

Inflammatory/Antiinflammatory Properties of High-Density Lipoprotein Distinguish Patients From Control Subjects Better Than High-Density Lipoprotein Cholesterol Levels and Are Favorably Affected by Simvastatin Treatment

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Background—The inflammatory/antiinflammatory properties of HDL were compared with HDL cholesterol in 2 groups of patients and in age- and sex-matched control subjects.

Methods and Results—Group 1 consisted of 26 patients not yet taking a statin who presented with coronary heart disease (CHD) or CHD equivalents by National Cholesterol Education Program Adult Treatment Panel III criteria studied before and 6 weeks after 40 mg/d of simvastatin. Group 2 consisted of 20 patients with documented CHD and HDL cholesterol ≥ 84 mg/dL. The inflammatory/antiinflammatory properties of HDL were determined by the ability of the subject's HDL to alter LDL-induced monocyte chemotactic activity (MCA) in a human artery wall coculture. Induction of MCA by a control LDL was determined in the absence or presence of the subject's HDL. Values in the absence of HDL were normalized to 1.0. Values >1.0 after the addition of HDL indicated proinflammatory HDL; values <1.0 indicated antiinflammatory HDL. Group 1 values before simvastatin were LDL cholesterol, 118 ± 24 mg/dL; HDL cholesterol, 57 ± 13 mg/dL; triglycerides, 125 ± 64 mg/dL; and high-sensitivity C-reactive protein (hs-CRP), 1.7 ± 1.9 mg/L; and MCA values were 1.38 ± 0.91 , compared with 0.38 ± 0.14 for control subjects ($P=1.5 \times 10^{-5}$). After simvastatin, values were LDL cholesterol, 73 ± 24 mg/dL; HDL cholesterol, 61 ± 14 mg/dL; triglycerides, 99 ± 52 mg/dL; and hs-CRP, 1.3 ± 1.3 mg/L; and MCA values were 1.08 ± 0.71 . In group 2, values were LDL cholesterol, 108 ± 34 mg/dL; HDL cholesterol, 95 ± 14 mg/dL; triglycerides, 89 ± 44 mg/dL; and hs-CRP, 0.8 ± 0.7 mg/L; and MCA values were 1.28 ± 0.29 , compared with 0.35 ± 0.11 for control subjects ($P=1.7 \times 10^{-14}$). Similar results were obtained with the cell-free assay.

Conclusions—The inflammatory/antiinflammatory properties of HDL distinguished patients from control subjects better than HDL cholesterol and were improved with simvastatin. (*Circulation*. 2003;108:2751-2756.)

Key Words: PLEASE ■ SUPPLY ■ KEY ■ WORDS

High-density lipoprotein cholesterol is a powerful epidemiological predictor of risk for clinical events caused by coronary artery disease.¹ In the Air Force/Texas Coronary Prevention Study (AFCAPS/TexCAPS), subjects with "average" total cholesterol levels were followed up for an average of 5.2 years. Of those given placebo, the event rate during the study was 2.1%, 2.9%, and 3.4% for those with HDL cholesterol levels of ≥ 40 mg/dL, 35 to 39 mg/dL, and ≤ 34 mg/dL, respectively. Although the differences were highly significant, knowledge of the HDL cholesterol level in predicting whether a specific individual would or would not have an event was clearly of limited use.²

Similarly, in the original Framingham study, the incidence of coronary heart disease (CHD) was compared with HDL

cholesterol levels.³ With minimal assumptions, one can calculate from the published data that 44% of the events occurred in men with HDL cholesterol levels of ≥ 40 mg/dL and 43% of the events occurred in women with HDL cholesterol levels ≥ 50 mg/dL.

Because a significant number of CHD events occur in patients with normal LDL cholesterol levels and normal HDL cholesterol levels,^{3,4} there has been a continuing search for markers with better predictive value in an individual patient.⁵

We previously reported that the acute-phase response in humans converted HDL from antiinflammatory to proinflammatory.⁶ These studies⁶ compared HDL taken from humans before and after elective surgery. Before surgery, HDL was antiinflammatory in our coculture model, ie, it inhibited LDL

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oxidation and inhibited LDL-induced monocyte chemotactic activity (MCA). However, at the peak of the acute-phase response, 3 days after surgery, HDL from the same patient was proinflammatory (promoted LDL oxidation and MCA in the human artery wall coculture). One week after surgery, the HDL returned to an antiinflammatory state. Thus, these changes in HDL are consistent with a classic acute-phase response. Gabay and Kushner⁷ emphasized that the acute-phase response can become chronic. Apolipoprotein E-null mice on a chow diet and LDL receptor-null mice on a high-fat diet maintained elevated levels of the positive acute-phase reactant apolipoprotein J and showed persistent decreases in the negative acute-phase reactant paraoxonase.⁸ In humans, the persistent elevation (highest tertile) of the positive acute-phase reactant C-reactive protein (CRP) in the absence of a detectable infection or other acute stress is also evidence of a "chronic" acute-phase response.⁵

In preliminary studies, we reported that the inflammatory/antiinflammatory properties of HDL from 27 normolipidemic patients clearly separated the patients from 31 age- and sex-matched control subjects (controls).⁹ Each of the patients had a $\geq 50\%$ narrowing of a coronary artery, none smoked, none were diabetic, none were taking hypolipidemic medications, and all had normal blood lipids. The patient HDL, in contrast to control HDL, was proinflammatory both in our coculture model and in a cell-free assay (CFA).⁹ These patients had no evidence of an acute illness that could explain an acute-phase response. Thus, we postulated that the inflammatory properties of HDL in these patients represented a chronic acute-phase response similar to that described by CRP levels in the top tertile of "normal."⁵

To test this hypothesis further, we have studied 2 additional patient groups. Group 1 included patients who presented with stable CHD or CHD equivalents by National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP-III) criteria¹⁰ who were not yet on a statin or on other hypolipidemic agents and whose physicians recommended treatment with a statin. The inflammatory/antiinflammatory properties of HDL from these patients was compared before and 6 weeks after starting statin therapy. A second group of patients with high HDL cholesterol levels and documented CHD were studied. The inflammatory/antiinflammatory properties of the HDL from both groups of patients were compared with age- and sex-matched healthy controls.

Methods

Materials

1-Palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphorylcholine (PAPC) (catalogue No. 850459) was purchased from Avanti Polar Lipids, and 1-palmitoyl-2-(5,6-epoxyisoprostane E(2))-*sn*-glycero-3-phosphocholine (PEIPC) was prepared from PAPC as described previously.^{11,12} Dichlorofluorescein diacetate (DCFH-DA) was from Molecular Probes. Simvastatin was a kind gift from Merck. All other materials were from previously cited sources.^{9,13}

Human Subjects

Human subjects were studied after written consent approved by the University of California at Los Angeles (UCLA) Institutional Review Board. Fasting blood was collected in heparinized tubes (Becton Dickinson). Sucrose solution was added at a ratio of 1 volume sucrose to 4 volumes of plasma,⁹ thoroughly mixed, divided

into aliquots, and kept frozen at -80°C until use. Two groups of patients were studied.

Group 1 consisted of 32 patients recruited from medical offices at UCLA who were not yet on a statin or other hypolipidemic agents and who had CHD or CHD risk equivalents by NCEP ATP-III criteria¹⁰ and whose physicians recommended statin therapy. After the first blood sample was collected, these patients were started on simvastatin 40 mg/d. After 6 weeks, a repeat blood sample was obtained. Exclusion criteria included previous use of any lipid-lowering medication in the 6 months before enrollment, hepatic transaminase levels above normal range within 2 months before dosing, known hepatic disease, evidence of drug abuse within the previous 6 months, history of acute coronary syndrome within 90 days before study enrollment, known hypersensitivity to any statin, and high-sensitivity (hs) CRP of >10 mg/L on repeat determination. Six of these 32 patients were subsequently found to have an hs-CRP value >10 mg/L after repeat determination on the same sample. Because the protocol for group 1 permitted only one blood sample before and one after statin therapy, patients with confirmed hs-CRP values >10 mg/L were excluded, as recommended by Ridker.¹⁴ These 6 did not otherwise differ from the remaining 26 patients. The remaining 26 patients (19 men and 7 women) constituted the first patient group. Ten of these patients had angiographic evidence of coronary atherosclerosis. Five had noninvasive evidence for significant atherosclerosis, 9 had established type II diabetes as their major criteria, 1 had type II diabetes and angiographic criteria, 1 had symptomatic peripheral vascular disease only, 3 others had symptomatic peripheral vascular disease in addition to another reason for statin therapy, and 1 was included because of a Framingham 10-year risk score of $>20\%$ (28%). Group 2 comprised 20 subjects (11 men and 9 women) referred by UCLA cardiologists because of documented CHD and high HDL cholesterol levels. Nineteen of the group 2 patients had CHD by coronary angiogram, and 1 had a myocardial infarction, which was not recent. None of the patients in group 2 were diabetic, and none were on a statin or other hypolipidemic medication. Age- and sex-matched healthy controls were recruited from the UCLA community for each of the 2 groups.

Determination of HDL Inflammatory/Antiinflammatory Properties

MCA Assay

Lipoproteins (isolated by fast performance liquid chromatography), human artery wall cocultures, and monocytes were prepared, and MCA was determined as previously described.¹⁵ Induction of MCA by a standard control LDL was determined in the absence or presence of the subject's HDL. Values in the absence of HDL were normalized to 1.0. Values >1.0 after the addition of HDL indicated proinflammatory HDL; values <1.0 indicated antiinflammatory HDL.

Cell-Free Assay

The CFA was a modification of a previously published method⁹ using PEIPC as the fluorescence-inducing agent. Briefly, HDL was isolated by the dextran sulfate method. Sigma HDL cholesterol reagent (catalog No. 352-3) containing dextran sulfate and magnesium ions was dissolved in distilled water (10.0 mg/mL). Dextran sulfate solution (50 μL) was mixed with 500 μL of the test plasma, incubated at room temperature for 5 minutes, and subsequently centrifuged at 8000g for 10 minutes. The supernatant containing HDL was used in the experiments after cholesterol determination by use of a cholesterol assay kit (catalog No. 2340-200, Thermo DMA Co). HDL isolated by this method inactivates bioactive phospholipids to a similar extent compared with HDL that has been isolated by gel electrophoresis or ultracentrifuge methods.⁹ To determine the inflammatory/antiinflammatory properties of HDL, the change in fluorescence intensity as a result of the oxidation of DCFH by PEIPC in the absence or presence of the test HDL was used. DCFH-DA was dissolved in fresh methanol at 2.0 mg/mL and was incubated at room temperature and protected from light for 30 minutes, resulting in the release of DCFH. PEIPC solution (10 μL) (final concentration of 50

TABLE 1. Plasma Lipid and hs-CRP Levels for Group 1 Patients Before Simvastatin Therapy and Their Age- and Sex-Matched Healthy Controls

	Patients						Controls					
	Age, y	Total Chol. Before, mg/dL	HDL Chol. Before, mg/dL	Trig. Before, mg/dL	LDL Chol. Before, mg/dL	hs-CRP Before, mg/L	Age	Total Chol., mg/dL	HDL Chol., mg/dL	Trig., mg/dL	LDL Chol., mg/dL	hs-CRP, mg/L
Mean	63.5	202	57	125	118	1.7	62.3	192	63.5	71	115	1.41
SD	13	28	13	64	24	1.9	11	5.2	6.1	26	7.9	1.28
Range	35–87	142–276	35–89	31–247	81–176	0.2–7.0	38–81	185–199	56–78	37–135	100–126	0.27–4.85
<i>P</i>			*	†					*	†		

Chol. indicates cholesterol; Trig., triglycerides.
 **P*=0.008; †*P*=0.0002.

μg/mL) and 90 μL of HDL-containing dextran sulfate supernatant (final concentration of 10 μg/mL cholesterol) were divided into aliquots into flat-bottom, black polystyrene microtiter plates (Microfluor2, catalog No. 14-245-176, Fisher) and mixed. The plates were then incubated at 37°C on a rotator for 1.0 hour. Ten microliters of DCFH solution (0.2 mg/mL) was then added to each well, mixed, and incubated for an additional 2 hours at 37°C with rotation. Fluorescence was determined with a plate reader (Spectra Max, Gemini XS; Molecular Devices) at an excitation wavelength of 485 nm, emission wavelength of 530 nm, and cutoff of 515 nm with the photomultiplier sensitivity set at medium. Values for intra-assay and interassay variability were 5.3±1.7% and 7.1±3.2%, respectively. Values in the absence of HDL were normalized to 1.0. Values >1.0 after the addition of the test HDL indicated proinflammatory HDL; values <1.0 indicated antiinflammatory HDL.

Other Procedures

Plasma levels of interleukin-6 and tumor necrosis factor-α were determined as previously described.^{16,17} Plasma total cholesterol, triglycerides, LDL cholesterol, HDL cholesterol, and glucose were determined as described previously⁸ using kits (Sigma). A sandwich enzyme immunoassay from Immunodiagnostik (ALPCO Diagnostics) was used to measure hs-CRP levels.¹⁸ HDL lipid hydroperoxide (LOOH) and paraoxonase activity were measured as previously described.¹⁹ Statistical significance was determined with model I ANOVA, and significance was defined as a value of *P*<0.05.

Results

The clinical characteristics of group 1 patients before simvastatin and the data for 26 age- and sex-matched healthy controls are shown in Table 1. Before simvastatin, the patients and controls did not differ significantly except for higher triglyceride levels in the patients (*P*=0.0002) and higher HDL cholesterol levels in the controls (*P*=0.008). Only 3 of the 26 patients had abnormally low HDL cholesterol levels (<50 mg/dL women; <40 mg/dL men). None of the controls had low HDL cholesterol levels. Fourteen of the

26 patients had elevated total cholesterol levels (>200 mg/dL). All controls had levels <200 mg/dL (Table 1). Nine of the 26 patients had triglyceride levels >150 mg/dL, whereas all controls had levels <150 mg/dL. Two patients had LDL cholesterol levels >160 mg/dL before treatment, whereas all controls had levels <160 mg/dL. Before simvastatin, 4 of the 26 patients had hs-CRP levels >3 mg/L; 3 of the controls had levels >3 mg/L. After simvastatin, there was a highly significant decrease in total cholesterol, LDL cholesterol, and triglycerides and a significant increase in HDL cholesterol (Table 2). After simvastatin, there was a reduction in hs-CRP levels (Table 2) (only 1 patient had an hs-CRP >3.0 mg/L after simvastatin), but this decrease did not reach statistical significance.

Table 3 shows the inflammatory/antiinflammatory properties of HDL from these 26 patients before and after simvastatin therapy compared with the controls. Before simvastatin, the values for patient HDL in the MCA assay were 1.38±0.91 (mean±SD) compared with the controls, whose values were 0.38±0.14 (*P*=1.5×10⁻⁵). Before simvastatin, only 6 of the 26 patients had MCA values <1.0, and only 1 patient had an MCA value <0.6, compared with all 26 of the controls, whose MCA values were <1.0. Twenty-four of the 26 controls had MCA values <0.6. The 6 patients excluded because of an hs-CRP value of >10 mg/L on repeat examination had MCA values before simvastatin similar to those of the other 26 patients (1.3±0.59). After simvastatin therapy, the patient MCA values for the 26 patients decreased to 1.08±0.71 (*P*=0.002). Twelve of the 26 patients had MCA values <1.0 and 4 had MCA values <0.6 after treatment. The 6 patients in group 1 who were excluded because of an hs-CRP value of >10 mg/L had MCA values after simvastatin of 0.83±0.29. As also shown in Table 3, the CFA yielded

TABLE 2. Plasma Lipid Levels and hs-CRP for Group 1 Patients Before and After Simvastatin Therapy

	Total Chol.		HDL Chol.		Trig.		LDL Chol.		hs-CRP	
	Before, mg/dL	After, mg/dL	Before, mg/dL	After, mg/dL	Before, mg/dL	After, mg/dL	Before, mg/dL	After, mg/dL	Before, mg/L	After, mg/L
Mean	202	154	57	61	125	99	118	73	1.7	1.3
SD	28	22	13	14	64	52	24	24	1.9	1.3
Range	142–276	120–193	35–89	41–91	31–247	39–228	81–176	38–123	0.2–7.0	0.2–4.8
<i>P</i>		*		0.03		0.005		†		0.86

Abbreviations as in Table 1.
 **P*=4×10⁻¹⁰; †*P*=4.2×10⁻¹².

TABLE 3. Inflammatory/Antiinflammatory Properties of HDL From Group 1 Patients Before and After Simvastatin Therapy and From Their Age- and Sex-Matched Healthy Controls

	Patients				Controls	
	MCA		CFA		MCA	CFA
	Before	After	Before	After		
Mean	1.38	1.08	1.19	0.91	0.38	0.53
SD	0.91	0.71	0.19	0.28	0.14	0.15
Range	0.5–4.7	0.2–3.7	0.9–1.8	0.3–1.3	0.13–0.63	0.31–0.85
<i>P</i>		0.002		*	†	‡

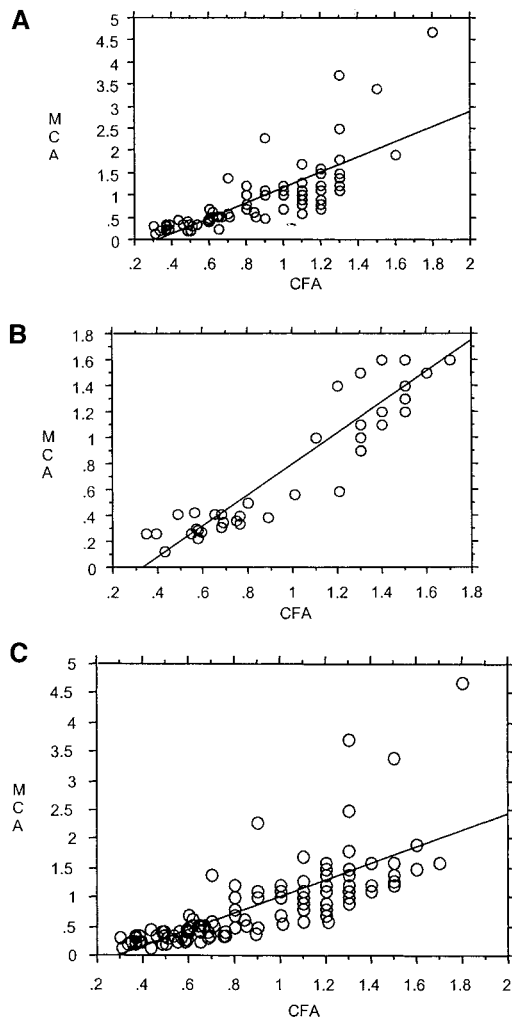
*CFA patients before simvastatin vs CFA patients after simvastatin, $P=2.3\times 10^{-6}$; †MCA for patients before simvastatin vs MCA for controls, $P=1.5\times 10^{-5}$; ‡CFA patients before simvastatin vs CFA controls, $P=7.4\times 10^{-14}$.

values of 1.19 ± 0.19 for the patients before simvastatin, compared with values of 0.53 ± 0.15 for the controls ($P=7.4\times 10^{-14}$). Before simvastatin, only 1 of 26 patients had a CFA value <1.0 , whereas all the normal controls had values <1.0 . After simvastatin therapy, the patients had CFA values of 0.91 ± 0.28 ($P=2.3\times 10^{-6}$), and 15 of the 26 patients had values <1.0 .

A comparison of the 3 subgroups of group 1 with sufficient numbers to permit statistical analysis (angiographic evidence of CHD [$n=10$], established type 2 diabetes [$n=9$], and noninvasive evidence for significant atherosclerosis [$n=5$]), accounting for 24 of the 26 patients, revealed no statistically significant differences except for lower MCA values after simvastatin for the diabetics compared with MCA values for the angiographic CHD group after simvastatin ($P=0.021$).

The Figure, A shows the correlation between MCA and CFA values for the 26 patients before and after simvastatin and their age- and sex-matched healthy controls. The correlation coefficient was 0.760 ($P=0.00008$). There was no significant difference in plasma interleukin-6 or tumor necrosis factor- α levels before or after simvastatin, and the differences in these cytokine levels between patients and controls were not significantly different (data not shown).

HDL LOOH levels were correlated with both MCA and CFA values in the group 1 patients. The correlation of MCA and HDL-LOOH was significant before ($r=0.214$; $P=0.039$) and after ($r=0.454$; $P=0.031$) simvastatin. Similarly, the correlation of CFA and HDL-LOOH was significant before ($r=0.589$; $P=0.024$) and after ($r=0.725$; $P=0.021$) simvastatin. There was also a significant correlation between HDL-LOOH and MCA ($r=0.734$; $P=0.018$) and CFA ($r=0.593$; $P=0.022$) in the controls. HDL-LOOH levels in the patients before simvastatin were 20.6 ± 5.7 ng LOOH/ μ g cholesterol, and after simvastatin, the values decreased to 17.1 ± 7.7 ng. This difference did not quite reach statistical significance ($P=0.062$). However, there was a highly significant difference between the patients and their healthy controls, whose HDL-LOOH levels were 10.8 ± 4.7 ng LOOH/ μ g cholesterol ($P=1\times 10^{-8}$ for patients before simvastatin versus controls; $P=9\times 10^{-4}$ for patients after simvastatin versus controls).



Correlation between values for inflammatory/antiinflammatory properties of HDL as determined by MCA assay vs those obtained using CFA. A, Group 1 patients before and after simvastatin therapy and their age- and sex-matched healthy controls. B, Group 2 patients with documented CHD and high HDL cholesterol levels and their age- and sex-matched healthy controls. C, All patients and controls, ie, combination of data from A and B.

Paraonase activity was not significantly different before or after simvastatin and was not significantly different between patients and controls (data not shown).

Table 4 shows the clinical characteristics of the 20 patients with CHD and high HDL cholesterol levels and their controls. The patient values were significantly different only for total cholesterol ($P=0.0008$) and HDL cholesterol ($P=1.2\times 10^{-10}$).

As shown in Table 5, the patient MCA values were 1.28 ± 0.29 , compared with 0.35 ± 0.11 in the controls ($P=1.7\times 10^{-14}$). Only 2 of the 20 patients had an MCA value <1.0 , whereas all of the controls had an MCA value <1.0 . None of the patients had MCA values <0.6 , whereas all of the controls had MCA values <0.6 . CFA values for the patients were 1.37 ± 0.19 , compared with values of 0.66 ± 0.21 for the controls ($P=4.4\times 10^{-12}$). Only 1 of the 20 patients had a CFA value <1.0 , whereas 18 of the 20 controls had CFA values <1.0 . As shown in the Figure, B, the correlation for

TABLE 4. Plasma Lipid and hs-CRP Levels for Group 2 Patients With CAD and High HDL Cholesterol Levels and Their Age- and Sex-Matched Healthy Controls

	Age, y	Total Chol., mg/dL	HDL Chol., mg/dL	Trig., mg/dL	LDL Chol., mg/dL	hs-CRP, mg/L	Age, y	Total Chol., mg/dL	HDL Chol., mg/dL	Trig., mg/dL	LDL Chol., mg/dL	hs-CRP, mg/L
Mean	53.0	217	95	89	108	0.8	52.8	184	52.6	97.7	112	1.01
SD	15.5	35	14	44	34	0.7	14.7	8.8	4.9	24	10	0.45
Range	21–77	160–307	84–145	44–207	57–221	0.1–2.8	22–76	168–197	47–63	59–132	91–128	0.53–1.91
<i>P</i>								0.0008*	†	0.40	0.58	0.057

Abbreviations as in previous tables.

**P* value for total cholesterol between patients and controls; †*P*=1.2×10⁻¹⁰ for patient HDL cholesterol levels vs control HDL cholesterol levels.

MCA and CFA values for the patients with CHD and high HDL cholesterol levels and their controls gave a correlation coefficient of 0.930 (*P*=0.00006).

The Figure, C, shows the correlation for MCA and CFA for the combination of all subjects, patient groups 1 and 2 and their controls (Tables 3 and 5). The correlation coefficient was 0.751 (*P*=0.00009).

Discussion

Sampietro et al²⁰ reported that CRP was elevated in familial hypoalphalipoproteinemia and was most elevated in patients with documented coronary artery disease, suggesting that normal HDL was antiinflammatory. We previously hypothesized that HDL may have evolved as part of the innate immune system being antiinflammatory under basal conditions in normal subjects, becoming proinflammatory during an acute-phase reaction,^{6,21} and returning to an antiinflammatory state after the acute-phase reaction. Our preliminary data in humans with atherosclerosis⁹ suggested that persistent inflammatory HDL represented a chronic acute-phase response similar to that described by CRP levels in the top tertile of normal.⁵ The data from patient group 1 extend our previous study and show that the inflammatory/antiinflammatory properties of HDL were better correlated with the presence of CHD or CHD equivalency by NCEP ATP-III criteria than were HDL cholesterol levels. In group 1, only 3 of 26 patients had abnormally low HDL cholesterol levels before simvastatin therapy, whereas 20 of 26 patients had MCA values ≥1.0 and all 26 patients had MCA values >0.6, compared with all 26 of the controls, whose values were <1.0. Indeed, 24 of the 26 controls had MCA values <0.6. After 6 weeks of simvastatin therapy, there was a highly significant reduction in the inflammatory properties of the HDL in group 1 patients, but their HDL remained significantly more inflammatory than HDL from controls.

TABLE 5. Inflammatory/Anti-inflammatory Properties of HDL From Group 2 Patients and Their Age- and Sex-Matched Healthy Controls

	Patient MCA	Patient CFA	Control MCA	Control CFA
Mean	1.28	1.37	0.35	0.66
SD	0.29	0.19	0.11	0.21
Range	0.5–1.6	0.8–1.7	0.13–0.59	0.4–1.2
<i>P</i>			*	†

**P*=1.7×10⁻¹⁴ for patient MCA vs control MCA; †*P*=4.4×10⁻¹² for patient CFA vs control CFA.

In patient group 2, which was composed of patients referred for study because of CHD and high HDL cholesterol levels (95±14 mg/dL), only 1 patient had an elevated LDL cholesterol level (>160 mg/dL), only 2 patients had elevated triglycerides (>150 mg/dL), and none were diabetic (data not shown). None of these 20 patients were on a statin or other hypolipidemic agent. Eighteen of these 20 patients had MCA values ≥1.0, and only 1 had an MCA value <0.6, whereas all 20 of the controls had MCA values <0.6. Nineteen of the 20 group 2 patients had CFA values ≥1.0. Only 1 control for group 2 had a CFA value of 1.0, and another had a CFA value of 1.2. The other 18 controls had CFA values <1.0.

Bowry et al²² reported that HDL is a major carrier of LOOH in humans. Because there were significant correlations between HDL-LOOH, MCA, and CFA, one might ask whether MCA and CFA simply measure HDL-LOOH. If this were the case, adding normal HDL would always give a CFA value >1.0, because all of the controls had some LOOH in their HDL. However, of the 46 control subjects studied here (26 for group 1 and 20 for group 2), only 1 had a CFA value >1.0. We previously reported that MCA and CFA measure the net action of a large number of factors in HDL.²¹ These factors include oxidized phospholipids, LOOH, paraoxonase activity, platelet-activating factor acetyl hydrolase activity, lecithin cholesterol acyl transferase activity, possibly GSH peroxidase activity, apolipoprotein A-I, apolipoprotein J, serum amyloid A, ceruloplasmin, antioxidant vitamins, and probably products such as nitrotyrosine, which can be generated by myeloperoxidase. Thus, the tests used here to determine the inflammatory/antiinflammatory status of HDL most likely represent the net effect of all of these factors, and the LOOH content and the other factors in HDL are likely interdependent.

Together with our previously studied patient group⁹ and the 2 patient groups reported here, there are now 3 patient groups with differing characteristics in which the inflammatory/antiinflammatory properties of HDL seem to better differentiate the patients from controls compared with HDL cholesterol levels. The size of each of these 3 patient groups was small, 20 to 27 patients in each group (and their age- and sex-matched healthy controls). Thus, the true predictive value of the inflammatory/antiinflammatory properties of HDL must await large-scale testing. The use of hs-CRP has been found to be highly predictive in large epidemiological studies.¹⁴ However, in smaller studies, hs-CRP has been less predictive than some other markers, eg, nitrotyrosine levels.²³ Similarly, in the studies reported here, hs-CRP was less

discriminatory than the inflammatory/antiinflammatory properties of HDL. However, the number of subjects reported here is far too few to reach any conclusions regarding the relative usefulness of hs-CRP and the inflammatory/antiinflammatory properties of HDL. The data regarding HDL cholesterol concentrations versus the inflammatory/antiinflammatory properties of HDL seem much more compelling. However, even the conclusions drawn from this seemingly clear-cut comparison must await testing in large populations. The correlation between MCA and CFA reported here was highly significant (Figure), suggesting that this CFA may allow for large-scale testing of the inflammatory/antiinflammatory properties of HDL compared with HDL cholesterol levels in predicting risk for CHD.

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