

# Lipoprotein Subclasses Determined by Nuclear Magnetic Resonance Spectroscopy and Coronary Atherosclerosis in Patients with Rheumatoid Arthritis

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**ABSTRACT. Objective.** Patients with rheumatoid arthritis (RA) are at increased risk of atherosclerosis, but routine lipid measurements differ little from those of people without RA. We examined the hypothesis that lipid subclasses determined by nuclear magnetic resonance spectroscopy (NMR) differed in patients with RA compared to controls and are associated with disease activity and the presence of coronary-artery atherosclerosis.

**Methods.** We measured lipoprotein subclasses by NMR in 139 patients with RA and 75 control subjects. Lipoproteins were classified as large low-density lipoprotein (LDL; diameter range 21.2–27.0 nm), small LDL (18.0–21.2 nm), large high-density lipoprotein (HDL; 8.2–13.0 nm), small HDL (7.3–8.2 nm), and total very low-density lipoprotein (VLDL;  $\geq 27$  nm). All subjects underwent an interview and examination; disease activity was quantified by the 28-joint Disease Activity Score (DAS28) and coronary artery calcification (CAC) was measured with electron-beam computed tomography.

**Results.** Concentrations of small HDL particles were lower in patients with RA ( $18.2 \pm 5.4$  nmol/l) than controls ( $20.0 \pm 4.4$  nmol/l;  $p = 0.003$ ). In patients with RA, small HDL concentrations were inversely associated with DAS28 ( $\rho = -0.18$ ,  $p = 0.04$ ) and C-reactive protein ( $\rho = -0.25$ ,  $p = 0.004$ ). Concentrations of small HDL were lower in patients with coronary calcification ( $17.4 \pm 4.8$  nmol/l) than in those without ( $19.0 \pm 5.8$  nmol/l;  $p = 0.03$ ). This relationship remained significant after adjustment for the Framingham risk score and DAS28 ( $p = 0.025$ ). Concentrations of small LDL particles were lower in patients with RA ( $1390 \pm 722$  nmol/l) than in controls ( $1518 \pm 654$  nmol/l;  $p = 0.05$ ), but did not correlate with DAS28 or CAC.

**Conclusion.** Low concentrations of small HDL particles may contribute to increased coronary atherosclerosis in patients with RA. (First Release June 1 2010; *J Rheumatol* 2010;37:1633–8; doi:10.3899/jrheum.090639)

## Key Indexing Terms:

LIPOPROTEIN

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

CORONARY ATHEROSCLEROSIS

RHEUMATOID ARTHRITIS

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Supported by grants HL65082, HL67964, HL65709, 1UL1 RR024975, and P60 AR056116 from the National Institutes of Health.

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Accepted for publication March 12, 2010.

Dyslipidemia, as determined by conventional measurements of total cholesterol, triglycerides, and high-density (HDL) and low-density lipoprotein (LDL) cholesterol, is a widely recognized cardiovascular risk factor in the general population<sup>1,2</sup>. Because cardiovascular risk is increased in patients with rheumatoid arthritis (RA), substantial interest has been focused on the role of abnormal lipid concentrations. Concentrations of LDL cholesterol are generally not elevated in RA<sup>3</sup>, but in some studies HDL cholesterol concentrations are decreased<sup>1</sup>, even before RA becomes clinically apparent<sup>2</sup>. Nevertheless, conventional HDL and LDL cholesterol concentrations have limited capacity to predict cardiovascular risk in RA<sup>3</sup> and, as we have previously shown, are not associated with coronary artery atherosclerosis in these patients<sup>4</sup>.

Recent evidence suggests that concentrations of specific lipid subfractions, as determined by nuclear magnetic resonance spectroscopy (NMR), are important in the initiation and progression of atherosclerosis<sup>5</sup>, and measurement of

these subfractions may improve the prediction of coronary risk<sup>6</sup>. Individuals with similar conventional lipid profiles could have significant differences in the distribution of specific very low-density lipoprotein (VLDL), LDL, and HDL lipoprotein subfractions, possibly resulting in differences in cardiovascular risk<sup>6,7</sup>. The mechanisms underlying individual differences in lipid subfraction concentrations and size are not clear, but inflammation is one factor that can modify lipid subfractions and result in a more atherogenic profile<sup>8</sup>.

We examined the hypothesis that lipid subclasses differed in patients with RA compared to control subjects and that these differences were associated with disease activity and with the presence of coronary artery atherosclerosis.

## MATERIALS AND METHODS

**Patients.** Our subjects were part of a cohort participating in studies to characterize the relationship between RA and atherosclerosis<sup>9</sup>. A total of 139 patients who met the classification criteria for RA<sup>10</sup> and 75 controls with no inflammatory disease were included in this study. All subjects were older than 18 years of age and were not taking lipid-lowering agents.

As described<sup>4,9,11,12</sup>, patients were recruited from clinical RA cohorts, an early RA registry, local rheumatologists, and by advertisements. Controls did not have RA or inflammatory arthritis and were recruited from patients' acquaintances, by advertisement, and from a database of volunteers maintained by the Vanderbilt General Clinical Research Center. Patients with RA and controls were frequency-matched for age, sex and race. The study was approved by the Institutional Review Board of Vanderbilt University Hospital. All subjects gave written informed consent.

**Clinical assessment.** Patient assessment included a structured interview, self-report questionnaires, physical examination, laboratory tests, and electron-beam computed tomography (CT), and in patients, review of medical records. Height and weight were measured and body mass index (BMI) calculated. Blood pressure was determined as the average of 2 measurements obtained 5 minutes apart after subjects had rested for at least 10 minutes. Hypertension was defined as current use of antihypertensive agents, or systolic blood pressure  $\geq 140$  mm Hg, or diastolic pressure  $\geq 90$  mm Hg. In patients, disease activity was measured using the Disease Activity Score based on 28 joints (DAS28)<sup>13</sup>. Functional capacity was measured using the modified Health Assessment Questionnaire (MHAQ)<sup>14</sup>.

**Lipoprotein subclasses.** After an overnight fasting period, blood was drawn. Plasma samples, stored at  $-70^{\circ}\text{C}$ , were analyzed by commercial proton NMR spectroscopy assay at Liposcience Inc., Raleigh, NC, USA. Quantitation is based on the spectral signals emitted by the amplitudes of the characteristic lipid methyl group NMR signals that they emit<sup>15</sup>. Concentrations of large LDL (diameter range 21.2–27.0 nm), small LDL (18.0–21.2 nm), large HDL (8.2–13.0 nm), small HDL (7.3–8.2 nm), and total VLDL ( $\geq 27$  nm) and mean particle size (in nanometer diameter units) were measured.

**Coronary artery calcification.** All subjects underwent imaging with an Imatron C-150 scanner (Imatron, South San Francisco, CA, USA) in order to derive an Agatston score, as described<sup>16</sup>. This is a noninvasive imaging technique to detect coronary artery calcification as a measure of coronary atherosclerosis burden. One investigator (PR), blinded to any clinical information, read all the scans and provided an overall calcium score for each subject based on the sum of the scores of each individual coronary artery.

**Other laboratory tests.** Total cholesterol, HDL and LDL cholesterol, and triglycerides were measured in all subjects. Insulin concentrations were measured using multiplex ELISA (Lincoplex, Millipore, St. Louis, MO, USA) and the homeostasis model assessment (HOMA) index [defined as

fasting glucose (mmol/l)  $\times$  fasting insulin ( $\mu\text{U/ml}$ )/22.5] was calculated to quantify insulin sensitivity. C-reactive protein (CRP) was measured in patients with RA.

**Statistical methods.** Demographic characteristics are presented as mean and standard deviation (SD) for continuous variables, and frequency (percentage) for categorical variables. The differences among cases and controls were determined by Wilcoxon rank-sum or Fisher's exact test, as appropriate. Analysis was performed in 2 steps: first, lipid subparticle concentrations and size were compared by Wilcoxon rank-sum test in RA patients and controls, and in RA patients with and without coronary artery calcification. Spearman correlations were calculated to examine the association between lipoprotein subfractions and metabolic and inflammatory variables. Second, in patients with RA, a multivariate logistic regression was modeled to examine the association between small HDL concentrations and coronary calcification after adjustment for the Framingham risk score and disease activity. All analyses used a 2-sided significance level of 5% and were performed with Stata 10.0 (Stata Corp., College Station, TX, USA).

## RESULTS

**Patients and controls.** Patients with RA and controls were of similar age ( $54 \pm 12$  yrs and  $52 \pm 12$  yrs, respectively;  $p = 0.23$ ) and sex (70.5% and 65.0% female, respectively;  $p = 0.44$ ). There were no significant differences between the groups in cumulative pack-years of smoking, diabetes, BMI, total, HDL or LDL cholesterol, or triglyceride concentrations. More patients with RA were current smokers (23% compared to 11% of controls), and there was a trend toward higher systolic and diastolic blood pressure in patients with RA (Table 1). The mean disease duration of RA was  $10.3 \pm 11.1$  years and the mean DAS28 was  $3.7 \pm 1.6$ . There were 77 (55.4%) patients taking corticosteroids, 98 (71%) taking methotrexate, and 28 (20%) taking anti-tumor necrosis factor (TNF) drugs. The median (interquartile range) Agatston score for patients with a calcium score greater than zero was 129 (range 32–425).

*Lipoprotein subclasses in patients with RA and controls.*

Table 1. Clinical characteristics of 139 patients with RA and 75 controls. Data are n (%) or mean  $\pm$  SD.

| Characteristics                         | RA,<br>n = 139  | Controls,<br>n = 75 | p    |
|---|-----------------|---------------------|------|
| General characteristics                 |                 |                     |      |
| Age, yrs                                | 54 $\pm$ 12     | 52 $\pm$ 12         | 0.23 |
| Female, %                               | 98 (70.5)       | 49 (65)             | 0.44 |
| Caucasian, %                            | 122 (87.8)      | 63 (84.0)           | 0.23 |
| Traditional cardiovascular risk factors |                 |                     |      |
| Systolic blood pressure, mm Hg          | 133 $\pm$ 20    | 128 $\pm$ 17        | 0.05 |
| Diastolic blood pressure, mm Hg         | 75 $\pm$ 11     | 72 $\pm$ 9          | 0.07 |
| Body mass index, kg/m <sup>2</sup>      | 28.7 $\pm$ 6.1  | 28.1 $\pm$ 5.4      | 0.54 |
| Cumulative smoking, pack-yr             | 12.9 $\pm$ 22.0 | 10.9 $\pm$ 23.3     | 0.29 |
| Diabetes (%)                            | 11 (7.9)        | 3 (4)               | 0.39 |
| Traditional lipid profile               |                 |                     |      |
| Total cholesterol, mg/dl                | 188 $\pm$ 40    | 192 $\pm$ 34        | 0.31 |
| High-density lipoprotein, mg/dl         | 49 $\pm$ 21     | 47 $\pm$ 13         | 0.86 |
| Low-density lipoprotein, mg/dl          | 114 $\pm$ 33    | 122 $\pm$ 30        | 0.06 |
| Triglycerides, mg/dl                    | 135 $\pm$ 183   | 115 $\pm$ 62        | 0.37 |
| Glucose, mg/dl                          | 90 $\pm$ 16     | 93 $\pm$ 38         | 0.69 |

Table 2 and Figure 1 show the concentrations of lipoprotein subclasses among patients with RA and controls. Concentrations of small HDL particles were significantly lower in patients with RA ( $18.2 \pm 5.4$  nmol/l) than in controls ( $20.0 \pm 4.4$  nmol/l;  $p = 0.003$ ). Concentrations of small LDL particles were lower in patients with RA ( $1390 \pm 722$  nmol/l) than in controls ( $1518 \pm 654$  nmol/l;  $p = 0.05$ ). There were no other significant differences in lipid subclasses among patients with RA and controls.

*Association between NMR lipoprotein subclasses and metabolic variables and markers of inflammation.* Table 3 shows the associations between NMR lipoprotein subclass and the traditional lipid profile with BMI, HOMA index, CRP, disease activity, and coronary calcium score in patients with RA. Small LDL concentrations were positively correlated with BMI and insulin resistance, whereas large LDL con-

Table 2. Lipoprotein particle concentration and size in patients with RA and controls, by nuclear magnetic resonance spectroscopy. Data are n (%), or mean  $\pm$  SD.

| Characteristics             | RA,<br>n = 139  | Controls,<br>n = 75 | p     |
|-----------------------------|-----------------|---------------------|-------|
| <b>LDL particles</b>        |                 |                     |       |
| Large LDL particles, nmol/l | 442 $\pm$ 175   | 397 $\pm$ 157       | 0.10  |
| Small LDL particles, nmol/l | 1390 $\pm$ 722  | 1518 $\pm$ 654      | 0.05  |
| LDL size, nm                | 21.0 $\pm$ 0.7  | 20.8 $\pm$ 0.7      | 0.10  |
| <b>HDL particles</b>        |                 |                     |       |
| Large HDL particles, nmol/l | 10.7 $\pm$ 4.8  | 10.7 $\pm$ 5.3      | 0.88  |
| Small HDL particles, nmol/l | 18.2 $\pm$ 5.4  | 20.0 $\pm$ 4.4      | 0.003 |
| HDL size, nm                | 9.1 $\pm$ 0.5   | 9.0 $\pm$ 0.4       | 0.11  |
| <b>VLDL particles</b>       |                 |                     |       |
| Total VLDL, nmol/l          | 65.7 $\pm$ 38.1 | 63.4 $\pm$ 25.9     | 0.86  |
| VLDL size, nm               | 50.4 $\pm$ 8.9  | 49.2 $\pm$ 6.9      | 0.58  |

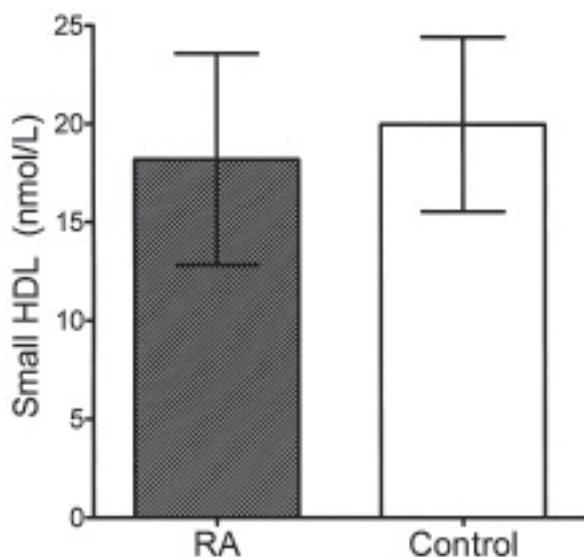


Figure 1. Small HDL cholesterol concentrations in patients with RA and controls. Error bars represent mean and SD;  $p = 0.003$

centrations were negatively correlated with these variables. Neither small nor large LDL concentrations were significantly correlated with DAS28, MHAQ, or CRP. Small HDL concentrations were inversely associated with DAS28 ( $\rho = -0.18$ ,  $p = 0.04$ ), MHAQ ( $\rho = -0.18$ ,  $p = 0.04$ ), and CRP ( $\rho = -0.25$ ,  $p = 0.004$ ).

*Association between NMR lipoprotein measurements and coronary calcium in patients with RA.* Small HDL concentrations were the only lipid subfraction correlated with the coronary calcium score and there was a weak inverse relationship ( $\rho = -0.18$ ,  $p = 0.03$ ; Table 3). In patients with RA who had coronary calcification, concentrations of small HDL ( $17.4 \pm 4.8$  nmol/l) were significantly lower than in those without coronary calcification ( $19.0 \pm 5.8$  nmol/l;  $p = 0.03$ ; Table 4, Figure 2). This association remained significant after adjustment for Framingham risk score and disease activity ( $p = 0.025$ ). A sensitivity analysis that excluded individuals with diabetes yielded results that were similar to those of the main analysis.

Patients with coronary calcification also tended to have lower HDL cholesterol concentrations ( $47 \pm 14$  mg/dl) than those without calcification ( $51 \pm 26$  mg/dl;  $p = 0.07$ ). There were no other statistically significant differences in lipid subclasses among RA patients with and those without coronary calcification. Lipid concentrations measured by conventional methods were not associated with coronary calcium score (Table 3), nor was the total cholesterol/HDL ratio ( $\rho = 0.06$ ,  $p = 0.48$ ).

*Association between NMR lipoprotein measurements and disease-specific treatment in patients with RA.* Patients with RA taking prednisone had higher concentrations of large LDL ( $470 \pm 180$  nmol/l vs  $400 \pm 159$  nmol/l;  $p = 0.02$ ) and lower concentrations of small LDL ( $1225 \pm 640$  nmol/l vs  $1607 \pm 797$  nmol/l;  $p = 0.005$ ) than those who were not. Small ( $17.8 \pm 5.5$  nmol/l vs  $18.9 \pm 5.1$  nmol/l;  $p = 0.22$ ) and large HDL ( $11.4 \pm 5.1$  nmol/l vs  $9.8 \pm 4.2$  nmol/l;  $p = 0.08$ ) concentrations did not differ significantly among patients taking or not taking prednisone. However, no significant differences in lipoprotein subclasses were found among patients receiving anti-TNF therapy, conventional disease-modifying antirheumatic drugs, and both therapies in combination (all  $p > 0.40$ ).

## DISCUSSION

Our study shows that patients with RA have lower concentrations of small HDL cholesterol, and to our knowledge is the first to suggest that in these patients, lower concentrations of small HDL cholesterol are associated with increased risk of atherosclerosis as measured by coronary calcification.

Clinical screening for traditional cardiovascular risk includes measurement of total cholesterol, triglycerides, and HDL cholesterol<sup>17</sup>. In the general population, coronary artery disease risk is associated with both high concentrations of LDL and low concentrations of HDL cholesterol.

Table 3. Spearman correlation analyses between lipoprotein measurements by nuclear magnetic resonance spectroscopy and metabolic and clinical variables and coronary artery calcification in patients with RA.

| Characteristic            | Body Mass Index     | HOMA               | CRP                | DAS28              | MHAQ               | Coronary Calcium   |
|---------------------------|---------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| Lipid subfractions        |                     |                    |                    |                    |                    |                    |
| Large LDL                 | -0.22 <sup>†</sup>  | -0.19 <sup>†</sup> | 0.001              | -0.05              | -0.09              | 0.09               |
| Small LDL                 | 0.21 <sup>†</sup>   | 0.26 <sup>†</sup>  | 0.16               | 0.12               | -0.06              | 0.02               |
| Large HDL                 | -0.13               | -0.15              | 0.01               | 0.04               | 0.15               | -0.07              |
| Small HDL                 | 0.20 <sup>†</sup>   | -0.12              | -0.25 <sup>†</sup> | -0.18 <sup>†</sup> | -0.18 <sup>†</sup> | -0.18 <sup>†</sup> |
| Traditional lipid profile |                     |                    |                    |                    |                    |                    |
| Total cholesterol         | -0.06               | -0.01              | -0.03              | -0.06              | -0.17 <sup>†</sup> | 0.08               |
| LDL cholesterol           | -0.003              | 0.07               | 0.01               | -0.01              | -0.21 <sup>†</sup> | 0.16               |
| HDL cholesterol           | -0.29 <sup>††</sup> | -0.24 <sup>†</sup> | -0.12              | -0.16              | 0.02               | -0.03              |
| Triglycerides             | 0.12                | 0.06               | 0.01               | 0.07               | -0.01              | -0.05              |

HOMA (homeostasis model assessment index): fasting glucose (mmol/l) × fasting insulin (μU/ml)/22.5. CRP: C-reactive protein; DAS28: Disease Activity Score 28-joint count; MHAQ: Modified Health Assessment Questionnaire. <sup>†</sup> p < 0.05, <sup>††</sup> p < 0.001.

Table 4. Lipoprotein particle concentration and size measured by nuclear magnetic resonance spectroscopy in patients with RA with and without coronary calcification. Data are mean ± SD.

| Characteristic                  | With Coronary Calcification, n = 68 | Without Coronary Calcification, n = 71 | p    |
|---------------------------------|-------------------------------------|--|------|
| LDL particles                   |                                     |  |      |
| Large LDL particles, nmol/l     | 458 ± 185                           | 426 ± 164                              | 0.44 |
| Small LDL particles, nmol/l     | 1411 ± 712                          | 1370 ± 736                             | 0.60 |
| LDL size, nm                    | 21.0 ± 0.7                          | 21.0 ± 0.7                             | 0.85 |
| HDL particles                   |                                     |  |      |
| Large HDL particles, nmol/l     | 10.7 ± 4.9                          | 10.7 ± 4.7                             | 0.71 |
| Small HDL particles, nmol/l     | 17.4 ± 4.8                          | 19.0 ± 5.8                             | 0.03 |
| HDL size, nm                    | 9.1 ± 0.5                           | 9.1 ± 0.5                              | 0.67 |
| VLDL particles                  |                                     |  |      |
| Total VLDL, nmol/l              | 63.2 ± 31.2                         | 68.1 ± 43.7                            | 0.63 |
| VLDL size, nm                   | 50.8 ± 9.4                          | 50.1 ± 8.4                             | 0.92 |
| Conventional lipid profile      |                                     |  |      |
| Total cholesterol, mg/dl        | 190 ± 38                            | 186 ± 43                               | 0.31 |
| High-density lipoprotein, mg/dl | 47 ± 14                             | 51 ± 26                                | 0.07 |
| Low-density lipoprotein, mg/dl  | 119 ± 33                            | 109 ± 33                               | 0.67 |
| Triglycerides, mg/dl            | 121 ± 66                            | 148 ± 248                              | 0.87 |
| Total cholesterol/HDL ratio     | 4.2 ± 1.3                           | 4.1 ± 1.6                              | 0.40 |

However, concentrations of LDL cholesterol generally have not been found to be elevated in RA<sup>18</sup>. In our study, LDL concentrations were marginally lower in patients with RA than controls, whereas HDL cholesterol concentrations did not differ. Some investigators have reported lower HDL cholesterol concentrations in RA; for example, a recent study comparing patients with and without RA in a US national sample aged 60 years or older found that HDL cholesterol was approximately 2.5 mg/dl lower in patients with RA meeting 3 or more American College of Rheumatology criteria, and 8.8 mg/dl lower in those meeting 4 or more criteria<sup>19</sup>. However, despite minor differences in HDL cholesterol reported, changes in the traditional cholesterol profile do not account for the increased atherosclerosis in patients with RA<sup>4</sup>.

Recent evidence suggests that the significant variation in cardiovascular risk observed in individuals with similar lipid profiles by conventional testing may be explained in part by variations in the distribution of lipid subclass concentrations<sup>6,7</sup>. Each individual lipoprotein class consists of particles of different diameter, density, and composition. The concentration of these subparticle fractions is associated with different cardiovascular risk. Higher concentrations of small LDL have been associated consistently with increased risk of atherosclerosis and coronary heart disease in many studies<sup>20</sup>, but less information is available regarding the clinical significance of HDL particle size. Studies have yielded conflicting results, with higher concentrations of small HDL associated with both increased and decreased risk of coronary heart disease<sup>21,22</sup>.

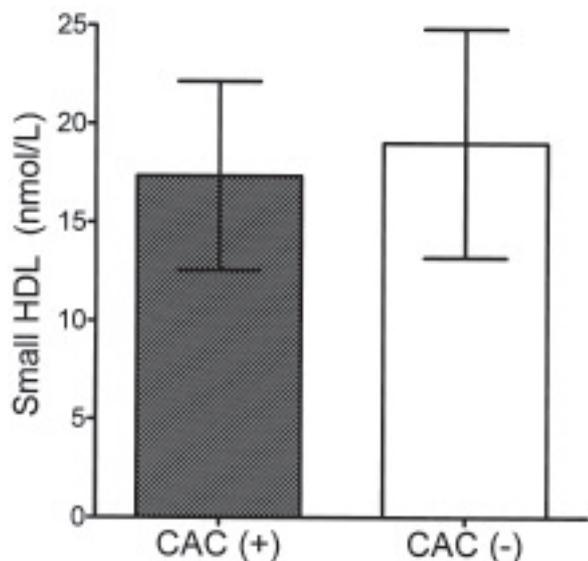


Figure 2. Small HDL cholesterol concentrations in RA patients with (+) and without (-) coronary artery calcification (CAC). Error bars represent mean and SD;  $p = 0.03$ .

Our results demonstrating differences in LDL and HDL cholesterol subclasses in RA are partly concordant with a previous report in patients with RA. In a cross-sectional study, Hurt-Camejo and colleagues<sup>23</sup> found that patients with RA ( $n = 31$ ) and controls ( $n = 28$ ) had similar concentrations of triglycerides and total and HDL cholesterol, whereas concentrations of small dense LDL were higher, and small HDL lower, in patients with RA. Also, Rizzo, *et al*<sup>24</sup> reported increased concentrations of small dense LDL in 25 patients with untreated RA but did not measure HDL subclasses. In contrast, we found that concentrations of small LDL tended to be lower in patients with RA compared to controls. It is difficult to explain the lower concentration of small LDL in our patients with RA. They had relatively well controlled RA, with a median DAS28 score of  $3.7 \pm 1.6$ , whereas patients in the studies of both Hurt-Camejo, *et al*<sup>23</sup> (mean erythrocyte sedimentation rate 65 mm/h, mean number of swollen joints 13) and Rizzo, *et al*<sup>24</sup> (average DAS28 score 6.2) had more active disease. Nevertheless, one would expect that the concentration of small LDL in patients with well controlled RA would be at least equivalent to that of controls. However, the effects of prednisone on lipoprotein subclasses are unclear; we found that current use of prednisone was associated with lower concentrations of small LDL. Our study and that of Hurt-Camejo, *et al*<sup>23</sup> found that concentrations of small HDL cholesterol were lower in patients with RA than in controls. Our study extends these observations and suggests that HDL particle size may be a more informative marker for atherosclerosis than traditional lipids in RA.

Several possible mechanisms may explain the association of low concentrations of small, dense HDL with increased atherosclerosis. First, since small HDL is a potent

antioxidant, then low levels might inadequately prevent oxidation of LDL and thus promote atherogenesis<sup>25</sup>. Second, the inverse association between small HDL concentrations and markers of active inflammation such as CRP and DAS28 suggests that this subfraction may link inflammation and vascular disease in RA. A potential mechanism for this could be serum amyloid A, which is increased by inflammation and impairs cellular cholesterol efflux to small HDL<sup>26</sup>. Third, small HDL may be the major subfraction mediating the antiinflammatory and antioxidant effects of HDL<sup>26</sup>. HDL is usually antiinflammatory, but can become proinflammatory under certain circumstances. In support of this idea, McMahon, *et al* reported that patients with RA have higher concentrations of proinflammatory HDL than controls<sup>27</sup>. It is thus possible that a contraction of the pool of small HDL signals a switch to a proinflammatory phenotype. However, possible relationships between proinflammatory HDL and particular lipoprotein subfractions remain poorly characterized. Fourth, complement regulatory proteins, protease inhibitors, and acute-phase response proteins have been identified in HDL, suggesting common pathways between this lipoprotein and inflammation and the innate immune system<sup>28</sup>.

Some limitations of this study should be considered. First, we used samples that were frozen at  $-70^{\circ}\text{C}$  for a period ranging between 6 and 36 months. However, previous studies showed stable concentrations of lipoprotein particles over 6 years under the same conditions<sup>6</sup>. Second, although some significant associations were seen, differences in the lipid profiles between patients and controls were small. Further, the significant differences among patients with and without coronary calcification were also small. Thus, the findings provide clues for further research rather than an explanation for the increased cardiovascular morbidity and mortality in RA. Third, this was a cross-sectional study and thus the temporal sequence of the events is unknown. Fourth, hyperglycemia and drugs to control diabetes could influence the HDL size. However, a sensitivity analysis that excluded individuals with diabetes yielded results that were similar to those of the main analysis. Fifth, given the number of different regimens of antiinflammatory and disease-modifying drugs used by patients, the individual effect of these on lipid profiles could not be examined in this study. Finally, NMR lipid analysis does not provide information about the function of lipoproteins.

In summary, low concentrations of small HDL particles may contribute to increased coronary atherosclerosis in patients with RA.

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