

Cholesteryl Ester Transfer Protein in Patients with Rheumatoid Arthritis

Iván Ferraz-Amaro, Miguel A. González-Gay, José A. García-Dopico, and Federico Díaz-González

ABSTRACT. Objective. To investigate how cholesteryl ester transfer protein (CETP), one of the enzymes involved in the reverse cholesterol transfer, is expressed in patients with rheumatoid arthritis (RA) and its potential relationship with both dyslipidemia and the risk of cardiovascular mortality observed in these patients.

Methods. Plasma CETP concentrations and CETP activity were measured in 101 patients with RA and 115 sex- and age-matched controls. A multivariable analysis adjusted for standard cardiovascular risk factors, including high-density lipoprotein cholesterol, was performed to evaluate the influence of CETP on dyslipidemia and cardiovascular mortality risk, as assessed by the Systematic Coronary Risk Evaluation (SCORE) risk function.

Results. Patients with RA showed lower CETP activity [β coefficient = -10.82 (95% CI -19.56 to 2.07) pmol/3 h; $p = 0.02$] and an inferior CETP mass [$\beta = -0.85$ (95% CI -1.64 to 0.05) $\mu\text{g/ml}$; $p = 0.03$] versus controls. Divided into those taking and those not taking glucocorticoids, patients taking glucocorticoids revealed lower CETP activity and mass [$\beta = -8.98$ (95% CI -14.55 to 3.41) pmol/3 h; $p = 0.00$, for CETP activity; and $\beta = -0.77$ (95% CI -1.46 to 0.08) $\mu\text{g/ml}$; $p = 0.03$, for CETP mass]. Patients with RA not taking glucocorticoids showed no differences versus controls in either CETP activity or mass. Both current prednisone intake [$\beta = -16.14$ (95% CI -24.87 to 7.41) pmol/3 h; $p = 0.00$] and average daily prednisone intake during the last 3 months [$\beta = -0.36$ (95% CI -0.54 to 0.18) $\mu\text{g/ml}$; $p = 0.01$] were strongly and inversely correlated with CETP activity and mass, respectively. CETP activity showed an inverse trend compared to SCORE risk, demonstrating that lower levels were effective predictors of total mortality when a higher SCORE risk was found [$\beta = -4.7$ (95% CI -9.3 to 0.02) pmol/3 h; $p = 0.04$] in patients with RA.

Conclusion. CETP is downregulated in patients with RA who are taking glucocorticoids. Low CETP activity is associated with an increased level of cardiovascular risk in patients with RA. (J Rheumatol First Release May 15 2013; doi:10.3899/jrheum.121507)

Key Indexing Terms:

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Rheumatoid arthritis (RA) is a chronic, systemic, inflammatory disorder of unknown etiology that, left uncontrolled, can lead to joint destruction and deformity because of

From the Rheumatology Division, Hospital Universitario de Canarias, Tenerife; Rheumatology Division, Hospital Universitario Marqués de Valdecilla, IFIMAV, Santander; Servicio de Laboratorio, Hospital Universitario de Canarias, Tenerife; and Departamento de Medicina, Facultad de Medicina, Universidad de La Laguna, Tenerife, Spain.

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I. Ferraz-Amaro, MD, Rheumatology Division, Hospital Universitario de Canarias; M.A. González-Gay, MD, Rheumatology Division, Hospital Universitario Marqués de Valdecilla, IFIMAV; J.A. García-Dopico, MD, Servicio de Laboratorio, Hospital Universitario de Canarias; F. Díaz-González, MD, Rheumatology Division, Hospital Universitario de Canarias, Departamento de Medicina, Facultad de Medicina, Universidad de La Laguna.

Address correspondence to Dr. I. Ferraz-Amaro, Servicio de Reumatología, Hospital Universitario de Canarias, Ofra s/n La Cuesta, La Laguna, S/C de Tenerife 38320, Spain.

E-mail: iferrazamaro@hotmail.com

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erosion of cartilage and bone. Epidemiological data suggest that RA is an independent risk factor for cardiovascular disease (CVD) and may be a major contributor to increased mortality among such patients^{1,2,3}. Dyslipidemia is one of the established and emerging cardiovascular risk factors with an increased prevalence among patients with RA⁴. In this regard, cardiovascular prevention using statins is required in a high proportion of patients with RA referred for cardiovascular risk stratification⁵.

Lipid profile in active or untreated RA often shows reduced levels of total cholesterol, low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol^{6,7,8,9}. It should be noted, however, that decreases in inflammation may coincide with increases in serum lipid values^{10,11}. This is consistent with findings observed in other pathologies/conditions associated with inflammation or infection, such as sepsis, cancer, trauma, or postoperative recovery⁷. The dyslipidemia that occurs in RA appears to stem from the systemic release of inflammatory cytokines

such as tumor necrosis factor (TNF) and interleukin 1 and 6. In addition, it is dependent upon disease activity, and seems already to be present during the preclinical phase of the disease^{12,13}. Nevertheless, alternative explanations are required to definitively link inflammation with dyslipidemia.

Reverse cholesterol transport refers to the flux of cholesterol from the whole body periphery to the liver and then finally to fecal excretion. One of the most important enzymatic modulations of this flux involves cholesteryl ester transfer protein (CETP). CETP is a molecule that mediates the transfer of cholesteryl esters from HDL particles to the triglyceride-rich lipoproteins LDL and very LDL (VLDL); triglycerides are simultaneously transferred in the opposite direction. During this process, HDL cholesterol decreases, while the cholesterol content in VLDL increases, causing LDL particles to become smaller and denser. Intracellular CETP in both the periphery and the liver appears to promote cholesterol removal from peripheral cells and uptake by the liver. Recently, CETP has gained widespread interest as a therapeutic target for increasing HDL cholesterol levels, and synthetic molecules have been designed to inhibit its function. However, elevated CETP activity has been documented in normotriglyceridemic men with low HDL cholesterol levels¹⁴ and some studies have found that higher CETP reduces the risk of coronary atherosclerosis¹⁵. However, in a recent prospective investigation involving a moderate-size community-based sample, lower plasma CETP activity was associated with a greater risk of CVD¹⁶. This finding has called into question the strategy of pharmacologically inhibiting CETP to lower the risk of CVD. Thus, the role of CETP in lipoprotein metabolism seems to be complex, and the influence of CETP on CVD is still not well understood¹⁷.

CETP activity has not been assessed in a chronic inflammatory human model such as RA. The objective of our study was to evaluate the role of CETP in inflammation-related RA dyslipidemia and its relationship with the increased cardiovascular risk found in patients with RA.

MATERIALS AND METHODS

Study participants. Two hundred sixteen subjects, 101 patients with RA and 115 controls matched for age and sex, were recruited for a cross-sectional study. Eligible RA patients in this study were enrolled consecutively between October 2011 and May 2012 from our service, where patients with RA are seen roughly every 4 months. The control group consisted of patients attending our center consulting for osteoarthritis, living in the same area during the same time period of the study, who were also matched for age, sex, and comorbidity. Patients with RA were men or women aged 18 years or older who were diagnosed by a rheumatologist and who fulfilled the 2010 American College of Rheumatology/European League Against Rheumatism classification criteria¹⁸. Disease duration had to be ≥ 1 year. Patients taking disease-modifying antirheumatic drugs were enrolled in the present study. However, RA patients undergoing anti-TNF- α treatment or other biologic therapies were excluded. The presence of glucocorticoid intake was not an exclusion criterion. Glucocorticoid-naïve RA patients were those who had not received any glucocorticoid for the last 3 months. The glucocorticoid dose was established as the equivalent dose for

prednisone in mg/day/3-month period. Controls and patients with diabetes were also excluded. In this regard, all patients and controls had a measure of glucemia < 7 mmol/l. No patient or control was taking statin or lipid-lowering therapy. Patients and controls were also excluded if they had a history of myocardial infarction, angina, stroke, severe renal failure, cancer, or any other chronic disease or evidence of infection. None of the controls was taking glucocorticoid treatment. The study protocol was approved by the Institutional Review Committee at Hospital Universitario de Canarias, and all subjects provided written informed consent.

Data collection. Data collection was the same in patients with RA and controls, except for additional questions asked to patients that were specifically related to the disease. Patients and controls filled in a medication usage questionnaire and underwent examination to assess anthropometric measures and blood pressure. Medical records were reviewed to ascertain diagnosis and medication. Weight was assessed to the nearest 100 g with the participant standing on a portable digital scale (Seca). Standing height was measured to the nearest 1 cm with a stadiometer (Seca). Waist circumference was measured at the smallest circumference between the rib cage and the iliac crest, with the subject in the standing position. Hip circumference was measured at the widest circumference between the waist and the thighs. The waist-to-hip ratio was then calculated. In patients with RA, disease activity was measured using the Disease Activity Score in 28 joints (DAS28)¹⁹, while the degree of disease disability was determined using the Health Assessment Questionnaire (HAQ)²⁰. Metabolic syndrome was defined using the 2005 National Cholesterol Education Program Adult Treatment Panel III (ATP III) criteria²¹. The Systematic Coronary Risk Evaluation (SCORE) was calculated as described²² to estimate the 10-year risk for cardiovascular death in both patients and controls. The Spanish population was included in the European group of low cardiovascular risk regions. Therefore, SCORE was calculated to determine the 10-year risk of fatal CVD in a population at low CVD risk (as it was considered for the Spanish population). Afterward, individuals were categorized as defined²³ into very high, high, moderate, and low risk, as follows: (1) Very high risk: subjects with any of the following: (a) documented CVD by invasive or noninvasive testing (coronary angiography, nuclear imaging, stress echocardiography, carotid plaque on ultrasound), previous myocardial infarction, acute coronary syndrome, coronary revascularization and other arterial revascularization procedures, ischemic stroke, or peripheral artery disease; (b) diabetes mellitus type 1 or type 2 with one or more cardiovascular risk factors and/or target organ damage (such as microalbuminuria 30–300 mg/24 h); (c) severe chronic kidney disease (glomerular filtration rate = 30 ml/min/1.73 m²); (d) calculated SCORE $\geq 10\%$. (2) High risk: subjects with any of the following: (a) markedly elevated single risk factors such as familial dyslipidemias and severe hypertension; (b) diabetes mellitus type 1 or type 2, but without cardiovascular risk factors or target organ damage; (c) moderate chronic kidney disease (glomerular filtration rate = 30–59 ml/min/1.73 m²); (d) calculated SCORE $\geq 5\%$ and $< 10\%$ for 10-year risk of fatal cardiovascular disease. (3) Moderate risk: when SCORE is $\geq 1\%$ and $< 5\%$ at 10 years. (4) Low risk applies to individuals with SCORE $< 1\%$ and free of qualifiers that would put them at moderate risk. SCORE was adapted for patients with RA by introducing a 1.5 multiplier factor when at least 2 of the following 3 criteria were met: disease duration > 10 years, rheumatoid factor or anticitrullinated protein antibodies positivity, and presence of certain extraarticular manifestations.

Assessments. Fasting serum samples were collected and frozen at -20°C until analysis of circulating lipids. Cholesterol, triglycerides, and HDL cholesterol were measured using the enzymatic colorimetric assay (Roche). Cholesterol ranged from 0.08 to 20.7 mmol/l (intraassay coefficient of variation 0.3%); triglycerides ranged from 4 to 1,000 mg/dl (intraassay coefficient of variation 1.8%); and HDL cholesterol ranged from 3 to 120 mg/dl (intraassay variation coefficient 0.9%). LDL cholesterol was calculated using the Friedewald formula²⁴. Apolipoprotein A1 and B and lipoprotein(a) were measured with the immunoturbidimetric method (Roche). CETP levels (mass) were determined through a quantitative ELISA (Alpco Diagnostics). CETP activity was measured by fluorometry

using a Roar CETP Activity Assay Kit (Roar Biomedical). This assay uses a proprietary substrate for detection of CETP-mediated transfer of neutral lipid from the substrate to a physiological acceptor. This transfer activity results in an increase in fluorescence intensity. In a total volume of 200 μ l, the assay remains linear in 0.2 to 0.8 μ l of normal human plasma during a 3-hour incubation at 37°C. All measurements were made in duplicate, and intraassay and interassay coefficients of variation were both < 3%. For our study purposes we chose to analyze both CETP concentration and activity.

Statistical analysis. In an initial step, we found a CETP mass of 2.0 ± 0.5 μ g/ml in a series of 20 patients with RA. On the basis of previous findings²⁵ we assumed a normal CETP mass of 2.2 ± 0.4 μ g/ml in controls. Following this procedure, using a 1:1 relation and according to a Student T test with $\alpha = 0.05$ and $\beta = 0.15$, we estimated that we would have to enroll 146 subjects, 73 patients, and 73 controls. The relationship between mass and activity was calculated using univariate analysis (Pearson) with mass and activity as continuous variables. For reliability analysis, we were unable to calculate the intraclass correlation coefficient because CETP mass and activity are expressed in different values. For this reason, CETP mass and activity were divided into quintiles, and a quadratic weighted kappa index was calculated. Demographic and clinical characteristics of patients with RA and controls shown in Table 1 were compared using chi-square tests for categorical variables or a Student t test for continuous variables (data

expressed as mean \pm SD). The association of CETP activity or mass with clinical features of RA or cardiovascular risk levels, as well as comparisons between RA patients and controls, were performed through multivariate analysis adjusting for factors known to be associated with cardiovascular risk levels. Linear trends shown by CETP activity through SCORE risk stratification were tested using linear regression with orthogonal polynomial contrasts. All analyses used a 2-sided significance level of 5% and were performed using SPSS software, version 19 (IBM). A p value < 0.05 was considered statistically significant.

RESULTS

Lipid profile: subjects' characteristics. A total of 216 participants, 101 patients with RA and 115 controls, mean ages 55 ± 10 years and 56 ± 11 years ($p = 0.49$), respectively, were studied. Demographic data, lipid profile, and disease-related characteristics of participants are shown in Table 1. Patients had a moderate-active disease level by DAS28 (3.99 ± 1.41). There were no differences between patients and controls with regard to body mass index, waist circumference and presence of hypertension, or metabolic

Table 1. Demographic data and disease-related characteristics of patients with rheumatoid arthritis (RA) and controls. Data are mean (\pm SD) or median (interquartile range). Dichotomous variables are expressed as n (%).

Characteristics	RA, n = 101		p [†]	Controls, n = 115	p	
	Glucocorticoids, n = 46	No Glucocorticoids, n = 55				
Demographic data						
Age, yrs	55 \pm 10	53 \pm 10	57 \pm 9	0.05	56 \pm 11	0.49
Women, n (%)	86 (85)	38 (67)	48 (87)	0.51	103 (90)	0.33
Smokers, n (%)	19 (19)	6 (13)	13 (24)	0.19	11 (10)	0.05
Body mass index, kg/m ²	29.0 \pm 5.8	28.0 \pm 4.6	29.8 \pm 6.6	0.12	30.1 \pm 5.9	0.23
Waist circumference, cm	97 \pm 16	94 \pm 15	99 \pm 16	0.11	93 \pm 12	0.12
Hip circumference, cm	107 \pm 16	106 \pm 11	109 \pm 20	0.37	102 \pm 18	0.10
Waist-hip ratio	0.90 (0.85–0.94)	0.89 (0.83–0.94)	0.90 (0.86–0.94)	0.35	0.87 (0.83–0.92)	0.65
Hypertension, n (%)	36 (36)	14 (25)	22 (40)	0.51	44 (38)	0.96
Metabolic syndrome, n (%)	32 (32)	15 (27)	17 (31)	0.96	28 (24)	0.25
ESR, mm/h	25 (16–39)	25 (15–41)	26 (18–39)	0.78	19 (13–24)	0.00*
CRP, mg/l	3.8 (1.4–9.5)	4.5 (1.3–13.7)	3.2 (1.6–9.0)	0.60	1.9 (0.9–4.8)	0.00*
Lipid profile						
Total cholesterol, mg/dl	210 \pm 40	204 \pm 30	216 \pm 46	0.08	207 \pm 38	0.49
Triglycerides, mg/dl	118 (85–151)	122 (85–139)	135 (91–152)	0.28	104 (77–135)	0.10
HDL cholesterol, mg/dl	59 \pm 15	57 \pm 13	60 \pm 16	0.62	55 \pm 13	0.04*
LDL cholesterol, mg/dl	127 \pm 33	128 \pm 28	127 \pm 37	0.66	129 \pm 34	0.73
Cholesterol-HDL ratio	3.77 \pm 1.14	3.74 \pm 0.83	3.84 \pm 1.34	0.53	3.88 \pm 0.86	0.33
Non-HDL cholesterol, mg/dl	152 \pm 42	148 \pm 29	157 \pm 50	0.21	152 \pm 36	0.99
Apolipoprotein A1	158 \pm 28	155 \pm 24	161 \pm 32	0.63	185 \pm 35	0.00*
Apolipoprotein B	91 \pm 22	93 \pm 19	89 \pm 25	0.98	109 \pm 25	0.00*
Lipoprotein(a)	46 \pm 44	54 \pm 46	37 \pm 40	0.12	64 \pm 56	0.05
Disease characteristics						
Disease duration, yrs	7 \pm 6	8.7 \pm 4.5	6.2 \pm 6.8	0.06		
DAS28-ESR	3.99 \pm 1.41	4.11 \pm 1.56	3.18 \pm 1.27	0.44		
HAQ	0.625 (0.250–1.250)	0.82 \pm 0.75	0.80 \pm 0.77	0.93		
Positive rheumatoid factor, n (%)	64 (63.4)	21 (38)	43 (78)	0.01*		
Current nonbiologic DMARD use, n (%)	76 (75.3)	41 (73)	47 (85)	0.22		
Current prednisone, n (%)	55 (54.5)					
Prednisone, mg/day/last 3 mo	6.5 \pm 2.7					

* $p < 0.05$ between controls and patients. [†] Comparing patients with and those without glucocorticoid therapy. ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; HAQ: Health Assessment Questionnaire; DAS28: Disease Activity Score; DMARD: disease-modifying antirheumatic drug; HDL: high-density lipoprotein; LDL: low-density lipoprotein.

syndrome. As expected, analyses of erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) values revealed statistically significant differences between controls and patients. When patients were divided into those taking glucocorticoids and glucocorticoid-naive subjects, no differences were found in population characteristics or disease features (data not shown). DAS28 was not different between them (4.11 ± 1.56 in glucocorticoid-naive vs 3.18 ± 1.27 in those taking glucocorticoids; $p = 0.45$).

Patients with RA had higher levels of HDL compared to controls (59 ± 15 vs 55 ± 13 mg/dl; $p = 0.04$), but total cholesterol, triglycerides, and LDL cholesterol were not different between patients and controls. Apolipoprotein A1 was higher in controls compared to patients (185 vs 158 mg/dl; $p < 0.01$), while RA patients showed lower levels of apolipoprotein B (91 vs 109 mg/dl; $p < 0.01$). When the lipid profile was compared between patients taking glucocorticoids and those not, no statistically significant difference was found (Table 1).

CETP activity and mass. Plasma CETP activity and concentration showed a high correlation for both patients and controls ($r = 0.50$, $p = 0.00$), and when data were examined separately, patients with RA exhibited values of $r = 0.53$, $p < 0.01$ versus controls ($r = 0.44$, $p < 0.01$). CETP activity and mass concentration quintiles also showed agreement, with a quadratic weight kappa index of 0.43 (95% CI 0.15 – 0.71 ; $p < 0.01$).

CETP activity and plasma concentration. Patients with RA showed lower CETP activity [β coefficient = -10.82 (95% CI -19.56 to 2.07) pmol/3 hour; $p = 0.02$] and an inferior CETP mass [$\beta = -0.85$ (95% CI -1.64 to 0.05) $\mu\text{g/ml}$; $p = 0.03$] compared to controls, with adjustment for sex, age, total cholesterol, smoking, diabetes, and hypertension including HDL cholesterol levels (Table 2). When patients were divided into those who took glucocorticoids and those who did not, only the former group exhibited lower CETP activity and mass [$\beta = -8.98$ (95% CI -14.55 to 3.41) pmol/3 h; $p < 0.01$, for CETP activity; and $\beta = -0.77$ (95% CI -1.46 to 0.08) $\mu\text{g/ml}$; $p = 0.03$, for CETP mass] compared with controls. Glucocorticoid-naive RA patients showed no differences compared with controls in terms of CETP activity or mass.

CETP activity and plasma concentration in glucocorticoid users and glucocorticoid-naive patients. In the RA intra-group comparison, those on glucocorticoid treatment showed lower levels of CETP activity [$\beta = -14.8$ (95% CI -23.9 to 5.8) pmol/3 hour; $p < 0.01$] than glucocorticoid-naive patients. This difference was not found to be related to CETP mass.

Disease characteristics and CETP. Current prednisone intake was strongly associated with lower levels of CETP activity [$\beta = -16.14$ (95% CI -24.87 to 7.41) pmol/3 h; $p = 0.00$], but not with CETP mass [$\beta = -0.65$ (95% CI -1.65 to

0.35) $\mu\text{g/ml}$; $p = 0.20$]. Similarly, prednisone intake (daily average/last 3 mo) was inversely correlated with CETP mass [$\beta = -0.36$ (95% CI -0.54 to 0.18) $\mu\text{g/ml}$; $p = 0.01$]; this relation was not maintained for CETP activity. On the other hand, ESR, CRP levels, and disease activity indexes such as DAS28, HAQ, disease duration or positive rheumatoid factor were not associated with CETP activity or mass.

CETP activity and cardiovascular risk. When SCORE risk assessments of patients with RA and controls were divided into categories (low, moderate, high, and very high risk), independently of glucocorticoid intake, trend analysis showed that they had a statistically significant inverse relationship with CETP activity (Table 3).

DISCUSSION

The major findings of our study are as follows. (1) The mass and activity of CETP in patients with RA is reduced compared to healthy controls. (2) Among patients with RA, those who take glucocorticoids have significantly lower CETP mass and activity. (3) CETP activity showed an inverse trend with cardiovascular risk assessed by the SCORE index, suggesting that low levels of CETP might be considered predictors of cardiovascular mortality in RA.

Hypercholesterolemia typically stems from a combination of environmental factors such as obesity and diet in tandem with genetic factors and/or a number of secondary causes including diabetes mellitus type 2, alcohol abuse, nephrotic syndrome, hypothyroidism, and medications. Hypercholesterolemia and coronary heart disease have been linked in industrialized societies and there is a direct relation between plasma levels of total and LDL cholesterol and the risk of coronary heart disease and coronary mortality²⁶. Similarly, HDL cholesterol concentration is inversely related to absolute coronary heart disease event rates. Hypercholesterolemia is thus one of the major modifiable risk factors for coronary heart disease, and lowering LDL cholesterol levels in patients with moderate to high risk has been shown to lead to a reduction in cardiovascular events. Recently, a new approach has gained increasing interest, that is, raising HDL cholesterol by inhibiting CETP, a plasma protein that promotes the transfer of cholesteryl esters from HDL and other lipoprotein fractions. However, the development of torcetrapib, the first CETP inhibitor to be tested in a clinical trial, was terminated because it resulted in an excessive number of deaths and cardiovascular events²⁷. Because RA is associated with an increase in cardiovascular risk and with changes in the lipid profile, we studied how CETP was expressed in patients with RA (compared to controls), as well as its potential relationship with cardiovascular risk in these individuals.

Our result disclosing that CETP activity is influenced by glucocorticoids has not previously been reported, and to our knowledge this is the first report that glucocorticoids have been linked to a specific enzymatic effect on lipid meta-

Table 2. Activity and mass expression of cholesteryl ester transfer protein (CETP) in patients with rheumatoid arthritis (RA) and controls, compared with RA disease characteristics. Data are β coefficient (95% CI).

	CETP Activity, pmol/3 h	p	CETP Mass, μ g/ml	p
Comparison between RA patients and controls (n = 216)*				
RA patients (101) vs controls (115)	-10.82 (-19.56 - 2.07)	0.02	-0.85 (-1.64 - 0.05)	0.03
Glucocorticoid-naive patients (46) vs controls (115)	-2.99 (-14.75 - 8.77)	0.62	-0.22 (-1.51 - 1.08)	0.74
Patients taking glucocorticoids (55) vs controls (115)	-8.98 (-14.55 - 3.41)	0.00	-0.77 (-1.46 - 0.08)	0.03
Patients taking glucocorticoids (55) vs naive patients (46)	-14.8 (-23.9 - 5.8)	0.00	-0.65 (-1.65 - 0.35)	0.20
Univariate analysis for patients and controls (n = 216)				
Age, yrs	-0.5 (-0.8 - 10.1)	0.01	-0.03 (-0.07 - 0.01)	0.09
Sex	4.6 (-17.1 - 7.8)	0.47	-0.49 (-1.76 - 0.79)	0.45
Hip circumference, cm	0.1 (-0.2 - 0.3)	0.33	-0.01 (-0.06 - 0.04)	0.61
Waist circumference, cm	-0.0 (-0.3 - 0.3)	0.81	0.00 (-0.04 - 0.04)	0.92
Waist-hip ratio	-2.0 (-5.4 - 1.4)	0.23	4.65 (-2.24 - 11.53)	0.18
Body mass index, kg/m ²	0.4 (-0.3 - 1.1)	0.26	0.01 (-0.06 - 0.08)	0.78
Metabolic syndrome	-8.4 (-16.4 - 0.4)	0.04	-0.25 (-1.16 - 0.67)	0.59
ESR, mm/h	-0.0 (-0.3 - 0.3)	0.98	0.01 (-0.02 - 0.05)	0.52
CRP, mg/l	-0.1 (-0.4 - 0.3)	0.73	0.00 (-0.05 - 0.05)	0.98
Lipid profile, n = 216				
HDL cholesterol, mg/dl	-0.2 (-0.3 - 0.3)	0.90	-0.00 (-0.03, - 0.03)	0.97
LDL cholesterol, mg/dl	0.1 (-0.0 - 0.2)	0.14	0.01 (-0.00 - 0.02)	0.14
Total cholesterol, mg/dl	0.0 (-0.1 - 0.1)	0.89	0.01 (-0.01 - 0.02)	0.35
Disease characteristics (n = 101)				
ESR	-0.13 (-0.53 - 0.28)	0.52	0.03 (-0.01 - 0.07)	0.11
CRP	-0.05 (-0.39 - 0.29)	0.78	-0.04 (-0.12 - 0.04)	0.36
Disease duration, yrs	0.26 (-0.81 - 1.33)	0.62	0.03 (-0.07 - 0.14)	0.52
DAS28-ESR	-2.16 (-5.96 - 1.65)	0.26	0.16 (-0.26 - 0.57)	0.45
HAQ	-3.60 (-11.35 - 4.16)	0.36	0.17 (-0.59 - 0.94)	0.66
Positive rheumatoid factor	-7.49 (-17.14 - 2.16)	0.13	-0.69 (-1.66 - 0.29)	0.16
Current nonbiologic DMARD use	0.36 (-22.86 - 23.60)	0.97	-0.03 (-2.40 - 2.34)	0.97
Current prednisone	-16.14 (-24.87 - 7.41)	0.00	-0.65 (-1.65 - 0.35)	0.20
Prednisone: average mg/day/previous 3 mo	2.62 (-2.23 - 7.47)	0.25	-0.36 (-0.54 - 0.18)	0.01

* Adjusted for sex, age, total cholesterol, smoking, diabetes mellitus, and hypertension, including HDL cholesterol. ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; HAQ: Health Assessment Questionnaire; DAS28: Disease Activity Score; DMARD: disease-modifying antirheumatic drug; HDL: high-density lipoprotein; LDL: low-density lipoprotein.

Table 3. Activity of cholesteryl ester transfer protein (CETP) compared with Systematic Coronary Risk Evaluation (SCORE) risk levels. No patient from the glucocorticoid-naive group fulfilled the definition for very high SCORE risk. CEPT activity expressed as median (25th-75th percentile) pmol/3 h.

CETP Activity	n	SCORE Risk Category						Linear Trend		
		Low Risk	n	Moderate Risk	n	High Risk	n	Very High Risk	β (95% CI)	p*
Controls	62	49.2 (36.9-61.3)	42	34.6 (26.0-53.5)	6	31.9 (21.8-42.9)	5	8.7 (1.5-15.8)	-9.3 (-14.8 - 3.7)	0.00
RA patients	51	38.3 (27.9-50.0)	31	26.8 (20.5-45.2)	15	22.3 (16.2-44.3)	4	17.7 (13.3-22.1)	-4.7 (-9.3 - 0.2)	0.04
GC-negative RA patients	32	38.8 (30.4-50.7)	9	28.4 (24.6-55.4)	5	20.3 (16.5-39.6)	0	—	-4.9 (-11.2 - 0.8)	0.04
GC-positive RA patients	19	37.8 (24.7-45.7)	22	21.9 (17.1-39.7)	10	16.3 (9.9-19.3)	4	17.7 (13.3-22.1)	-5.7 (-10.8 - 0.5)	0.03

* p value refers to a trend analysis model considering independent variable (SCORE risk) as orthogonal polynomials. GC: glucocorticoid; RA: rheumatoid arthritis.

bolism. Classically, glucocorticoid use has been associated with an increased risk of serious adverse cardiovascular events, particularly ischemic heart disease and heart failure, in a way similar to Cushing disease, in which atherosclerosis becomes exacerbated and cardiovascular risk increases in patients with hypercortisolism. The risk in the latter case is dose-dependent and it remains unclear whether such risk is

low or absent in patients on low-dose glucocorticoid therapy²⁸. Studies to analyze the effect of glucocorticoids on atherosclerotic vascular disease have often yielded contradictory results, and glucocorticoids have been shown to confer some beneficial effects on dyslipidemia. In one report, moderate- to low-dose prednisone (20 mg/day tapered to 5 mg/day over 3 months) had no significant

adverse effect on lipoprotein levels if other risk factors were taken into account²⁹. Another observational study concluded that glucocorticoid use was associated with a more favorable lipid profile in older adults³⁰. Studies in patients with systemic lupus erythematosus demonstrated that the adverse effects exerted by glucocorticoids on lipid profiles are dose-dependent, and occur only at prednisone doses > 10 mg/day³¹. The relative risks of ischemic heart disease, heart failure, or stroke may also differ depending upon the exact condition being treated with glucocorticoids³².

Interestingly, Vis, *et al*³³ stated that prednisone use is associated with high HDL cholesterol levels in patients with RA. Similarly, García-Gómez, *et al*³⁴ suggested that low-dose glucocorticoid therapy in patients with RA is associated with an increase in HDL cholesterol, without increasing LDL cholesterol or triglycerides. Although we did not find higher plasma HDL cholesterol concentrations in patients with RA taking glucocorticoids, these data could be in agreement with our report, because patients with RA taking glucocorticoids show CETP suppression. However, it should be noted that previous studies^{35,36,37,38} showed that CETP mass and activity were not related with plasma HDL cholesterol concentrations. Therefore, the relationship between glucocorticoid use and cardiovascular risk is biased by the fact that these drugs tend to be used more often in patients with severe disease; consequently, it is difficult to determine whether the disease or the treatment increases the risk. However, when patients in our study were divided into those groups according to glucocorticoid intake, no difference was found regarding DAS28 results. Thus, our findings do not indicate that higher disease activity may be associated with glucocorticoid treatment in all cases. Further, we did not observe an association between CETP activity and disease activity scores, rheumatoid factor status, and disease duration.

We acknowledge that we did not find changes in the lipid profiles of patients taking glucocorticoids and those not taking such medication. This means that, although CETP activity was influenced by glucocorticoid therapy, there were no evident differences in the classic lipid measures such as HDL cholesterol, LDL cholesterol, triglycerides, or total cholesterol when patients with RA were stratified according to glucocorticoid intake. As emphasized, any interpretation of these data is biased by the difficulty in distinguishing effects due to disease activity, which itself may be associated with increased cardiovascular risk and lipid abnormalities, from those directly related to the glucocorticoids themselves. It must also be kept in mind that the use of glucocorticoids may gradually lead to peripheral insulin resistance, hyperinsulinemia, and increased hepatic VLDL synthesis. The lipoprotein effects of glucocorticoids are unlikely to be the sole cause (if they have any role at all) of the influence of these drugs on cardiovascular risk.

In addition, with respect to cardiovascular risk, we found

that patients and controls maintained a negative relationship with CETP activity levels, and this was not influenced by glucocorticoids. Indeed, glucocorticoid use among the entire group of patients with RA, as well as on those who specifically took this drug, showed this negative relation. When SCORE risk was considered as an ordinal (risk stratification) variable, CETP activity continued to be negatively correlated with cardiovascular risk, showing that low activity levels are associated with a higher total mortality risk. Our data are consistent with a recent report¹⁶ that also showed, in a moderate-size community-based sample, that lower plasma CETP activity is associated with a greater risk of CVD.

Our investigation has both strengths and limitations that warrant discussion. First, assessment of plasma CETP activity *in vitro* is challenging. We used a standardized assay but *ex vivo* assessment of CETP activity may not reflect *in vivo* activity. Although the assay we used has VLDL as acceptor particles, CETP has been implicated in lipid transfer reactions involving several different lipid particles³⁹. Second, our finding that CETP activity and mass are correlated was in agreement with previous reports^{35,40,41}. Third, as discussed above, glucocorticoids exert pleiotropic metabolic effects that may be difficult to interpret in the setting of a chronic inflammatory disease such as RA. Finally, our study had a cross-sectional design. Other studies that assessed the predictive value of a marker on cardiovascular risk generally had a prospective design with cardiovascular events as endpoints. For these reasons, we consider that this could be a potential limitation to generalize our results to all patients with RA.

Our data show that CETP activity is downregulated in patients with RA taking glucocorticoids. CETP activity in patients with RA has a consistent inverse relationship with the SCORE index, which suggests that CETP activity could serve as a surrogate marker of cardiovascular death risk in these patients.

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