Nuclear Magnetic Resonance Spectroscopy of Lipoproteins and Risk of Coronary Heart Disease in the Cardiovascular Health Study

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Objectives—Relationships between incident cardiovascular disease and lipoprotein subclass measurements by nuclear magnetic resonance spectroscopy were evaluated in the Cardiovascular Health Study (CHS) in a nested case-cohort analysis.

Methods and Results—The case group consisted of 434 participants with incident myocardial infarction (MI) and angina diagnosed after entry to the study (1990 to 1995) and the comparison group, 249 “healthy” participants with no prevalent clinical or subclinical disease. By univariate analysis, the median levels for healthy participants versus participants with incident MI and angina were 0 versus 7 mg% for small low density lipoprotein (LDL), 1501 versus 1680 nmol/L for the number of LDL particles, and 21.6 versus 21.3 for LDL size, and these values were significantly different between “healthy” participants and those with incident MI and angina for women but not men. The levels of less dense LDL, which is most of the total LDL cholesterol among women, was not related to incident MI and angina. For women, large high density lipoprotein cholesterol (HDLc), but not small HDLc, levels were significantly higher for healthy participants compared with levels for participants with MI and angina. For men and women, levels of total and very low density lipoprotein triglycerides were higher for the case group than for the healthy group. In multivariate models for women that included triglycerides and HDLc, the number of LDL particles (but not LDL size) remained significantly related to MI and angina.

Conclusions—Small LDL, the size of LDL particles, and the greater number of LDL particles are related to incident coronary heart disease among older women. (Arterioscler Thromb Vasc Biol. 2002;22:1175-1180.)

Key Words: lipoproteins ■ women ■ subclinical disease ■ aging

The relationship between lipoprotein levels, especially LDL cholesterol (LDLc), and the risk of clinical cardiovascular disease (CVD) is much weaker in older than in younger individuals.1 There are several possible reasons for the decline in the magnitude of relative risk with age.

First, lipid levels may decline in some individuals with advancing age.2,3 The decline in LDLc is often associated with various chronic diseases, inflammation, and weight loss.2 Therefore, the LDLc levels measured in older individuals may not reflect a true estimate of their usual adult LDLc levels.

Second, the LDLc levels may be primary determinants of the extent of atherosclerosis rather than clinical events.4 The presence of subclinical disease, such as carotid intima–medial wall thickness, high coronary calcium scores, and decreased ankle-brachial blood pressure, all increase with advancing age.5 Comparison of incident cases of coronary heart disease (CHD) and noncases (controls) may be substantially modified among older individuals by the amount of subclinical disease.

Third, the measurement of total cholesterol, LDLc, and HDL cholesterol (HDLc), for instance, may not accurately reflect the levels of the specific lipoprotein subclasses that are associated with increased risk of CVD among older individuals.6–8 A distribution favoring small dense LDL particles, called pattern B, has been associated with an increased risk of CHD.7 It is possible, for example, that although LDLc levels decrease with advancing age, the subclass distribution of the LDL particles might also change, possibly toward a more favorable distribution. The correlation between the small LDL particles and total LDLc may be relatively weak so that measurement of total LDLc does not provide a good estimate of risk.

We have evaluated the relationship of VLDL, LDL, and HDL concentrations and subclass levels measured by nuclear
magnetic resonance (NMR) spectroscopy to the risk of CHD among Cardiovascular Health Study (CHS) participants. We have compared incident cases with a group with minimal subclinical atherosclerosis as well as with a traditional control group. We have further determined whether lipoprotein subclasses add prediction above and beyond traditional measurements of LDLc, HDLc, and triglycerides.

The present study will focus, primarily, on LDL subclasses (L1, small LDL; L2, medium LDL; and L3, large LDL), average LDL size, and concentration of LDL particles. The primary end points in the present study are myocardial infarction (MI) and angina combined. Results, however, are similar for MI and angina separately.

The specific hypotheses that we have tested are as follows: (1) the measures of lipoproteins by NMR spectroscopy, specifically, the number of LDL particles and size, are related to risk of CHD; (2) these lipoprotein markers are related to measures of subclinical atherosclerosis; (3) the lipoproteins are associated with risk of CHD, independent of LDLc measurement, by chemical measurement; and (4) the lipoproteins are associated, independent of other cardiovascular risk factors, with the risk of CHD.

Methods

Subjects

The original sample of the CHS included 5201 adults aged ≥65 years recruited from a defined sample of Medicare files in 4 communities in the United States. The eligible participants were noninstitutionalized and were expected to remain in the area for at least 3 years. They were able to give informed consent and did not require a proxy to respond at baseline. Participants were recruited to the study between June 1989 and May 1990. The original sample of 5201 participants included 57% women and was 95% white. In 1992 to 1993, 687 additional African American participants were recruited to the study in 3 of the 4 communities by methods similar to those used for the original recruitment from the Medicare files.

A case-cohort evaluation of NMR spectroscopic analysis of lipoproteins was implemented by using cardiovascular events through June 1995. At that time, we had insufficient follow-up on the African Americans recruited in 1992 to 1993; thus, only the original cohort members were selected for this case-cohort study. Anyone who had clinical CVD at baseline (defined as angina, stroke, MI, or transient cerebral ischemia) was excluded from the study. We created several case groups for analysis.

An incident MI group consisted of all participants with an incident MI before June 30, 1995, and no stroke before the MI (n=217). An incident angina group included participants with angina but no MI or stroke (n=226).

There were 2 comparison groups. One was a “healthy subgroup.” which, in addition to having no clinical disease, also had no brain infarcts ≥3 mm on MRI, had maximum carotid intima–medial wall thickness <20th percentile, had an ankle-arm index >1, had maximum carotid stenosis <25%, had no major ECG abnormalities by Minnesota code, had no abdominal aneurysm ≥3 cm on ultrasound, and had normal wall motion abnormality on echocardiogram. Only 68 men satisfied the criteria for this group, so all were included. Of the 213 women eligible, 182 were randomly selected in this group to bring the total to 250. We call this the healthy group.

The second comparison group (labeled controls), consisting of 500 individuals, was randomly sampled from 2516 of the individuals remaining who had no prevalent or incident CHD and did not meet the criteria of the healthy group.

To test the hypothesis that the NMR lipoprotein levels were related to subclinical atherosclerosis, the control group was then further subclassified according to whether they had subclinical or no subclinical CVD at the time of entry to the CHS on the basis of prior criteria of the CHS. This group was then compared with the healthy subgroup.

Laboratory Methods

In the CHS, plasma total cholesterol, HDLc, and triglycerides were measured by enzymatic methods as described. The CHS central laboratory was standardized by the Centers for Disease Control for lipoprotein measurements. The coefficients of variation for total cholesterol, triglycerides, and HDLc were 1.66%, 1.78%, and 2.15%, respectively.

Aliquots (0.5 mL) of EDTA plasma stored at −80°C at the CHS central laboratory were shipped on dry ice to Lipomed, Inc. for NMR lipoprotein subclass analysis. In brief, the NMR method uses the characteristic signals broadcast by lipoprotein subclasses of different size as the basis of their quantification. Each measurement produces the concentrations of 6 VLDL, 4 LDL (including IDL), and 5 HDL subclasses. VLDL subclass levels are given in mass concentration units of milligrams per deciliter triglyceride, and LDL and HDL subclass levels are given as milligrams per deciliter cholesterol. From the subclass levels are calculated weighted-average VLDL, LDL, and HDL particle sizes (nanometer diameter), LDL particle concentration (nanomolar), and estimates of total cholesterol, HDLc, LDLc total triglyceride, and VLDL triglyceride. To simplify the data analysis, we grouped some of the subclasses to yield the following 10 subclass categories: large VLDL (60 to 200 nm), medium VLDL (35 to 60 nm), small VLDL (27 to 35 nm), IDL (23 to 27 nm), L3 (21.3 to 23 nm), L2 (19.8 to 21.2 nm), L1 (18.3 to 19.7 nm), large HDL (8.8 to 13.0 nm), medium HDL (8.2 to 8.8 nm), and small HDL (7.3 to 8.2 nm). LDL and HDL subclass distributions determined by NMR and gradient gel electrophoresis are highly correlated. LDL subclass diameters, which are consistent with electron microscopy data, are uniformly ~5 nm smaller than those estimated by gradient gel electrophoresis. There was no prior evidence of changes in NMR measurements related to the storage of samples over time.

Statistical Methods

Many NMR lipoprotein values were not normally distributed. Two of the subclass variables, L1 and L2, had values of 0 for many participants. Therefore, medians and interquartile ranges were used to describe the measurements, and nonparametric correlations were used to examine bivariate associations.

Logistic regression analysis was used to determine odds ratios (ORs) for each of the lipid or lipoprotein measurements comparing cases with either the control group or the healthy group. Unadjusted analyses were performed for each of LDLc (CHS and NMR), LDL particle concentration, LDL size, and L1. Then, models were built adjusting for conventional CVD risk factors in 3 models; built adjusting for LDLc (CHS), LDL size, and L1. Then, models were built adjusting for conventional CVD risk factors in 3 models. To simplify the data analysis, we grouped some of the subclasses to yield the following 10 subclass categories: large VLDL (60 to 200 nm), medium VLDL (35 to 60 nm), small VLDL (27 to 35 nm), IDL (23 to 27 nm), L3 (21.3 to 23 nm), L2 (19.8 to 21.2 nm), L1 (18.3 to 19.7 nm), large HDL (8.8 to 13.0 nm), medium HDL (8.2 to 8.8 nm), and small HDL (7.3 to 8.2 nm). LDL and HDL subclass distributions determined by NMR and gradient gel electrophoresis are highly correlated. LDL subclass diameters, which are consistent with electron microscopy data, are uniformly ~5 nm smaller than those estimated by gradient gel electrophoresis. There was no prior evidence of changes in NMR measurements related to the storage of samples over time.

Analysis was performed with the use of SPSS, version 10. Only ~5% of the participants were on lipid-lowering drugs at the time of their entry to the CHS (1989 to 1990). Exclusions of individuals on lipid-lowering drugs at entry did not affect the results.

Results

At entry, the mean age of the participants was 73 (SD 5.5 years), and the median age was 71 years. There was a high correlation between the levels of LDLc measured by the CHS laboratory and the levels derived by NMR spectroscopy (r=0.85). The L3 subclass was correlated less strongly with the LDLc (CHS) value (r=0.31), as was L2 (r=0.18) and L1 (r=0.10).
The number of LDL particles was also correlated with LDLc (CHS, \(r = 0.72\)). However, there was no significant association of LDL size and LDLc (CHS). The median levels and interquartile ranges of LDLc were higher as estimated by NMR, 149 (125 to 175) mg/dL, compared with those measured by the CHS, 128 (106 to 152) mg/dL.

The levels of LDLc (CHS and NMR), LDL size, and LDL particles were significantly different between the incident angina and MI case group and the healthy group (Table) for women, but not men.

Levels of L1 were significantly higher for the case group than for the healthy group among women. Levels of L1 and L2 were very low for women compared with men (Table). Triglyceride levels (CHS) and VLDL triglycerides (NMR) were higher for the case group compared with the healthy group for both men and women. The differences between the MI and angina case group and the control group, which included participants with extensive subclinical CVD, were much smaller than those for the healthy group.

In women, after adjustment for age and race, there was a statistically significant positive linear relationship in the odds ratio of MI and angina for LDL particles and for total LDLc (NMR or CHS) and an inverse relationship with LDL size. These associations are illustrated in Figure 1 by ORs for each quartile of the several LDL measurements. Similarly, there was a significant positive relationship between total triglycerides (CHS or NMR) and VLDL subclass levels and the MI, angina cases compared with the healthy group for women (Figure 2). A weaker, generally nonsignificant trend for men was also identified. Levels of the large HDL subclass and HDLc, as measured by the CHS chemical methods, were inversely related to MI and angina cases compared with the healthy CHS group for both men and women (Figure 3).

In women, in bivariate analysis, LDL particle concentration (OR 1.11/100 mmol/L, 1.03 to 1.09) was significantly different between the MI and angina case group and the healthy group, even when LDLc (CHS) was included in the analysis. Similarly, LDL size (OR 0.68, 0.51 to 0.91), the presence of any L1 (OR 1.77, 1.12 to 2.30), and CHS LDLc (OR 1.13/10 mg, 1.06 to 1.21) were all significant in a bivariate analysis.

To test the hypothesis that the lipoprotein levels and distribution were related to subclinical disease in the absence of clinical CVD, we next compared the healthy group with the control group stratified by the presence or absence of subclinical disease (see Methods). We evaluated 4 groups: (1) the healthy group, excluding a few that had subclinical disease by our more stringent CHS criteria; (2) the controls with subclinical disease by prior CHS criteria but no clinical CVD, incident stroke, or transient ischemic attack; (3) the controls without subclinical disease or incident clinical CVD,
stroke, or transient ischemic attack (they were similar to the healthy group except that they could have abdominal aneurysm or MRI infarcts because these were not included in the original CHS subclinical disease criteria; the hypothesis, therefore, is that the controls without subclinical disease would have lipoprotein levels similar to those of the healthy participants, whereas controls with subclinical disease would have levels similar to those of the cases with angina and MI); and (4) those previously described as experiencing incident angina and MI.

In women, lipid levels in the subclinical disease group were higher than lipid levels in the healthy group, whereas lipid levels in the group without subclinical disease were similar to those in the healthy group. For example, median LDLc (NMR) for women was 142 mg/dL for the healthy group, 152 mg/dL for the group with no subclinical disease, 161 mg/dL for the group with subclinical disease, and 159 mg/dL for the incident MI and angina case group. These differences among the 4 groups was statistically significant. The healthy group, compared with the group with subclinical disease, had lower numbers of LDL particles (Figure 4) and larger LDL size (Figure 5) for women but not for men.

LDL subclass levels, particle size, and particle concentration are associated with factors such as waist circumference, glucose, and insulin, which may also be important determinants of the risk of CHD. For both men and women, L1 and L2 were directly correlated (by Spearman test, data not shown) with fasting glucose, insulin, waist circumference, and CRP. The levels of L3 were inversely correlated with glucose, insulin, waist circumference, and CRP in women and men. LDL size was inversely related and the number of LDL particles was directly related to these 4 variables mentioned above. The inclusion of these risk factors in multivariate models (and, in addition, chemically measured LDLc, HDLc, and triglycerides) did not eliminate the association between the number of LDL particles in women and the odds of angina and MI compared with the healthy group (OR 1.11, 1.04 to 1.18 per 100 particles). The association of LDL size, however, was no longer statistically significant after adjustment for these risk factors.

Discussion

In the present study, we have shown that the number of LDL particles and LDL size are risk factors in women for the angina and MI group compared with the healthy group. This approach is similar to traditional analyses among younger and middle-aged individuals because of the lower prevalence of subclinical disease for controls without clinical CVD. There were practically no men (n=67) in the healthy group.

We have previously reported that measures of subclinical disease in the CHS are related to cardiovascular risk factors and are strongly related to the risk of clinical disease. Among those with diabetes, measures of subclinical disease
were the primary determinants of clinical disease.\textsuperscript{13} A measure of coronary atherosclerosis, such coronary calcium, would be a more direct test of the association of lipoprotein with subclinical disease. In a recent study,\textsuperscript{17} we showed that there is a very strong association ($P=0.0001$) in the CHS of subclinical measures and the extent of coronary calcium: median numbers were 683 (clinical), 374 (subclinical), and 122 (with no subclinical disease). In preliminary analysis in a small subsample, NMR lipoprotein level of L1 (but not L2 or L3) and number of LDL particles were strongly and significantly related to coronary calcium in women but not in men.

There is strong evidence that small dense LDL particles are important determinants of the risk of CHD.\textsuperscript{18–21} Higher levels of small LDL are usually associated with higher levels of triglycerides and lower levels of HDLc.\textsuperscript{22} Whether cardiovascular risk is independent of other risk factors is still controversial.\textsuperscript{23–27}

The CHS, in an older population, has shown that total LDLc is a relatively weak predictor of CVD, especially when the comparison group (controls) includes a large number of older individuals who have subclinical CVD. The levels of total LDLc and other lipids (ie, triglycerides and HDLc) are important risk factors for the extent of subclinical atherosclerosis. Therefore, it is not unreasonable to expect that atherosclerosis risk factors may be important CVD event risk factors when cases are compared with controls with little subclinical disease (as is true in most studies of younger people) but that they may not be potent CVD event risk factors in older people or in those with clinical CHD,\textsuperscript{28} a condition in which controls have significant subclinical disease. This appears to be the case in many instances and is the case in the present study of CHD risk predicted by NMR lipoprotein markers.\textsuperscript{1–3} To our knowledge, the present study is the first to use a rich database to provide controls (the minimal subclinical disease group) in a unique way that allows the separation of atherosclerosis risk from event risk in the elderly.

In recent reports from the Healthy Women Study, which involves women in their early 60s, we reported that NMR-measured LDL size and the number of LDL particles were strongly related to the extent of carotid intima–medial wall thickness and the extent of coronary calcium as measured by electron beam computed tomography.\textsuperscript{29–31} The large LDL subclass (L3) was not related to the extent of subclinical disease, either by carotid or coronary calcium assessment; this was similar to the results in the CHS. The L3 subclass is the primary component of LDL in women and, apparently, is unrelated to atherosclerosis. The difference in lipoprotein subclass distribution between men and women\textsuperscript{32} (with men having more small LDL than women) may account for the higher rates of MI among men than postmenopausal women in spite of similar total LDLc levels.

Determination of whether measurements of LDL subclass levels, particle size, or particle number are of clinical importance in evaluating an individual patient will depend on further clinical and epidemiological studies, including the evaluation of these LDL markers, in ongoing longitudinal studies that also include measurements of underlying subclinical atherosclerosis. In addition, more data are needed about the effects exerted by pharmacological and nonpharmacological therapies on LDL subclasses and particle concentrations and the relations of these effects on subsequent outcomes in clinical trials, especially in primary prevention. Our data suggest that at least for women, measurement of number and size of LDL particles may be important.

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