different methods. A reference method needs to be widely accepted as appropriate, accurate and reliable. However, even with a consensus reference method, it may not be possible to standardize or harmonize all of the methods because their measurement principles are so different. Possible approaches to reference measurements would include developing reference materials that are at a minimum are characterized and defined by composition, density and size.

No study reported day-to-day variability in individuals. Four studies reported on intra- and interassay variability (measures of the same serum sample). In three studies, variability was small, up to 0.2 percent for intraassay variability and up to 1.4 percent for interassay variability; though one study found a interassay variability of 13 percent among 19 samples assayed over a week. A possible reason for the larger variability of the interassay test (where the same sample is being run on different days after storage at –70° C) is that storage of the samples may have altered their characteristics. However, given that only a small subset of studies evaluated variability, it is difficult to assessed their generalizability to other studies.

A major limitation of the studies comparing methodologies and assessing the tests' variability is the small number of studies; thus the accuracy of their findings is hard to assess. In addition, many of the studies evaluated only small numbers of patients (or serum samples) and they frequently did not adequately describe the subjects who donated samples. Furthermore, regarding the variability, the studies tested variability as a secondary analysis, with the purpose of demonstrating the accuracy of the test that is being studied for a different purpose. Therefore, the reporting of the analyses tended to be brief and incomplete.

Association between LDL subfractions and CVD

A large number of studies have evaluated the putative association between LDL subfractions and CVD. However, relatively few of these have been performed with either of the clinically available methods of measuring LDL subfractions. In addition, overall, most studies have compared LDL subfractions to prevalent disease. Together, these issues limit the applicability of the studies to address the question of whether there is clinical value of measuring LDL subfractions for helping clinicians and patients to assess both cardiovascular risk and potential need for treatment. The studies were clinically heterogeneous in terms of age – where generally the large majority of patients were under age 65 years – sex ratio, smoking status, comorbidities and other past medical history. Overall, the applicability of these studies to the Medicare population may be somewhat limited, particularly if age, comorbidities, or other factors alter any associations between LDL subfraction and CVD.

None of the studies of LipoPrint® GE, only six studies of NMR, and only one study of gradient GE performed at HeartLab® evaluated incident CVD or progression of CVD. These evaluated a wide range of CVD outcomes including CAD death, new CAD diagnosis, MI, stroke, change in minimum lumen diameter of the coronary arteries, and concurrent rate of coronary artery stenosis. They also evaluated a wide range of LDL subfraction measures including two or three subfractions with size thresholds at 180 Å or 183 Å for the lower limit, 197 Å and 212 Å or 213 Å for between-subfraction thresholds, and 227 Å or 230 Å for the upper size limit; LDL particle concentration, and LDL particle size. The studies of incident CVD using NMR to measure LDL subfractions used considerably more uniform specific methods of measuring subfraction than across the other studies (primarily GE). These studies generally found that LDL particle concentration and particle numbers (NMR-specific measurements) are associated with incident CVD, but LDL particle size and small LDL particle fraction were not as consistently associated with incident disease. Among four out of the five studies, LDL particle concentration remained significantly associated with CVD events after adjustment for LDL or other traditional cardiovascular risk factors. An important caveat, though, is that each study used different methods (where reported) for choosing which other risk factors to adjust for and thus adjusted for different risk factors. Where reported, LDL size or small LDL were not significantly associated with incident disease after adjustment. The one Berkeley HeartLab® gradient GE study found an association between the smallest LDL subfraction (IVb, 220-233 Å) and progression of coronary artery stenosis; however, this study is difficult to interpret clinically since the investigators used an average of baseline and year 4 lipid levels instead of baseline data alone as a predictor. Notably, though, the association was stronger for artery segments with less than 30 percent stenosis at baseline.

Among the studies that evaluated the clinically available methods of measuring LDL subfraction and prevalent CVD (including all the studies of LipoPrint® GE) findings were mixed with about half finding a statistically significant association after adjustment for LDL and/or other risk factors but half not. These studies were likewise varied in their specific measures of LDL subfractions and in which prevalent CVD were evaluated.

These findings held for the analyses of all the different methods for measuring LDL subfractions. Only LDL particle concentration, as measured by NMR, was consistently found to be associated with incident CVD after adjustment for lipids (and other risk factors) in four studies. A wide range of other specific measures of LDL subfraction (primarily by GE or ultracentrifugation) have been found to be associated with incidence or progression of CVD by only a minority of studies (6 of 20 studies). Among the 6 "positive" studies that found associations between LDL subfraction measures (other than particle concentration), no consistent measure or outcome differentiated these from the remaining 14 "negative" studies.

LDL subfraction data, most commonly from LipoPrint® or other GE, was more commonly associated with prevalent CVD, though the studies were very heterogeneous in their measurements and outcomes. Overall, though, about two-thirds of studies found some statistically significant association between LDL subfraction measures (usually pattern) and prevalent CVD. The clinical utility of this association, however, is unclear. These studies fail to address whether the abnormal LDL subfraction profile is related to the development of CVD or whether it is a response to the presence of CVD. Furthermore, its only potential clinical value would be as a treatable risk factor for incident or progressing CVD.

The question of the relative or incremental value of LDL subfraction measurement as a predictor for CVD compared to traditional risk factors (such as lipoprotein cholesterol, blood pressure, demographics, smoking, and comorbidities) was not a specific question of interest of any of the evaluated studies. No study evaluated any cardiovascular risk assessment technologies and measured the incremental increase in diagnostic performance. At best, studies reported sufficient details from baseline (unadjusted) or adjusted models for cardiovascular outcomes that relative strengths of associations could be gleaned. Only three studies, two using NMR and one using GE, came closest to directly addressing the question of relative value by reporting on the association between cardiovascular risk factors and incident disease in a multivariable model together with LDL subfraction data. All found that other CVD risk factors had stronger associations with incident coronary disease than LDL subfraction. Among the remaining less clinically relevant analyses (univariable analyses or prevalent CVD), about equal numbers of studies found that LDL subfraction was most strongly associated with CVD, was similarly associated as more traditional risk factors, or were less strongly (or not) associated with CVD, regardless of the specific CVD outcome. Overall, the data do not adequately answer the question of how strongly LDL subfraction information is associated with CVD, in relation to other known and putative risk factors. In summary, none of the LDL subfraction measurements have definitively been demonstrated to add to the ability to discriminate between individuals who are at higher versus lower risks of cardiovascular events compared to commonly used predictors, such as LDL and HDL cholesterol.

Close to 300 articles provide some data on the effect of various treatments or regimens on LDL subfraction profiles (this number, though, is likely to be a high estimate, as these articles were not thoroughly screened). Only seven of these studies also reported CVD outcomes. All were secondary analyses of randomized trials of lipid reduction interventions or (in one trial) a multifactorial cardiovascular risk reduction regimen. All were aimed at secondary prevention in specific groups of patients at increased risk of second CVD events or with abnormal lipoprotein cholesterol patterns. Based on demographics – particularly age – and comorbidities, these trials are all of relatively limited applicability to the Medicare population. Furthermore, as discussed in depth in the preface and results for Question 4.4, none of the analyses directly addressed the question of whether treatment based on LDL subfractions is associated with reduction in cardiovascular events or improvement in clinical severity. A single study, though, found that patients with small dense LDL had reduced progression of carotid MLD on intensive therapy compared to usual care, in contrast with those with more buoyant LDL Three of the studies may suggest that the risk of CVD that is associated with abnormal LDL subfractions may be mitigated by pravastatin or more general risk reduction, but this finding is inconclusive at best. The studies were inconsistent regarding whether changes in LDL subfractions are associated with improved CVD outcomes; these analyses were also hampered by their failure to stratify their analyses based on intervention, so that it is unclear whether the treatments played a role in risk reduction.

Limitations

There were several limitations to the review process beyond the limitations of the evidence itself. As described, the principle portions of this review focus on two methods available for clinical use for measuring LDL subfractions. This approach was agreed upon with CMS and AHRQ, though it may provide undue emphasis on two commercial entities, while minimizing potentially unique features of tests run with other tests. It is unclear how the financial interests of these two companies may have impacted on the studies that have been performed or published using these methods. Ideally, to reduce bias, or at a minimum the perception of bias, it would be preferable to have truly independent studies of these methods. To the best of our understanding from the literature and documents available on the internet, the LipoPrint® GE kit is clinically available and can be used by any research laboratory. Notably, the studies that used this kit all evaluated prevalent CVD, used a variety of different definitions for LDL subfractions, and found a mix of statistically significant and nonsignificant associations. In contrast, again to the best of our understanding, all of the studies that used NMR sent their samples to LipoScience® for processing. It is not clear whether bias due to availability of this test may have been introduced. This approach had the advantage of having more consistent definitions for LDL subfractions, but notably, this test provided the only subfraction measurement that consistently was found to be significantly associated with incident CVD. Across studies, though, we were unable to adequately judge the nuances of the different methods based on the reported (or unreported) technical details. We therefore could not evaluate how these differences may have impacted the differences of results across the studies.

However, except for the issue of potential publication bias, these issues may be of relatively minor importance compared to the large degree of heterogeneity in test methods and measures, populations evaluated, and outcomes assessed. From the perspective of assessing whether these tests may be of value for CVD risk assessment in the general population, the details of the tests and the changes (potentially advances) in the techniques are of lesser interest than a determination of whether any of the test measures could be useful predictors of CVD risk and potential treatment targets.

Future research

Given the large number of studies that have evaluated LDL subfractions, it is unfortunate that it remains true that future research is needed to address the questions posed for this systematic review. In part, this conclusion is based on the difficulties encountered in summarizing the evidence due to the large heterogeneity of the methods used to measure LDL subfractions and on the relative paucity of studies of clinically relevant CVD incidence or progression. Of the clinically available methods, only NMR-based particle concentration has consistently been found to be associated with incident CVD, but only in four disparate studies. The lack of consistent associations among other measures is partly due to lack of data (ie, LipoPrint® GE as a predictor of incident disease) or the great heterogeneity of measurement methods (among other studies). Even among the NMR studies and the LipoPrint® GE studies (of prevalent disease) researchers do not consistently use the same types or definitions of measures. In part this may be due to each research laboratory attempting to determine what is the "best" measure (or threshold) for predicting an outcome, but this has the effect that achieving consensus across studies is difficult, if not impossible.

With the exception of research into bringing a new LDL subfraction measurement technique (or kit) to market, currently there is little clinical value to testing a methodology that is not available for clinical use or that can be performed only in the setting of a research study. There are many such studies that find (or fail to find) an association with a specific CVD outcome. The addition of more studies of measurement techniques that are not clinically available will not assist clinicians, patients, payers, or regulators to determine whether measurement of LDL subfractions is worthwhile. Likewise, further research into whether LDL subfractions are associated with prevalent disease is of limited clinical value. There are adequate data that such an association may exist (despite the majority of studies finding no such association) and future research should focus only on CVD incidence (both primary or secondary prevention) and progression. Since LDL subfractions, like other risk factors, would not be used to diagnose a patient with CVD, it is unlikely that small dense LDL subfractions would be sufficiently predictive of prevalent disease to instigate an investigation for CVD in the absence of signs of symptoms of disease.

Current research has adequately found a potential association between LDL subfractions and CVD (both heterogeneously defined), but this is insufficient for clinical use. Thus future research regarding the putative association of LDL subfractions and CVD should focus on uniformly (and universally) defined measures of subfractions using available tests for CVD incidence or progression. From a clinical perspective (as opposed to a laboratory perspective), it is more important that a given laboratory measurement that is common and standardized across laboratories is a good predictor of the clinical outcome of interest. It is less important whether that laboratory measurement is correlated to other available (or unavailable) measurements. We are not in a position to provide specific recommendations for how the best measurement is chosen, how it is standardized, or how it is characterized, except to suggest that the clinical utility (its strength as a predictor of CVD) is most important. An update of this systematic review should likewise focus on these clinically relevant questions.

Under the assumption that LDL subfractions are associated with progression or incidence of CVD, research is needed into its relative and incremental value as a risk factor. Various risk factors, including the Framingham score, are currently used to assess people's future risk of CVD. Several of these factors, such as LDL cholesterol, blood pressure, obesity, and smoking are amenable to intervention. The available data provide little insight into whether the addition of LDL subfraction information would affect intervention decisions (whether they have incremental value) or would ultimately result in better outcomes. The few secondary analyses of randomized trials of CVD risk factor interventions do not address whether treatment based on, or specific to, LDL subfractions would result in improved clinical outcomes compared to current standard practice. An additional aspect that may be necessary to clarify whether LDL subfraction measurement might be clinically useful is research into whether putative associations with incidence or severity of CVD exist in specific groups of individuals (such as those with abnormal glucose tolerance, kidney disease, or normal LDL cholesterol concentrations).

There are relatively few studies comparing different methods for measuring LDL subfractions. From a clinical perspective, it would be helpful to have some additional studies that directly compare the two clinically available methods. The primary purpose of these studies should be to assess whether classification or other standard measure of LDL subfractions are comparable when measured with either test. In particular, the finding in Ensign 2006[17] of an approximately 50 Å difference in size measurement between NMR and GE needs to be confirmed (or refuted) before definitive size thresholds are established. If the differential is confirmed, it may be necessary to adjust the (calculated) size of the NMR measurements to conform more to GE measurements. This study also provided some evidence that the size differential may not be consistent across populations. This requires further investigation. However, from a clinical perspective, the important question remains which (if any) of the methods is the most useful in predicting CVD. From this perspective, the question of the agreement of methods regarding specific test metrics may be less important, except to help standardize the tests.

The remaining area covered by this review that requires further research is on the within-subject variability of LDL subfractions. The potential variability may be due either to day-to-day changes within individuals or to laboratory variability. If there is a large degree of variability within individuals then different approaches may be necessary to testing patients and measuring the associations with CVD. Batched or repeated measures may be necessary. If the within-subject variability is large this might in part explain the lack of consistency across studies as to the strength of associations with CVD. Several studies, in different populations, that are designed specifically to address this question are needed. Additional studies evaluating the within-sample (intraassay and interassay) variability would also be helpful to confirm the findings of a small number of studies in relatively few subjects. Improved reporting of the subject characteristics are needed to properly evaluate the studies.

Summary

In summary, despite a large number of studies evaluating the association of LDL subfractions and CVD (that have led to a very large number of studies of potential interventions to alter subfraction patterns), the clinically useful evidence regarding whether measurement of LDL subfractions may be a helpful tool for assessing cardiovascular risk (or altering treatment of cardiovascular risks) is lacking. This is largely due to the relative paucity of studies that have evaluated clinically available tests and their associations with incidence or progression of CVD.

Only one measure (LDL particle concentration measured by NMR) was consistently significantly associated with CVD events, after adjustment for lipoproteins and other cardiovascular risk factors. The strength of the associations varied, with RR or OR ranging from 1.11 to 2.90. Other NMR measures were not consistently associated with incident or progressive disease. LipoPrint® GE, the other clinically available test, has not been tested as a predictor for incident or progressive CVD. Studies of the remaining (not clinically available) tests are inconsistent, but mostly find no association with incident CVD (before or after adjustment for lipoproteins and other risk factors). More well-conducted research is needed. Future studies should focus on the clinically available tests and incidence or progression of CVD, and should aim to use standard test metrics and classifications to allow for comparison across studies. The current evidence suggests that LDL subfractions is not a consistently strong predictor of CVD compared to other known risk factors, but this question has not been properly evaluated by any study.

The small number of trials of cardiovascular interventions that have been secondarily analyzed to evaluate LDL subfractions suggest a possible role for the subfractions in predicting outcomes with treatment, but fail to address the clinical question of whether treating patients based on LDL subfractions would reduce their risk of CVD.

The small number of studies that directly compared different tests generally found fair to good agreement, though not all studies consistently agreed. These studies need to be reproduced to assess their validity. This is particularly true for the one study that found a difference in size measurements between NMR and GE since this is frequently cited among other studies. Within-subject and within-sample variability have not been adequately evaluated to definitely determine the tests' accuracy. It is possible that the day-to-day variability found by one study may partly account for the heterogeneity of results regarding the value of the test as a predictor of CVD.

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Appendix A. Search Strategy

LDL Lipoprotein Subfractions **Aug 22, 2007**

Ovid MEDLINE(R) (mesz), CAB Abs (caba), CCTR, CDSR (coch)

#	Search History	Results
1	(ldl or ldl-c).mp. or exp Cholesterol, LDL/ or exp Lipoproteins, LDL/	65262
2	ldl cholesterol.mp.	18455
3	or/1-2	65262
4	particle size.mp. or exp Particle Size/	43827
5	(subfraction$ or subclass$).mp.	27004
6	particle density.mp.	982
7	exp Nuclear Magnetic Resonance, Biomolecular/ or exp Magnetic Resonance Spectroscopy/	132032
8	(nuclear magnetic resonance or nmr or magnetic resonance spectroscopy).mp.	145366
9	exp Chromatography, High Pressure Liquid/ or exp Chromatography/	484708
10	(chromatography or hplc or fplc).mp.	570172
11	ultracentrifugation.mp. or exp Ultracentrifugation/	62272
12	centrifugation.mp. or exp Centrifugation/	95595
13	exp Electrophoresis/ or electrophoresis.mp.	428986
14	or/4-13	1183751
15	3 and 14	9676
16	limit 15 to (humans and english language) [Limit not valid in: CAB Abs,CCTR,CDSR; records were retained]	7591
17	remove duplicates from 16	**6369**
	Ovid MEDLINE(R) <1950 to June Week 4 2007>	(5997)
	CAB Abstracts <1973 to May 2007>	(321)
	EBM Reviews - Cochrane Central Register of Controlled Trials <3rd Quarter 2007>	(47)
	EBM Reviews - Cochrane Database of Systematic Reviews <3rd Quarter 2007>	(4)

Appendix B. Rejected Articles

Adler L, Hill JS, Frohlich J. Chemical precipitation of apolipoprotein B-containing lipoproteins facilitates determination of LDL particle size. Clinical Biochemistry. 2000. UI 10913516
comparison of different techniques of the same method

Akanji AO, Suresh CG, Fatania HR, et al. Associations of apolipoprotein E polymorphism with low-density lipoprotein size and subfraction profiles in Arab patients with coronary heart disease. Metabolism: Clinical & Experimental. 2007. UI 17379005
LDL immediately after CAD event

Alabakovska SB, Todorova BB, Labudovic DD, Tosheska KN. Gradient gel electrophoretic separation of LDL and HDL subclasses on BioRad Mini Protean II and size phenotyping in healthy Macedonians. Clinica Chimica Acta. 2002. UI 11814466
No relevant info

Ala-Korpela M, Lankinen N, Salminen A, et al. The inherent accuracy of 1H NMR spectroscopy to quantify plasma lipoproteins is subclass dependent. Atherosclerosis. 2007. UI 16730730
Not comparison of 2 methods

Alvarez JJ, Lasuncion MA, Olmos JM, Herrera E. Interindividual variation in the partition of lipoprotein(a) into lipoprotein subfractions. Clinical Biochemistry. 1993. UI 8299210
Not LDL subfractions

Austin MA, Hokanson JE, Edwards KL. Hypertriglyceridemia as a cardiovascular risk factor. American Journal of Cardiology. 1998. UI 9526807
No relevant info

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: Clinical & Experimental. 1982. UI 6952064
No relevant info

Bathen TF, Engan T, Krane J. Principal component analysis of proton nuclear magnetic resonance spectra of lipoprotein fractions from patients with coronary heart disease and healthy subjects. Scandinavian Journal of Clinical & Laboratory Investigation. 1999. UI 10533847
LDL immediately after CAD event

Baumstark MW, Kreutz W, Berg A, et al. Structure of human low-density lipoprotein subfractions, determined by X-ray small-angle scattering. Biochimica et Biophysica Acta. 1990. UI 2294970
No relevant info

Berneis K, Jeanneret C, Muser J, et al. Low-density lipoprotein size and subclasses are markers of clinically apparent and non-apparent atherosclerosis in type 2 diabetes. Metabolism: Clinical & Experimental. 2005. UI 15690318
N<10 per arm

Berneis K, La BM, Blanche PJ, Krauss RM. Analysis and quantitation of biotinylated apoB-containing lipoproteins with streptavidin-Cy3. Journal of Lipid Research. 2002. UI 12091501
Not LDL subfractions

Bickerstaffe R, Desmond FB,. Lipoprotein classification by analytical ultracentrifugation. Pathology. 1982. UI 7099724
No relevant info

Bittolo-Bon G, Cazzolato G,. Analytical capillary isotachophoresis of total plasma lipoproteins: a new tool to identify atherogenic low density lipoproteins. Journal of Lipid Research. 1999. UI 9869664
No relevant info

Bokemark L, Wikstrand J, Attvall S, et al. Insulin resistance and intima-media thickness in the carotid and femoral arteries of clinically healthy 58-year-old men. The Atherosclerosis and Insulin Resistance Study (AIR). Journal of Internal Medicine. 2001. UI 11168785
Same dataset as other study

Bozoky Z, Fulop L, Kohidai L. A short-run new analytical ultracentrifugal micromethod for determining low-density lipoprotein sub-fractions using Schlieren refractometry. European Biophysics Journal. 2001. UI 11288837
Not LDL subfractions

Braun LT, Rosenson RS,. Asessing coronary heart disease risk and managing lipids. Nurse Practitioner. 2001. UI 11809040
No relevant info

Brook RD, Bard RL, Rubenfire M, et al. Usefulness of visceral obesity (waist/hip ratio) in predicting vascular endothelial function in healthy overweight adults. American Journal of Cardiology. 2001. UI 11728354
No clinical CVD outcome

Brook RD, Kansal M, Bard RL, et al. Usefulness of low-density lipoprotein particle size measurement in cardiovascular disease prevention. Clinical Cardiology. 2005. UI 16450798
No clinical CVD outcome

Busbee DL, Payne DM, Jasheway DW, et al. Separation and detection of lipoproteins in human serum by use of size-exclusion liquid chromatography: a preliminary report. Clinical Chemistry. 1981. UI 6171365
Not LDL subfractions

Camejo G, Rosengren B, Olsson U, Bondjers G. Agarose isoelectric focusing of plasma low and very low density lipoproteins using the PhastSystem. Analytical Biochemistry. 1989. UI 2604050
No relevant info

Campbell DJ, Neal BC, Chalmers JP, et al. Low-density lipoprotein particles and risk of intracerebral haemorrhage in subjects with cerebrovascular disease. European Journal of Cardiovascular Prevention & Rehabilitation. 2007. UI 17568241
LDL immediately after CAD event

Cazzolato G, Avogaro P, Bittolo-Bon G. Characterization of a more electronegatively charged LDL subfraction by ion exchange HPLC. Free Radical Biology & Medicine. 1991. UI 1937142
No relevant info

Ceriotti L, Shibata T, Folmer B, et al. Low-density lipoprotein analysis in microchip capillary electrophoresis systems. Electrophoresis. 2002. UI 12412132
Not LDL subfractions

Felmeden DC, Spencer CG, Blann AD, et al. Low-density lipoprotein subfractions and cardiovascular risk in hypertension: relationship to endothelial dysfunction and effects of treatment. Hypertension. 2003. UI 12623954
No clinical CVD outcome

Fonda M, Semolic AM, Soranzo MR, Cattin L. Production of polyacrylamide gradient gel for lipoprotein electrophoretic separation. Clinica Chimica Acta. 2003. UI 14637269
No relevant info

Foucar E. Diagnostic certainty is sometimes certainly an error. American Journal of Clinical Pathology. 2003. UI 12645348
Letter

Garvey WT, Kwon S, Zheng D, et al. Effects of insulin resistance and type 2 diabetes on lipoprotein subclass particle size and concentration determined by nuclear magnetic resonance. Diabetes. 2003. UI 12540621
No relevant info

Griffin BA, Caslake MJ, Yip B, et al. Rapid isolation of low density lipoprotein (LDL) subfractions from plasma by density gradient ultracentrifugation. Atherosclerosis. 1990. UI 2390137
N<10 per arm

Gylling H, Miettinen TA,. Cholesterol absorption and lipoprotein metabolism in type II diabetes mellitus with and without coronary artery disease. Atherosclerosis. 1996. UI 8902158
N<10 per arm

Hirano T, Ito Y, Saegusa H, Yoshino G. A novel and simple method for quantification of small, dense LDL. Journal of Lipid Research. 2003. UI 12897184
sdLDL fraction only

Hulthe J, Wiklund O, Olsson G, et al. Computerized measurement of LDL particle size in human serum. Reproducibility studies and evaluation of LDL particle size in relation to metabolic variables and the occurrence of atherosclerosis. Scandinavian Journal of Clinical & Laboratory Investigation. 1999. UI 10691057
No relevant info

Inano K, Tezuka S, Miida T, Okada M. Capillary isotachophoretic analysis of serum lipoproteins using a carrier ampholyte as spacer ion. Annals of Clinical Biochemistry. 2000. UI 11026526
No relevant info

Jaakkola O, Solakivi T, Tertov VV, et al. Characteristics of low-density lipoprotein subfractions from patients with coronary artery disease. Coronary Artery Disease. 1993. UI 8261211
No relevant info

Jungner I, Sniderman AD, Furberg C, et al. Does low-density lipoprotein size add to atherogenic particle number in predicting the risk of fatal myocardial infarction?. American Journal of Cardiology. 2006. UI 16563891
Not LDL subfractions

Kahlon TS, Adamson GL, Glines LA, et al. Partial specific volume and preferential hydration of low density lipoprotein subfractions. Lipids. 1986. UI 3702615
No relevant info

Kahlon TS, Adamson GL, Shen MM, Lindgren FT. Sedimentation equilibrium of human low density lipoprotein subfractions. Lipids. 1982. UI 7098773
No relevant info

Krauss RM, Burke DJ,. Identification of multiple subclasses of plasma low density lipoproteins in normal humans. Journal of Lipid Research. 1982. UI 7057116
No relevant info

Kulkarni KR. Cholesterol profile measurement by vertical auto profile method. Clinics in Laboratory Medicine. 2006. UI 17110240
Technical description of specific measure

la-Korpela M, Hiltunen Y, Bell JD. Quantification of biomedical NMR data using artificial neural network analysis: lipoprotein lipid profiles from 1H NMR data of human plasma. NMR in Biomedicine. 1995. UI 8732179
No relevant info

la-Korpela M, Korhonen A, Keisala J, et al. 1H NMR-based absolute quantitation of human lipoproteins and their lipid contents directly from plasma. Journal of Lipid Research. 1994. UI 7897326
No relevant info

la-Korpela M, Pentikainen MO, Korhonen A, et al. Detection of low density lipoprotein particle fusion by proton nuclear magnetic resonance spectroscopy. Journal of Lipid Research. 1998. UI 9717732
No relevant info

Lamarche B, St-Pierre AC, Ruel IL, et al. A prospective, population-based study of low density lipoprotein particle size as a risk factor for ischemic heart disease in men. Canadian Journal of Cardiology. 2001. UI 11521128
Same dataset as other study

Lamarche B, Tchernof A, Mauriege P, et al. Fasting insulin and apolipoprotein B levels and low-density lipoprotein particle size as risk factors for ischemic heart disease. JAMA. 1998. UI 9643858
Same dataset as other study

Lamarche B, Tchernof A, Moorjani S, et al. Small, dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men. Prospective results from the Quebec Cardiovascular Study. Circulation. 1997. UI 8994419
Same dataset as other study

Le GD, Nouvelot A, Chermant JL. Determination of size and molecular weight distributions of lipoproteins using automatic image analysis and density gradient ultracentrifugation. Journal of Biochemical & Biophysical Methods. 1990. UI 2345267
No relevant info

Lee DM, Alaupovic P,. Apolipoproteins B, C-III and E in two major subpopulations of low-density lipoproteins. Biochimica et Biophysica Acta. 1986. UI 3768392
No relevant info

Lee DM, Alaupovic P,. Physiocochemical properties of low-density lipoproteins of normal human plasma. Evidence for the occurrence of lipoprotein B in associated and free forms. Biochemical Journal. 1974. UI 4363108
No relevant info

Lee LT, Lefevre M, Wong L, et al. Gradient acrylamide/agarose gels for electrophoretic separation of intact human very low density lipoproteins, intermediate density lipoproteins, lipoprotein a, and low density lipoproteins. Analytical Biochemistry. 1987. UI 2440347
No relevant info

Lee LT, Lefevre M, Wong L, et al. Gradient acrylamide/agarose gels for electrophoretic separation of intact human very low density lipoproteins, intermediate density lipoproteins, lipoprotein a, and low density lipoproteins. Analytical Biochemistry. 1987. UI 2440347
Not LDL subfractions

Leonsson M, Hulthe J, Oscarsson J, et al. Intima-media thickness in cardiovascularly asymptomatic hypopituitary adults with growth hormone deficiency: relation to body mass index, gender, and other cardiovascular risk factors. Clinical Endocrinology. 2002. UI 12460325
Very atypical population

Liu ML, Ylitalo K, Salonen R, et al. Circulating oxidized low-density lipoprotein and its association with carotid intima-media thickness in asymptomatic members of familial combined hyperlipidemia families. Arteriosclerosis, Thrombosis & Vascular Biology. 2004. UI 15205217
Same dataset as other study

Liu MY, McNeal CJ, Macfarlane RD. Charge density profiling of circulating human low-density lipoprotein particles by capillary zone electrophoresis. Electrophoresis. 2004. UI 15349939
Not LDL subfractions

Luc G, De Gennes JL, Chapman MJ. Further resolution and comparison of the heterogeneity of plasma low-density lipoproteins in human hyperlipoproteinemias: type III hyperlipoproteinemia, hypertriglyceridemia and familial hypercholesterolemia. Atherosclerosis. 1988. UI 3401287
No relevant info

Lupattelli G, Lombardini R, Schillaci G, et al. Flow-mediated vasoactivity and circulating adhesion molecules in hypertriglyceridemia: association with small, dense LDL cholesterol particles. American Heart Journal. 2000. UI 10966556
No clinical CVD outcome

Lyons TJ, Jenkins AJ, Zheng D, et al. Nuclear magnetic resonance-determined lipoprotein subclass profile in the DCCT/EDIC cohort: associations with carotid intima-media thickness. Diabetic Medicine. 2006. UI 16922701
Subfraction as "predictor" of 4 yo CVD outcomes

Mackey RH, Kuller LH, Sutton-Tyrrell K, et al. Hormone therapy, lipoprotein subclasses, and coronary calcification: the Healthy Women Study. Archives of Internal Medicine. 2005. UI 15767525
Same dataset as other study

Makimattila S, Liu ML, Vakkilainen J, et al. Impaired endothelium-dependent vasodilation in type 2 diabetes. Relation to LDL size, oxidized LDL, and antioxidants. Diabetes Care. 1999. UI 10372251
No clinical CVD outcome

McNamara JR, Jenner JL, Li Z, et al. Change in LDL particle size is associated with change in plasma triglyceride concentration. Arteriosclerosis & Thrombosis. 1992. UI 1420088
Not day-to-day comparison, No clinical CVD outcome

Melish JS, Waterhouse C,. Concentration gradient electrophoresis of plasma from patients with hyperbetalipoproteinemia. Journal of Lipid Research. 1972. UI 4335796
No relevant info

Menys VC, Liu Y, Mackness MI, et al. Isolation of plasma small-dense low-density lipoprotein using a simple air-driven ultracentrifuge and quantification using immunoassay of apolipoprotein B. Clinical Chemistry & Laboratory Medicine. 2004. UI 15061377
sdLDL fraction only

Menys VC, Liu Y, Mackness MI, et al. Measurement of plasma small-dense LDL concentration by a simplified ultracentrifugation procedure and immunoassay of apolipoprotein B. Clinica Chimica Acta. 2003. UI 12867279
sdLDL fraction only

Nosadini R, Manzato E, Solini A, et al. Peripheral, rather than hepatic, insulin resistance and atherogenic lipoprotein phenotype predict cardiovascular complications in NIDDM. European Journal of Clinical Investigation. 1994. UI 8050454
No relevant info

Ohmori R, Momiyama Y, Tanaka N, et al. LDL fractions assessed by anion-exchange high-performance liquid chromatography in patients with coronary artery disease. Atherosclerosis. 2006. UI 16620833
No relevant info

Okabe M. The high occurrence of low density lipoprotein subfractions in coronary heart disease. Japanese Circulation Journal. 1979. UI 232192
LDL subfraction analysis not comparable to modern methods

Okada M, Matsui H, Ito Y, et al. Low-density lipoprotein cholesterol can be chemically measured: a new superior method. Journal of Laboratory & Clinical Medicine. 1998. UI 9735925
No relevant info

Okazaki M, Usui S, Ishigami M, et al. Identification of unique lipoprotein subclasses for visceral obesity by component analysis of cholesterol profile in high-performance liquid chromatography. Arteriosclerosis, Thrombosis & Vascular Biology. 2005. UI 15637308
No relevant info

Olsson AG, Eklund B,. Studies in asymptomatic primary hyperlipidaemia. V. Peripheral circulation. Acta Medica Scandinavica. 1975. UI 170797
No clinical CVD outcome

Opplt JJ, Chick LL, Opplt MA. Correlative design of electrophoretic and ultracentrifugal investigation of metabolic effects of probucol. Artery. 1982. UI 7092580
Not LDL subfractions

Opplt JJ, Holzberg ES,. Ultracentrifugal subclasses of low and intermediate density lipoproteins. Journal of Lipid Research. 1994. UI 8014586
No relevant info

O'Sullivan JJ, Matthew A, Conroy RM, et al. Relation of angiographically defined coronary artery disease to serum lipoprotein levels. Clinical Cardiology. 1990. UI 2282727
Not LDL subfractions

Otvos JD, Jeyarajah EJ, Bennett DW, Krauss RM. Development of a proton nuclear magnetic resonance spectroscopic method for determining plasma lipoprotein concentrations and subspecies distributions from a single, rapid measurement. Clinical Chemistry. 1992. UI 1326420
No relevant info

Packard CJ. LDL subfractions and atherogenicity: an hypothesis from the University of Glasgow. Current Medical Research & Opinion. 1996. UI 8862937
Not original data

Park I, Paeng KJ, Yoon Y, et al. Separation and selective detection of lipoprotein particles of patients with coronary artery disease by frit-inlet asymmetrical flow field-flow fractionation. Journal of Chromatography B: Analytical Technologies in the Biomedical & Life Sciences. 2002. UI 12401369
No relevant info

Pauciullo P, Carlson LA, Eklund B, et al. Concentration and chemical composition of plasma lipoprotein subfractions in patients with peripheral vascular disease. Evidence for normal apolipoprotein B but low cholesteryl ester content in small VLDL. Atherosclerosis. 1985. UI 4091876
No relevant info

Petersen M, Dyrby M, Toubro S, et al. Quantification of lipoprotein subclasses by proton nuclear magnetic resonance-based partial least-squares regression models. Clinical Chemistry. 2005. UI 15961551
No relevant info

Rainwater DL, Moore PH, Shelledy WR, et al. Characterization of a composite gradient gel for the electrophoretic separation of lipoproteins. Journal of Lipid Research. 1997. UI 9215553
No relevant info

Rainwater DL. Electrophoretic separation of LDL and HDL subclasses. Methods in Molecular Biology. 1998. UI 9918045
No relevant info

Rizzo M, Rini GB, Berneis K. The clinical relevance of LDL size and subclasses modulation in patients with type-2 diabetes. Experimental & Clinical Endocrinology & Diabetes. 2007. UI 17853329
No relevant info

Rubenstein B, Steiner G,. Fractionation of human low density lipoprotein by column chromatography. Canadian Journal of Biochemistry. 1976. UI 189881
No relevant info

Ruotolo G, Tettamanti C, Garancini MP, et al. Smaller, denser LDL particles are not a risk factor for cardiovascular disease in healthy nonagenarian women of the Cremona Population Study. Atherosclerosis. 1998. UI 9733216
No clinical CVD outcome

Sawle A, Higgins MK, Olivant MP, Higgins JA. A rapid single-step centrifugation method for determination of HDL, LDL, and VLDL cholesterol, and TG, and identification of predominant LDL subclass. Journal of Lipid Research. 2002. UI 11861676
No relevant info

Scheffer PG, Bakker SJ, Heine RJ, Teerlink T. Measurement of LDL particle size in whole plasma and serum by high performance gel-filtration chromatography using a fluorescent lipid probe. Clinical Chemistry. 1998. UI 9761248
comparison of different techniques of the same method

Schlenck A, Herbeth B, Siest G, Visvikis S. Characterization and quantification of serum lipoprotein subfractions by capillary isotachophoresis: relationships with lipid, apolipoprotein, and lipoprotein levels. Journal of Lipid Research. 1999. UI 10553016
No relevant info

Shahrul BS, Faridah AR,. Simplified gradient gel electrophoresis for quantification of low-density lipoprotein subclass. Clinica Chimica Acta. 2003. UI 14500048
No relevant info

Slyper AH, Zvereva S, Schectman G, et al. Low-density lipoprotein particle size is not a discriminating marker for atherogenic risk in male offspring of parents with early coronary artery disease. Metabolism: Clinical & Experimental. 1997. UI 9258281
No clinical CVD outcome

St-Pierre AC, Ruel IL, Cantin B, et al. Comparison of various electrophoretic characteristics of LDL particles and their relationship to the risk of ischemic heart disease. Circulation. 2001. UI 11696468
Same dataset as other study

Swinkels DW, Hak-Lemmers HL, Demacker PN. Single spin density gradient ultracentrifugation method for the detection and isolation of light and heavy low density lipoprotein subfractions. Journal of Lipid Research. 1987. UI 3681148
No relevant info

Terpstra AH, Woodward CJ, Sanchez-Muniz FJ. Improved techniques for the separation of serum lipoproteins by density gradient ultracentrifugation: visualization by prestaining and rapid separation of serum lipoproteins from small volumes of serum. Analytical Biochemistry. 1981. UI 6165257
Not LDL subfractions

Tornvall P, Karpe F, Carlson LA, Hamsten A. Relationships of low density lipoprotein subfractions to angiographically defined coronary artery disease in young survivors of myocardial infarction. Atherosclerosis. 1991. UI 1799399
No relevant info

Tsukamoto H, Takei I, Ishii K, Watanabe K. Simplified method for the diameter sizing of serum low-density lipoprotein using polyacrylamide gradient gel electrophoresis. Clinical Chemistry & Laboratory Medicine. 2004. UI 15497465
No relevant info

Usui S, Nakamura M, Jitsukata K, et al. Assessment of between-instrument variations in a HPLC method for serum lipoproteins and its traceability to reference methods for total cholesterol and HDL-cholesterol. Clinical Chemistry. 2000. UI 10620573
Not day-to-day comparison, Not repeated measures

Vakkilainen J, Makimattila S, Seppala-Lindroos A, et al. Endothelial dysfunction in men with small LDL particles. Circulation. 2000. UI 10942737
No clinical CVD outcome

Warnick GR, McNamara JR, Boggess CN, et al. Polyacrylamide gradient gel electrophoresis of lipoprotein subclasses. Clinics in Laboratory Medicine. 2006. UI 17110241
No relevant info

Westhuyzen J, Graham SD, Rasiah RL, Saltissi D. Simplified sizing of low-density lipoprotein using polyacrylamide gradient gel electrophoresis of plasma. European Journal of Clinical Chemistry & Clinical Biochemistry. 1997. UI 9156560
No relevant info

Williams PT, Krauss RM, Nichols AV, et al. Identifying the predominant peak diameter of high-density and low-density lipoproteins by electrophoresis. Journal of Lipid Research. 1990. UI 2373962
Not comparison of 2 methods

Williams PT, Vranizan KM, Krauss RM. Correlations of plasma lipoproteins with LDL subfractions by particle size in men and women. Journal of Lipid Research. 1992. UI 1619368
No relevant info

Yohannes G, Sneck M, Varjo SJ, et al. Miniaturization of asymmetrical flow field-flow fractionation and application to studies on lipoprotein aggregation and fusion. Analytical Biochemistry. 2006. UI 16750506
No relevant info

Zhang B, Kaneshi T, Ohta T, Saku K. Relation between insulin resistance and fast-migrating LDL subfraction as characterized by capillary isotachophoresis. Journal of Lipid Research. 2005. UI 16061945
No relevant info

Zhang B, Maeda N, Okada K, et al. Association between fast-migrating low-density lipoprotein subfraction as characterized by capillary isotachophoresis and intima-media thickness of carotid artery. Atherosclerosis. 2006. UI 16236285
Not LDL subfractions

Zorn U, Haug C, Celik E, et al. Characterization of modified low density lipoprotein subfractions by capillary isotachophoresis. Electrophoresis. 2001. UI 11358140
Not LDL subfractions

Zorn U, Wolf CF, Wennauer R, et al. Separation of lipoproteins by capillary isotachophoresis combined with enzymatic derivatization of cholesterol and triglycerides. Electrophoresis. 1999. UI 10424488
No relevant info

Appendix C. Potential Treatment Studies

Abbasi F, Chu JW, McLaughlin T, et al. Effect of metformin treatment on multiple cardiovascular disease risk factors in patients with type 2 diabetes mellitus. Metabolism: Clinical & Experimental. 2004. UI 14767866
Metformin

Abbey M, Owen A, Suzakawa M, et al. Effects of menopause and hormone replacement therapy on plasma lipids, lipoproteins and LDL-receptor activity. Maturitas. 1999. UI 10656504
Hormone treatment

Aguilar-Salinas CA, Arita MO, Sauque RL, et al. Effects of estrogen/medrogestone therapy on the apoprotein B-containing lipoproteins in postmenopausal women with type 2 diabetes mellitus under satisfactory and non-satisfactory glycemic control. Israel Medical Association Journal: Imaj. 2001. UI 11344825
Hormone treatment

Alexandersen P, Haarbo J, Christiansen C. Impact of combined hormone replacement therapy on serum lipid metabolism: new aspects. Gynecological Endocrinology. 1997. UI 9272426
Hormone treatment

Almario RU, Vonghavaravat V, Wong R, Kasim-Karakas SE. Effects of walnut consumption on plasma fatty acids and lipoproteins in combined hyperlipidemia. American Journal of Clinical Nutrition. 2001. UI 11451720
Walnuts

Altena TS, Michaelson JL, Ball SD, et al. Lipoprotein subfraction changes after continuous or intermittent exercise training. Medicine & Science in Sports & Exercise. 2006. UI 16531908
Exercise

Ambring A, Friberg P, Axelsen M, et al. Effects of a Mediterranean-inspired diet on blood lipids, vascular function and oxidative stress in healthy subjects. Clinical Science. 2004. UI 14683522
Mediterranean diet

Anber V, Millar JS, McConnell M, et al. Interaction of very-low-density, intermediate-density, and low-density lipoproteins with human arterial wall proteoglycans. Arteriosclerosis, Thrombosis & Vascular Biology. 1997. UI 9409221
Fibrate

Andrade RJ, Garcia-Escano MD, Valdivielso P, et al. Effects of interferon-beta on plasma lipid and lipoprotein composition and post-heparin lipase activities in patients with chronic hepatitis C. Alimentary Pharmacology & Therapeutics. 2000. UI 10886050
Interferon-beta

Arosio M, Sartore G, Rossi CM, et al. LDL physical properties, lipoprotein and Lp(a) levels in acromegalic patients. Effects of octreotide therapy. Italian Multicenter Octreotide Study Group. Atherosclerosis. 2000. UI 10924734
Octreotide (acromegaly)

Ashton EL, Best JD, Ball MJ. Effects of monounsaturated enriched sunflower oil on CHD risk factors including LDL size and copper-induced LDL oxidation. Journal of the American College of Nutrition. 2001. UI 11506059
Sunflower oil

Avogaro P, Cazzolato G. Changes in the composition and physico-chemical characteristics of serum lipoproteins during ethanol-induced lipaemia in alcoholic subjects. Metabolism: Clinical & Experimental. 1975. UI 171538
Alcohol withdrawal

Ayaori M, Ishikawa T, Yoshida H, et al. Beneficial effects of alcohol withdrawal on LDL particle size distribution and oxidative susceptibility in subjects with alcohol-induced hypertriglyceridemia. Arteriosclerosis, Thrombosis & Vascular Biology. 1997. UI 9409225
Alcohol withdrawal

Bachen EA, Muldoon MF, Matthews KA, Manuck SB. Effects of hemoconcentration and sympathetic activation on serum lipid responses to brief mental stress. Psychosomatic Medicine. 2002. UI 12140348
Labetolol

Backes JM, Gibson CA. Effect of lipid-lowering drug therapy on small-dense low-density lipoprotein. Annals of Pharmacotherapy. 2005. UI 15671087
Lipid lowering

Baldassarre S, Scruel O, Deckelbaum RJ, et al. Beneficial effects of atorvastatin on sd LDL and LDL phenotype B in statin-naive patients and patients previously treated with simvastatin or pravastatin. International Journal of Cardiology. 2005. UI 16186066
Statin

Barnes JF, Farish E, Rankin M, Hart diabetes. A comparison of the effects of two continuous Hormone treatment regimens on cardiovascular risk factors. Atherosclerosis. 2002. UI 11755937
Hormone treatment

Baumstark MW, Frey I, Berg A. Acute and delayed effects of prolonged exercise on serum lipoproteins. II. Concentration and composition of low-density lipoprotein subfractions and very low-density lipoproteins. European Journal of Applied Physiology & Occupational Physiology. 1993. UI 8354253
Exercise

Bavirti S, Ghanaat F, Tayek JA. Peroxisome proliferator-activated receptor-gamma agonist increases both low-density lipoprotein cholesterol particle size and small high-density lipoprotein cholesterol in patients with type 2 diabetes independent of diabetic control. Endocrine Practice. 2003. UI 14715475
Troglitazone

Berg A, Baumstark MW, Frey I, et al. Clinical and therapeutic use of probucol. European Journal of Clinical Pharmacology. 1991. UI 2044650
Probucol

Blaha V, Zadak Z, Solichova D, et al. Hypocholesterolemic effect of pravastatin is associated with increased content of antioxidant vitamin-E in cholesterol fractions. Acta Medica (Hradec Kralove). 1998. UI 9729642
Pravastatin

Blake GJ, Albert MA, Rifai N, Ridker PM. Effect of pravastatin on LDL particle concentration as determined by NMR spectroscopy: a substudy of a randomized placebo controlled trial. European Heart Journal. 2003. UI 14563343
Pravastatin

Bos G, Poortvliet MC, Scheffer PG, et al. Dietary polyunsaturated fat intake is associated with low-density lipoprotein size, but not with susceptibility to oxidation in subjects with impaired glucose metabolism and type II diabetes: the Hoorn study. European Journal of Clinical Nutrition. 2007. UI 16943850
Dietary fat

Bradley K, Flack JM, Belcher J, et al. Chlorthalidone attenuates the reduction in total cholesterol and small, dense LDL cholesterol subclass associated with weight loss. American Journal of Hypertension. 1993. UI 8398006
Chlorthalidone

Bredie, SJ, Bosch FH, Demacker PN, et al. Effects of peritoneal dialysis with an overnight icodextrin dwell on parameters of glucose and lipid metabolism. Peritoneal Dialysis International. 2001. UI 11475343
Icodextrin

Bredie, SJ, de Bruin TW, Demacker PN, et al. Comparison of gemfibrozil versus simvastatin in familial combined hyperlipidemia and effects on apolipoprotein-B-containing lipoproteins, low-density lipoprotein subfraction profile, and low-density lipoprotein oxidizability. American Journal of Cardiology. 1995. UI 7856526
Gemfibrazole, Simvastatin

Brousseau ME, Goldkamp AL, Collins D, et al. Polymorphisms in the gene encoding lipoprotein lipase in men with low HDL-C and coronary heart disease: the Veterans Affairs HDL Intervention Trial. Journal of Lipid Research. 2004. UI 15292370
Gemfibrazole

Brousseau ME, Schaefer EJ, Wolfe ML, et al. Effects of an inhibitor of cholesteryl ester transfer protein on HDL cholesterol. New England Journal of Medicine. 2004. UI 15071125
Torcetrapib

Brussaard HE, Gevers Leuven JA, Kluft C, et al. Effect of 17 beta-estradiol on plasma lipids and LDL oxidation in postmenopausal women with type II diabetes mellitus. Arteriosclerosis, Thrombosis & Vascular Biology. 1997. UI 9081688
Estrogen

Byrne DJ, Neil HA, Vallance DT, Winder AF. A pilot study of garlic consumption shows no significant effect on markers of oxidation or sub-fraction composition of low-density lipoprotein including lipoprotein(a) after allowance for non-compliance and the placebo effect. Clinica Chimica Acta. 1999. UI 10481920
Garlic

Caixas A, Ordonez-Llanos J, de LA, et al. Optimization of glycemic control by insulin therapy decreases the proportion of small dense LDL particles in diabetic patients. Diabetes. 1997. UI 9200657
Insulin

Caixas A, Perez A, Payes A, et al. Effects of a short-acting insulin analog (Insulin Lispro) versus regular insulin on lipid metabolism in insulin-dependent diabetes mellitus. Metabolism: Clinical & Experimental. 1998. UI 9580247
Insulin

Calabresi L, Donati D, Pazzucconi F, et al. Omacor in familial combined hyperlipidemia: effects on lipids and low density lipoprotein subclasses. Atherosclerosis. 2000. UI 10657575
Omega 3

Campos H, Blijlevens E, McNamara JR, et al. LDL particle size distribution. Results from the Framingham Offspring Study. Arteriosclerosis & Thrombosis. 1992. UI 1450174
Dietary fat, cholesterol

Campos H, Dreon diabetes, Krauss RM. Associations of hepatic and lipoprotein lipase activities with changes in dietary composition and low density lipoprotein subclasses. Journal of Lipid Research. 1995. UI 7775858
Dietary fat

Campos H, Sacks FM, Walsh BW, et al. Differential effects of estrogen on low-density lipoprotein subclasses in healthy postmenopausal women. Metabolism: Clinical & Experimental. 1993. UI 8412768
Estrogen

Campos H, Wilson PW, Jimenez D, et al. Differences in apolipoproteins and low-density lipoprotein subfractions in postmenopausal women on and off estrogen therapy: results from the Framingham Offspring Study. Metabolism: Clinical & Experimental. 1990. UI 2120547
Estrogen

Charest A, Desroches S, Vanstone CA, et al. Unesterified plant sterols and stanols do not affect LDL electrophoretic characteristics in hypercholesterolemic subjects. Journal of Nutrition. 2004. UI 14988452
Sterols, Stanols

Charest A, Vanstone C, St-Onge MP, et al. Phytosterols in nonfat and low-fat beverages have no impact on the LDL size phenotype. European Journal of Clinical Nutrition. 2005. UI 15856068
Phytosterol

Cheung MC, Austin MA, Moulin P, et al. Effects of pravastatin on apolipoprotein-specific high density lipoprotein subpopulations and low density lipoprotein subclass phenotypes in patients with primary hypercholesterolemia. Atherosclerosis. 1993. UI 8257447
Pravastatin

Clifton PM, Noakes M, Nestel PJ. LDL particle size and LDL and HDL cholesterol changes with dietary fat and cholesterol in healthy subjects. Journal of Lipid Research. 1998. UI 9741692
Dietary fat, cholesterol

da Costa Vieira JL, Gomes ME, Almeida AB, Moriguchi EH. Changes in the profile of lipoprotein subfractions associated with hormone replacement therapy. Arquivos Brasileiros de Cardiologia. 2001. UI 11262568
Hormone treatment

Davidson MH, Bays HE, Stein E, et al. Effects of fenofibrate on atherogenic dyslipidemia in hypertriglyceridemic subjects. Clinical Cardiology. 2006. UI 16796078
Fenofibrate

Davy BM, Davy KP, Ho RC, et al. High-fiber oat cereal compared with wheat cereal consumption favorably alters LDL-cholesterol subclass and particle numbers in middle-aged and older men. American Journal of Clinical Nutrition. 2002. UI 12145006
Oat

de GJ, Demacker PN, Stalenhoef AF. The effect of simvastatin treatment on the low-density lipoprotein subfraction profile and composition in familial hypercholesterolaemia. Netherlands Journal of Medicine. 1993. UI 8107933
Simvastatin

de Graaf J, Hendriks JC, Demacker PN, Stalenhoef AF. Identification of multiple dense LDL subfractions with enhanced susceptibility to in vitro oxidation among hypertriglyceridemic subjects. Normalization after clofibrate treatment. Arteriosclerosis & Thrombosis. 1993. UI 8485123
Clofibrate

de Graaf J, Swinkels DW, Demacker PN, et al. Differences in the low density lipoprotein subfraction profile between oral contraceptive users and controls. Journal of Clinical Endocrinology & Metabolism. 1993. UI 8421088
Oral contraceptive

Desroches S, Mauger JF, Ausman LM, et al. Soy protein favorably affects LDL size independently of isoflavones in hypercholesterolemic men and women. Journal of Nutrition. 2004. UI 14988449
Soy protein

Dornbrook-Lavender KA, Joy MS, nu-Ciocca CJ, et al. Effects of atorvastatin on low-density lipoprotein cholesterol phenotype and C-reactive protein levels in patients undergoing long-term dialysis. Pharmacotherapy. 2005. UI 15843280
Atorvastatin

Dreon diabetes, Fernstrom HA, Campos H, et al. Change in dietary saturated fat intake is correlated with change in mass of large low-density-lipoprotein particles in men. American Journal of Clinical Nutrition. 1998. UI 9583838
Diets

Dreon diabetes, Fernstrom HA, Miller B, Krauss RM. Apolipoprotein E isoform phenotype and LDL subclass response to a reduced-fat diet. Arteriosclerosis, Thrombosis & Vascular Biology. 1995. UI 7749804
Diets

Dreon diabetes, Fernstrom HA, Miller B, Krauss RM. Low-density lipoprotein subclass patterns and lipoprotein response to a reduced-fat diet in men. FASEB Journal. 1994. UI 8299884
Diets

Dreon diabetes, Fernstrom HA, Williams PT, Krauss RM. A very low-fat diet is not associated with improved lipoprotein profiles in men with a predominance of large, low-density lipoproteins. American Journal of Clinical Nutrition. 1999. UI 10075324
Diets

Dreon diabetes, Fernstrom HA, Williams PT, Krauss RM. LDL subclass patterns and lipoprotein response to a low-fat, high-carbohydrate diet in women. Arteriosclerosis, Thrombosis & Vascular Biology. 1997. UI 9108784
Diets

Dumesnil JG, Turgeon J, Tremblay A, et al. Effect of a low-glycaemic index--low-fat--high protein diet on the atherogenic metabolic risk profile of abdominally obese men. British Journal of Nutrition. 2001. UI 11737954
Diets

Ebenbichler CF, Laimer M, Kaser S, et al. Relationship between cholesteryl ester transfer protein and atherogenic lipoprotein profile in morbidly obese women. Arteriosclerosis, Thrombosis & Vascular Biology. 2002. UI 12231567
Bariatric surgery

Elisaf MS, Petris C, Bairaktari E, et al. The effect of moxonidine on plasma lipid profile and on LDL subclass distribution. Journal of Human Hypertension. 1999. UI 10578224
Moxonidine

Empen K, Geiss HC, Lehrke M, et al. Effect of atorvastatin on lipid parameters, LDL subtype distribution, hemorrheological parameters and adhesion molecule concentrations in patients with hypertriglyceridemia. Nutrition Metabolism & Cardiovascular Diseases. 2003. UI 12929621
Atorvastatin

Farish E, Spowart K, Barnes JF, et al. Effects of postmenopausal hormone replacement therapy on lipoproteins including lipoprotein(a) and LDL subfractions. Atherosclerosis. 1996. UI 8879436
Estrogen

Feher MD, Caslake M, Foxton J, et al. Atherogenic lipoprotein phenotype in type 2 diabetes: reversal with micronised fenofibrate. Diabetes/Metabolism Research Reviews. 1999. UI 10634964
Fenofibrate

Forster LF, Stewart G, Bedford D, et al. Influence of atorvastatin and simvastatin on apolipoprotein B metabolism in moderate combined hyperlipidemic subjects with low VLDL and LDL fractional clearance rates. Atherosclerosis. 2002. UI 12119202
Atorvastatin, Simvastatin

Foulon T, Payen N, Laporte F, et al. Effects of two low-dose oral contraceptives containing ethinylestradiol and either desogestrel or levonorgestrel on serum lipids and lipoproteins with particular regard to LDL size. Contraception. 2001. UI 11535207
Oral contraceptive

Franceschini G, Cassinotti M, Vecchio G, et al. Pravastatin effectively lowers LDL cholesterol in familial combined hyperlipidemia without changing LDL subclass pattern. Arteriosclerosis & Thrombosis. 1994. UI 7918306
Pravastatin

Franceschini G, Lovati MR, Manzoni C, et al. Effect of gemfibrozil treatment in hypercholesterolemia on low density lipoprotein (LDL) subclass distribution and LDL-cell interaction. Atherosclerosis. 1995. UI 7605377
Gemfibrozil

Freed MI, Ratner R, Marcovina SM, et al. Effects of rosiglitazone alone and in combination with atorvastatin on the metabolic abnormalities in type 2 diabetes mellitus. American Journal of Cardiology. 2002. UI 12398960
Rosiglitazone

Frost RJ, Otto C, Geiss HC, et al. Effects of atorvastatin versus fenofibrate on lipoprotein profiles, low-density lipoprotein subfraction distribution, and hemorheologic parameters in type 2 diabetes mellitus with mixed hyperlipoproteinemia. American Journal of Cardiology. 2001. UI 11137832
Atorvastatin, Fenofibrate

Geiss HC, Otto C, Parhofer KG. Effect of ezetimibe on low-density lipoprotein subtype distribution: results of a placebo-controlled, double-blind trial in patients treated by regular low-density lipoprotein apheresis and statins. Metabolism: Clinical & Experimental. 2006. UI 16631435
Ezetimibe

Geiss HC, Otto C, Schwandt P, Parhofer KG. Effect of atorvastatin on low-density lipoprotein subtypes in patients with different forms of hyperlipoproteinemia and control subjects. Metabolism: Clinical & Experimental. 2001. UI 11474489
Atorvastatin

Geiss HC, Schwandt P, Parhofer KG. Influence of simvastatin on LDL-subtypes in patients with heterozygous familial hypercholesterolemia and in patients with diabetes mellitus and mixed hyperlipoproteinemia. Experimental & Clinical Endocrinology & Diabetes. 2002. UI 12058342
Simvastatin

Giri S, Thompson PD, Taxel P, et al. Oral estrogen improves serum lipids, homocysteine and fibrinolysis in elderly men. Atherosclerosis. 1998. UI 9622279
17beta-estradiol

Goldberg RB, Kendall diabetes, Deeg, MA, et al. A comparison of lipid and glycemic effects of pioglitazone and rosiglitazone in patients with type 2 diabetes and dyslipidemia. Diabetes Care. 2005. UI 15983299
Pioglitazone, Rosiglitazone

Goulet J, Lamarche B, Charest A, et al. Effect of a nutritional intervention promoting the Mediterranean food pattern on electrophoretic characteristics of low-density lipoprotein particles in healthy women from the Quebec City metropolitan area. British Journal of Nutrition. 2004. UI 15333160
Mediterranean diet

Granfone A, Campos H, McNamara JR, et al. Effects of estrogen replacement on plasma lipoproteins and apolipoproteins in postmenopausal, dyslipidemic women. Metabolism: Clinical & Experimental. 1992. UI 1435290
Estrogen

Greene CM, Waters D, Clark RM, et al. Plasma LDL and HDL characteristics and carotenoid content are positively influenced by egg consumption in an elderly population. Nutrition & Metabolism. 2006. UI 20073026003
Eggs

Griffin B, Farish E, Walsh D, et al. Response of plasma low density lipoprotein subfractions to oestrogen replacement therapy following surgical menopause. Clinical Endocrinology. 1993. UI 8287573
Estrogen

Griffin BA, Caslake MJ, Gaw A, et al. Effects of cholestyramine and acipimox on subfractions of plasma low density lipoprotein. Studies in normolipidaemic and hypercholesterolaemic subjects. European Journal of Clinical Investigation. 1992. UI 1633833
Cholestyramine, Acipimox

Griffin MD, Sanders TA, Davies IG, et al. Effects of altering the ratio of dietary n-6 to n-3 fatty acids on insulin sensitivity, lipoprotein size, and postprandial lipemia in men and postmenopausal women aged 45-70 y: the OPTILIP Study. American Journal of Clinical Nutrition. 2006. UI 17158408
Omega 3:Omega 6

Guerin M, Dolphin PJ, Talussot C, et al. Pravastatin modulates cholesteryl ester transfer from HDL to apoB-containing lipoproteins and lipoprotein subspecies profile in familial hypercholesterolemia. Arteriosclerosis, Thrombosis & Vascular Biology. 1995. UI 7670950
Pravastatin

Guerin M, Egger P, Soudant C, et al. Dose-dependent action of atorvastatin in type IIB hyperlipidemia: preferential and progressive reduction of atherogenic apoB-containing lipoprotein subclasses (VLDL-2, IDL, small dense LDL) and stimulation of cellular cholesterol efflux. Atherosclerosis. 2002. UI 12052475
Atorvastatin

Guerin M, Le GW, Frisdal E, et al. Action of ciprofibrate in type IIb hyperlipoproteinemia: modulation of the atherogenic lipoprotein phenotype and stimulation of high-density lipoprotein-mediated cellular cholesterol efflux. Journal of Clinical Endocrinology & Metabolism. 2003. UI 12915663
Ciprofibrate

Gylling H, Miettinen TA. Serum cholesterol and cholesterol and lipoprotein metabolism in hypercholesterolaemic NIDdiabetes patients before and during sitostanol ester-margarine treatment. Diabetologia. 1994. UI 7988779
Sitostanol ester

Halle M, Berg A, Garwers U, et al. Influence of 4 weeks' intervention by exercise and diet on low-density lipoprotein subfractions in obese men with type 2 diabetes. Metabolism: Clinical & Experimental. 1999. UI 10337867
Exercise, diet

Halverstadt A, Phares DA, Wilund KR, et al. Endurance exercise training raises high-density lipoprotein cholesterol and lowers small low-density lipoprotein and very low-density lipoprotein independent of body fat phenotypes in older men and women. Metabolism: Clinical & Experimental. 2007. UI 17378998
Exercise

Harder H, Dinesen B, Astrup A. The effect of a rapid weight loss on lipid profile and glycemic control in obese type 2 diabetic patients. International Journal of Obesity & Related Metabolic Disorders: Journal of the International Association for the Study of Obesity. 2004. UI 14610532
Diet, low calorie

Harper CR, Edwards MC, Jacobson TA. Flaxseed oil supplementation does not affect plasma lipoprotein concentration or particle size in human subjects. Journal of Nutrition. 2006. UI 17056811
Flaxseed

Hayashi K, Kurushima H, Kuga Y, et al. Comparison of the effect of bezafibrate on improvement of atherogenic lipoproteins in Japanese familial combined hyperlipidemic patients with or without impaired glucose tolerance. Cardiovascular Drugs & Therapy. 1998. UI 9607127
Bezafibrate

Hayashi T, Hirano T, Yamamoto T, et al. Intensive insulin therapy reduces small dense low-density lipoprotein particles in patients with type 2 diabetes mellitus: relationship to triglyceride-rich lipoprotein subspecies. Metabolism: Clinical & Experimental. 2006. UI 16784958
Intensive diabetes treatment

Hays JH, DiSabatino A, Gorman RT, et al. Effect of a high saturated fat and no-starch diet on serum lipid subfractions in patients with documented atherosclerotic cardiovascular disease. Mayo Clinic Proceedings. 2003. UI 14601690
Diet, Saturated fatty acids, Low starch

Herbst KL, Amory JK, Brunzell JD, et al. Testosterone administration to men increases hepatic lipase activity and decreases HDL and LDL size in 3 wk. American Journal of Physiology - Endocrinology & Metabolism. 2003. UI 12736156
Testosterone

Hermenegildo C, Garcia-Martinez MC, Tarin JJ, et al. The effect of oral hormone replacement therapy on lipoprotein profile, resistance of LDL to oxidation and LDL particle size. Maturitas. 2001. UI 11358646
Hormone treatment

Hermenegildo C, Garcia-Martinez MC, Valldecabres C, et al. Transdermal estradiol reduces plasma myeloperoxidase levels without affecting the LDL resistance to oxidation or the LDL particle size. Menopause. 2002. UI 11875328
Estradiol

Herron KL, Lofgren IE, Sharman M, et al. High intake of cholesterol results in less atherogenic low-density lipoprotein particles in men and women independent of response classification. Metabolism: Clinical & Experimental. 2004. UI 15164336
Eggs

Hirano T, Yoshino G, Kashiwazaki K, Adachi M. Doxazosin reduces prevalence of small dense low density lipoprotein and remnant-like particle cholesterol levels in nondiabetic and diabetic hypertensive patients. American Journal of Hypertension. 2001. UI 11587157
Doxazosin

Homma Y, Kobayashi T, Yamaguchi H, et al. Specific reduction of plasma large, light low-density lipoprotein by a bile acid sequestering resin, cholebine (MCI-196) in type II hyperlipoproteinemia. Atherosclerosis. 1997. UI 9105567
Cholebine

Homma Y, Kobayashi T, Yamaguchi H, et al. Decrease of plasma large, light LDL (LDL1), HDL2 and HDL3 levels with concomitant increase of cholesteryl ester transfer protein (CETP) activity by probucol in type II hyperlipoproteinemia. Artery. 1993. UI 8447724
Probucol

Homma Y, Moriguchi EH, Sakane H, et al. Effects of probucol on plasma lipoprotein subfractions and activities of lipoprotein lipase and hepatic triglyceride lipase. Atherosclerosis. 1991. UI 1892484
Probucol

Homma Y, Ohshima K, Yamaguchi H, et al. Effects of eicosapentaenoic acid on plasma lipoprotein subfractions and activities of lecithin:cholesterol acyltransferase and lipid transfer protein. Atherosclerosis. 1991. UI 1811549
Fish oil

Homma Y, Ozawa H, Kobayashi T, et al. Effects of bezafibrate therapy on subfractions of plasma low-density lipoprotein and high-density lipoprotein, and on activities of lecithin:cholesterol acyltransferase and cholesteryl ester transfer protein in patients with hyperlipoproteinemia. Atherosclerosis. 1994. UI 8060379
Bezafibrate

Homma Y, Ozawa H, Kobayashi T, et al. Effects of simvastatin on plasma lipoprotein subfractions, cholesterol esterification rate, and cholesteryl ester transfer protein in type II hyperlipoproteinemia. Atherosclerosis. 1995. UI 7605391
Simvastatin

Ikejiri A, Hirano T, Murayama S, et al. Effects of atorvastatin on triglyceride-rich lipoproteins, low-density lipoprotein subclass, and C-reactive protein in hemodialysis patients. Metabolism: Clinical & Experimental. 2004. UI 15334369
Atorvastatin

Ikewaki K, Noma K, Tohyama J, et al. Effects of bezafibrate on lipoprotein subclasses and inflammatory markers in patients with hypertriglyceridemia--a nuclear magnetic resonance study. International Journal of Cardiology. 2005. UI 15907413
Bezafibrate

Ikewaki K, Tohyama J, Nakata Y, et al. Fenofibrate effectively reduces remnants, and small dense LDL, and increases HDL particle number in hypertriglyceridemic men - a nuclear magnetic resonance study. Journal of Atherosclerosis & Thrombosis. 2004. UI 15557710
Fenofibrate

Jenkins DJ, Kendall CW, Vuksan V, et al. Effect of wheat bran on serum lipids: influence of particle size and wheat protein. Journal of the American College of Nutrition. 1999. UI 10204832
Wheat Bran

Kalogirou M, Tsimihodimos V, Gazi I, et al. Effect of ezetimibe monotherapy on the concentration of lipoprotein subfractions in patients with primary dyslipidaemia. Current Medical Research & Opinion. 2007. UI 17519084
Ezetimibe

Kasim-Karakas SE, Lane E, Almario R, et al. Effects of dietary fat restriction on particle size of plasma lipoproteins in postmenopausal women. Metabolism: Clinical & Experimental. 1997. UI 9109849
Dietary fat

Katzel LI, Coon PJ, Rogus E, et al. Persistence of low HDL-C levels after weight reduction in older men with small LDL particles. Arteriosclerosis, Thrombosis & Vascular Biology. 1995. UI 7749838
Weight loss

Kazama H, Usui S, Okazaki M, et al. Effects of bezafibrate and pravastatin on remnant-like lipoprotein particles and lipoprotein subclasses in type 2 diabetes. Diabetes Research & Clinical Practice. 2003. UI 12590014
Pravastatin, Bezafibrate

Kearney T, de Gallegos CN, Proudler A, et al. Effects of short- and long-term growth hormone replacement on lipoprotein composition and on very-low-density lipoprotein and low-density lipoprotein apolipoprotein B100 kinetics in growth hormone-deficient hypopituitary subjects. Metabolism: Clinical & Experimental. 2003. UI 12524662
Growth hormone

Keidar S, Goldberg AC, Cook K, et al. High carbohydrate fat-free diet modulates epitope expression of LDL-apoB-100 and interaction of LDL with human fibroblasts. Journal of Lipid Research. 1989. UI 2480987
Diet

Keidar S, Goldberg AC, Cook K, et al. High carbohydrate fat-free diet modulates epitope expression of LDL-apoB-100 and interaction of LDL with human fibroblasts. Journal of Lipid Research. 1989. UI 19911438981
Diet

Kelley DS, Rasooly R, Jacob RA, et al. Consumption of Bing sweet cherries lowers circulating concentrations of inflammation markers in healthy men and women. Journal of Nutrition. 2006. UI 16549461
Bing cherries

Kim MK, Campos H. Intake of trans fatty acids and low-density lipoprotein size in a Costa Rican population. Metabolism: Clinical & Experimental. 2003. UI 12800093
Trans fatty acids

Kondo A, Morita H, Nakamura H, et al. Influence of fibrate treatment on malondialdehyde-modified LDL concentration. Clinica Chimica Acta. 2004. UI 14687899
Fibrate

Kontopoulos AG, Athyros VG, Papageorgiou AA, et al. Effects of simvastatin and ciprofibrate alone and in combination on lipid profile, plasma fibrinogen and low density lipoprotein particle structure and distribution in patients with familial combined hyperlipidaemia and coronary artery disease. Coronary Artery Disease. 1996. UI 8993943
Simvastatin, Ciprofibrate

Kratz M, Gulbahce E, von EA, et al. Dietary mono- and polyunsaturated fatty acids similarly affect LDL size in healthy men and women. Journal of Nutrition. 2002. UI 11925466
Dietary fats

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Diet, low fat

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Pioglitazone, Metformin, Sulfphonylurea

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Step 2 Diet, Fish

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Cerivastatin

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Pioglitazone, Diet & Exercise

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Psyllium, Sterols

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Atorvastatin, Pravastatin

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Gemfibrozil, Omega 3

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Tobacco, Beta-blocker

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Omega 3, Exercise

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Gemfibrozil

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Nateglinide, Glibenclamide

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Atrovastatin, Simvastatin

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Estrogen, Simvastatin

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Estrogen

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Estrogen

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Estrogen

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Tamoxifen

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Estrogen, Simvastatin

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