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Original article

Impaired HDL cholesterol efflux capacity in systemic lupus erythematosus patients is related to subclinical carotid atherosclerosis

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Abstract

Objectives. Lipid profiles appear to be altered in SLE patients due to disease activity and inflammation. Cholesterol efflux capacity (CEC) is the ability of high-density lipoprotein cholesterol to accept cholesterol from macrophages. CEC has been linked to cardiovascular events in the general population and is impaired in SLE patients. The aim of this study was to establish whether CEC is related to subclinical carotid atherosclerosis in SLE patients.

Methods. The present report is of a cross-sectional study that encompassed 418 individuals: 195 SLE patients and 223 controls. CEC, using an *in vitro* assay, and lipoprotein serum concentrations were assessed in patients and controls. Carotid intima-media thickness and carotid plaques were evaluated in SLE patients. A multivariable analysis was performed to study the relationship of CEC to SLE-related data, lipid profile and subclinical carotid atherosclerosis.

Results. CEC was downregulated in SLE patients [8.1 (4.2) % *vs* 16.9 (10.4) %, $P = 0.004$). This occurred independently of traditional cardiovascular risk factors, statin use or other variations in the lipid profile related to the disease. Traditional cardiovascular risk factors, both in patients and controls, and SLE-related data such as activity, severity or damage were not associated with CEC. After multivariable regression analysis including lipid profile–related molecules, CEC was inversely and independently associated with the presence of carotid plaques in SLE patients [odds ratio 0.87 (95% CI: 0.78, 0.97), $P = 0.014$].

Conclusion. CEC is impaired in SLE patients independently of other inflammation-related lipid profile modifications that occur during the disease. CEC is associated with carotid plaques in SLE patients.

Key words: systemic lupus erythematosus, carotid plaque, cholesterol efflux capacity

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CLINICAL SCIENCE

Rheumatology key messages

- Cholesterol efflux capacity is impaired in SLE patients.
- Cholesterol efflux capacity is impaired in SLE patients independently of other inflammation-related lipid profile modifications.
- Cholesterol efflux capacity is independently associated with subclinical atherosclerosis in SLE patients.

Introduction

An increased prevalence of atherosclerosis is present among patients with SLE. The risk of cardiovascular disease (CVD) (which includes myocardial infarction, cerebrovascular disease, and peripheral vascular disease) in SLE patients has been found to be at least twice that of the general population [1]. The pathogenesis of this accelerated atherosclerosis in SLE is incompletely understood and likely multifactorial. In this sense, although traditional risk factors for atherosclerosis are common in patients with SLE [2], SLE itself confers the greatest risk for premature atherosclerosis [3, 4]. Among traditional atherosclerotic risk factors, it is accepted that dyslipidaemia has a clear impact on clinical CVD and surrogate markers for subclinical atherosclerosis in SLE [5]. In this regard, increasing evidence indicates that the systemic inflammatory load in SLE disrupts cholesterol homeostasis, increasing vulnerability to cholesterol accumulation in arterial wall cells, including macrophages and endothelial cells [6, 7]. However, the relationship between the inflammatory state and dyslipidaemia in SLE is complex and requires further study.

The high-density lipoprotein (HDL) particle has multiple potentially antiatherogenic properties. Much of its antiatherogenic effect is thought to be mediated by its participation in the removal of cholesterol from macrophages in atherosclerotic plaques during a process termed 'cholesterol efflux'. This is the first step in reverse cholesterol transport in which excess cholesterol is removed from the body, a process of well-known antiatherogenic significance [8]. Cholesterol efflux capacity (CEC) has a strong inverse association with both carotid intima-media thickness (cIMT) and the likelihood of angiographic coronary artery disease, independent of HDL cholesterol levels [9], and it has been inversely associated with the incidence of cardiovascular events in a population-based cohort [10].

Taking all of these findings into account and also considering that SLE is associated with early-onset cardiovascular disease and that it is often presented with dyslipidaemia, we aimed to study whether CEC is impaired in SLE patients and whether it is related to subclinical carotid atherosclerosis in SLE patients.

Methods

Study participants

This was a cross-sectional study that included 418 individuals: 195 patients with SLE and 223 controls. All SLE patients were 18 years old or older, had a clinical diagnosis of SLE, and had fulfilled $\geq$4 ACR classification criteria for SLE [11]. Moreover, they had been diagnosed by rheumatologists and were periodically followed-up at rheumatology outpatient clinics. For the purpose of inclusion in the present study, SLE disease duration was required to be $\geq$1 year. Controls included in the current study were subjects without any known condition or drug treatment history that could influence lipid levels, and they were not taking any lipid-lowering medications other than statins. None of the controls was receiving glucocorticoids. However, since glucocorticoids are often used in the management of SLE, patients taking prednisone or an equivalent dose of $\geq$10 mg/day were excluded. As previously mentioned, both patients and controls under statin treatment were allowed to participate in the study. Patients and controls were excluded if they had a history of myocardial infarction, angina, stroke, a glomerular filtration rate of $<$60 ml/min/1.73 m^2, a history of cancer, any other chronic disease, or evidence of active infection. Research was carried out in compliance with the Declaration of Helsinki. The study protocol was approved by the Institutional Review Committee at Hospital Universitario de Canarias and Hospital Doctor Negrín (both in Spain), and all subjects provided informed written consent (Approval Number 2015_84).

Data collection

The subjects, both patients and controls, completed a cardiovascular risk factor and medication use questionnaire and underwent a physical examination. Weight, height, BMI, waist circumference and systolic and diastolic blood pressure (measured with the participant in a supine position) were assessed under standardized conditions. Information regarding smoking status (current smoker *vs* non-smoker), diabetes, and hypertension was obtained from the questionnaire. Medical records were reviewed to ascertain specific diagnoses and medications. Dyslipidaemia was defined if one of the following was present: total cholesterol $>$ 200 mg/dl, triglycerides $>$ 150 mg/dl, HDL cholesterol $<$ 40 in men or $<$ 50 mg/dl in women, or low-density lipoprotein (LDL) cholesterol $>$ 130 mg/dl. SLE disease activity and damage were assessed using the SLEDAI-2000 (SLEDAI-2K) [12] and the SLICC/ACR Damage Index (SDI) [11], respectively. For the present study, the SLEDAI-2k index was broken down into none, mild, moderate, high, and very high activity, as previously described [13] (The SLEDAI category could not be calculated in 10 patients due to missing

data.) Disease severity was measured as well, using the Katz Index [14].

Lipids and cholesterol efflux assessments

Fasting serum samples were collected and frozen at –80°C until analysis of circulating lipids. Cholesterol, triglycerides, and HDL cholesterol were measured using the enzymatic colorimetric assay (Roche). Lipoprotein A and lipoproteins were assessed using a quantitative immunoturbidimetric assay (Roche). Cholesterol ranged from 0.08 to 20.7 mmol/l (intra-assay coefficient of variation of 0.3%); triglycerides ranged from 4 to 1.000 mg/dl (intra-assay coefficient of variation of 1.8%); and HDL cholesterol ranged from 3 to 120 mg/dl (intra-assay coefficient of variationof 0.9%). The atherogenic index was calculated using the total cholesterol : HDL cholesterol ratio according to the Castelli formula. LDL cholesterol was calculated using the Friedewald formula. A standard technique was used to measure high-sensitivity CRP.

Macrophage-specific CEC was measured using BODIPY cholesterol as previously described [10]. Briefly, J774 macrophages were seeded into a 96-well plate at 7×10^4 cells per well. The following day the cells were incubated for 1 h with BODIPY-tagged cholesterol (25 μM; Avanti Polar Lipids), 0.2% BSA, and 2 μg/ml ACAT inhibitor (Sandoz, Sigma-Aldrich) in Roswell Park Memorial Institute (RPMI) medium plus 1% fetal bovine serum (FBS). Following washing with MEM-HEPES, cells were incubated overnight in serum-free RPMI containing 0.3 mM cAMP, 0.2% BSA, and 2 μg/ml ACAT inhibitor. Apolipoprotein B-depleted plasma from study subjects was prepared using polyethylene glycol precipitation. After washing with MEM-HEPES, BODIPY cholesterol–labelled cells were incubated with 2.8% apolipoprotein B–depleted plasma in MEM-HEPES buffer, 0.15 mM cAMP and 2 μg/ml acyl-CoA cholesterol acyltransferase (ACAT) inhibitor for 4 h at 37°C. The resulting quantity of BODIPY cholesterol in the media was measured directly using a spectrofluorometer plate reader (Tecan, Trading AG, Switzerland) with an excitation wavelength of 485 nm and an emission detection at 530 nm. The CEC was calculated as the amount of effluxed BODIPY cholesterol expressed as a fraction of the initial cell content of BODIPY cholesterol. Each assay was performed in triplicate, and when the percentage of variation of every sample was higher than 7%, the sample was reassessed.

Carotid ultrasound assessment

A carotid US examination was performed to assess cIMT in the common carotid artery and to identify focal plaques in the extracranial carotid tree in patients with SLE [15]. A commercially available scanner, the Esaote Mylab 70 (Genoa, Italy), equipped with a 7–12 MHz linear transducer and an automated software-guided radiofrequency technique, Quality Intima Media Thickness in real-time (QIMT, Esaote, Maastricht, Holland), was used for this purpose. As previously reported [12], based on the Mannheim consensus, plaque criteria in the accessible extracranial carotid tree (common carotid artery, bulb and internal carotid artery) were defined as follows: a focal protrusion in the lumen measuring at least cIMT $>$ 1.5 mm; a protrusion at least 50% greater than the surrounding cIMT; or arterial lumen encroaching $>$0.5 mm [16].

Statistical analysis

Demographic and clinical characteristics were compared between SLE patients and controls using χ^2 tests for categorical variables or a Student's *t* test for continuous variables [data expressed as mean (S.D.)]. For non-continuous variables, either a Mann–Whitney *U* test was performed or a logarithmic transformation was made, and data were expressed as a median and IQR. Multivariable linear regression analyses were performed to establish the relationship of demographics, traditional cardiovascular risk factors, lipid profile and SLE-related data to CEC. The relationship of CEC to carotid assessments was determined through multivariable logistic regression analysis, adjusting for confounding factors. For the purpose of this study, confounding variables were those with a statistical *P* value lower than 0.20 in the association analysis between carotid assessment and CEC. To avoid collinearity, variables derived from a formula were excluded from the regression models [e.g. LDL:HDL ratio, atherogenic index, etc.]. Collinearity in the regression models was tested through the calculation of the variance inflation factor. All analyses used a 5% two-sided significance level and were performed using SPSS software, version 21 (IBM, Chicago, IL, USA) and STATA software, version 13/SE (Stata Corp., College Station, TX, USA). A *P*-value $<$ 0.05 was considered statistically significant.

Results

Demographic, laboratory and disease-related data

A total of 418 participants, 195 patients with SLE and 223 controls, were included in this study. Demographic and disease-related characteristics of the participants are shown in Table 1. SLE patients were younger (mean difference 7.8 years) and were more frequently women. There were no differences between patients and controls with regard to BMI or abdominal circumference. However, the presence of hypertension and current use of statins (39% *vs* 29%, $P = 0.023$) were more common in patients with SLE. Many differences were found in the lipid profile between patients and controls. In this sense, cholesterol, triglycerides, LDL cholesterol, the LDL:HDL cholesterol ratio, non-HDL cholesterol, apolipoprotein A1 and B, and atherogenic index were found to be lower in SLE patients. In contrast, lipoprotein A revealed higher serum levels in SLE patients. However, CRP, HDL cholesterol, and the apo A:apo B ratio did not differ between patients and controls. The mean CEC of HDL was significantly lower in SLE patients compared with

Table 1 Characteristics of SLE patients and controls

	Controls (n = 223)	SLE patients (n = 195)	P
Age, years	59 (9)	51 (11)	**<0.001**
Female, *n* (%)	155 (70)	185 (95)	**<0.001**
BMI, kg/m^2	28.2 (4.8)	27.4 (5.5)	0.11
Abdominal circumference, cm	93 (14)	92 (13)	0.55
Systolic blood pressure, mmHg	133 (15)	129 (21)	**0.042**
Diastolic blood pressure, mmHg	85 (41)	84 (51)	0.85
Cardiovascular co-morbidity			
Smoking, *n* (%)	45 (20)	46 (24)	0.44
Diabetes, *n* (%)	10 (4)	9 (5)	0.97
Hypertension, *n* (%)	64 (29)	77 (39)	**0.023**
Dyslipidemia, *n* (%)	165 (74)	138 (71)	0.12
Statins, *n* (%)	22 (10)	52 (27)	**<0.001**
Laboratory data, including lipid profile			
CRP, mg/dl	1.0 (1.0–3.0)	1.9 (0.9–4.9)	0.60
Cholesterol, mg/dl	218 (39)	200 (38)	**<0.001**
Triglycerides, mg/dl	105 (52)	127 (80)	**0.001**
HDL cholesterol, mg/dl	63 (17)	63 (21)	0.65
LDL cholesterol, mg/dl	134 (35)	111 (29)	**<0.001**
LDL:HDL cholesterol ratio	2.33 (0.94)	1.91 (0.75)	**<0.001**
Non-HDL cholesterol, mg/dl	155 (38)	136 (33)	**<0.001**
Lipoprotein A, mg/dl	16 (9–35)	38 (12–116)	**<0.001**
Apolipoprotein A1, mg/dl	191 (35)	180 (37)	**0.002**
Apolipoprotein B, mg/dl	102 (24)	95 (24)	**0.005**
Apo B:Apo A ratio	0.55 (0.16)	0.55 (17)	0.90
Atherogenic index	3.72 (1.14)	3.39 (1.08)	**0.004**
Cholesterol efflux capacity, %	16.9 (10.4)	8.1 (4.2)	**0.004**
SLE-related data			
Disease duration, years		17 (10)	
SLICC		1 (1–3)	
SLICC >=1, *n* (%)		145 (74)	
Katz Index		2 (1–3)	
Katz Index >= 3, *n* (%)		75 (38)	
SLEDAI		2 (0–5)	
SLEDAI activity categories, *n* (%)			
No activity, *n* (%)		78 (40)	
Mild, *n* (%)		63 (32)	
Moderate, *n* (%)		31 (16)	
High and Very high, *n* (%)		13 (7)	
Auto-antibody profile at inclusion			
Anti-DNA positive, *n* (%)		98 (50)	
ENA positive, *n* (%)		66 (34)	
Anti-Ro, *n* (%)		62 (32)	
Anti-La, *n* (%)		30 (15)	
Anti-RNP, *n* (%)		48 (25)	
Anti-Sm, *n* (%)		21 (11)	
Antiphospholipid battery, *n* (%)		71 (36)	
Lupus anticoagulant, *n* (%)		39 (20)	
ACA IgM, *n* (%)		20 (10)	
ACA IgG, *n* (%)		31 (16)	
Anti beta2 glycoprotein IgM, *n* (%)		13 (7)	
Anti beta2 glycoprotein IgG, *n* (%)		22 (11)	
C3, mg/dl		96 (27)	
C4, mg/dl		17 (7)	
Current prednisone, *n* (%)		99 (51)	
Prednisone, mg/day		5.0 (5.0–7.5)	
DMARDs, *n* (%)		152 (78)	
HCQ, *n* (%)		132 (68)	
MTX, *n* (%)		23 (12)	
MMF, *n* (%)		15 (8)	

(continued)

Table 1 Continued

	Controls ($n = 223$)	SLE patients ($n = 195$)	P
AZA, *n* (%)		27 (14)	
Rituximab, *n* (%)		6 (3)	
Belimumab, *n* (%)		3 (2)	
CYC, *n* (%)		1 (1)	
Carotid intima-media assessment			
Carotid plaques, *n* (%)		66 (34)	
bilateral, *n* (%)		34 (17)	
cIMT, microns		627 (122)	

Data represent mean (S.D.) or median (interquartile range) when data were not normally distributed. SLEDAI categories were defined as: 0, no activity; 1–5 mild; 6–10 moderate; >10 high activity. Dyslipidaemia was defined if one of the following was present: total cholesterol > 200 mg/dl, triglycerides > 150 mg/dl, HDL cholesterol < 40 in men or < 50 mg/dl in women, or LDL cholesterol > 130 mg/dl. C3 C4: complement; LDL: low density lipoprotein; ACA: anticardiolipin; HDL: high-density lipoprotein.

controls [16.9 (10.4) *vs* 8.1 (4.2), $P = 0.004$] when the univariate analysis was performed.

Most SLE patients were in the no activity (40%) or mild activity (32%) categories as shown by the SLEDAI score. Disease duration was 17 (10) years. SLICC and Katz Indexes were, respectively, 1 (IQR 1–3) and 2 (IQR 1–3). Seventy-four percent of the patients had a SLICC/ACR DI: damage index score equal to or higher than 1, and 38% had a Katz Index equal to or higher than 3. About half of the patients (51%) were taking prednisone [the median dose of those 99 patients on prednisone was 5 (IQR 5–7.5) mg/day at the time of the study]. At the time of the study, 98 (50%) patients were found to be positive for anti-DNA, and 34% were positive for ENA, with anti-Ro being the antibody the most frequently found (32%). DMARDs use was reported in 78% of the patients, and 68% were taking HCQ when the study was performed.

Multivariable analysis of the differences in lipid profiles between SLE patients and controls

Table 2 shows the differences in lipid-related molecules between patients and controls. After adjusting for age, sex, BMI, systolic pressure, hypertension, and statins (Model 1 in Table 2), most of these molecules showed differences between the two populations. In this sense, total cholesterol, LDL cholesterol, LDL:HDL ratio, non-HDL cholesterol, apoliproteins A1 and B, atherogenic index, and CEC were statistically significant downregulated in SLE patients. Only, triglycerides and lipoprotein A showed higher levels in SLE patients. Remarkably, HDL cholesterol did not differ between patients and controls.

Because lipid-related molecules are interrelated (they share metabolic pathways and it is not easy to separate the effect of one from that of the others), we performed a multivariable analysis adjusting for demographics and cardiovascular risk factors plus all the lipid-related molecules that were found to be different between patients and controls in Model 1 (Model 2 in Table 2). Because of collinearity, lipid molecules derived from a formula were excluded from the regression model (LDL cholesterol, LDL:HDL ratio, non-HDL cholesterol, apoB:apoA, and atherogenic index). Total cholesterol [beta coef. –10 (95% CI: −17, −4), $P = 0.001$], triglycerides [beta coef. 26 (95% CI: 11, 41), $P = 0.001$], and lipoprotein A [beta coef. 50 (95% CI: 34, 67), $P = <0.001$] maintained their differences between patients and controls. Interestingly, CEC [beta coef. −8 (95% CI: −10, −7) % , $P = <0.001$] conserved its downregulation in SLE patients after adjustment for other lipid profile–related molecules.

Relation of demographics, lipid profile, and disease-related data to efflux capability in SLE patients and controls

Demographics and lipid profiles were, in general, not related to CEC in either patients or controls. In this sense, only systolic blood pressure showed a negative association with CEC in controls [beta coefficient −0.12 (95% CI −0.23, 0.02), $P = 0.025$], but not in patients. Similarly, no traditional cardiovascular risk factors were related to CEC in either population. Regarding lipid profiles, with the exception of apolipoprotein B in patients with SLE, no correlations were identified between the standard lipid profile molecules and CEC. Lastly, concerning SLE-related data, no associations were identified with any manifestation of the disease. For example, none of disease duration, disease activity (SLEDAI), disease damage (SLICC), disease severity (Katz Index) or antibody patterns showed any correlations with CEC. Only patients on MTX showed a higher and statistically significant CEC [beta coefficient 2.60 (95% CI: 0.78, 4.42) %, $P = 0.005$] (Table 3).

Cholesterol efflux is associated with carotid plaque in SLE patients

In SLE patients, age, systolic blood pressure, the presence of hypertension, and current treatment with statins were univariately associated with the presence of carotid plaque. Similarly, triglycerides ($P = 0.021$) and lipoprotein A ($P = 0.047$) were higher in patients with

Table 2 Multivariable analysis of the differences in lipid profile between SLE patients and controls

	Controls	SLE patients	Univariate model	Model 1	Model 2 beta coef (95% CI)	
Lipid profile						
Cholesterol, mg/dl	218 (39)	200 (38)	**<0.001**	**<0.001**	−10 (−17, −4)	**0.001**
Triglycerides, mg/dl	105 (52)	127 (80)	**0.001**	**<0.001**	26 (11, 41)	**0.001**
HDL cholesterol, mg/dl	63 (17)	63 (21)	0.65			
LDL cholesterol, mg/dl	134 (35)	111 (29)	**<0.001**	**<0.001**	–	–
LDL:HDL cholesterol ratio	2.33 (0.94)	1.91 (0.75)	**<0.001**	**0.001**	–	–
Non-HDL cholesterol, mg/dl	155 (38)	136 (33)	**<0.001**	**<0.001**	–	–
Lipoprotein A, mg/dl	16 (9–35)	38 (12–116)	**<0.001**	**<0.001**	50 (34, 67)	**<0.001**
Apolipoprotein A1, mg/dl	191 (35)	180 (37)	**0.002**	**<0.001**	−6 (−13, 2)	0.14
Apolipoprotein B, mg/dl	102 (24)	95 (24)	**0.005**	0.10	−1 (−5, 4)	0.79
Apo B:Apo A ratio	0.55 (0.16)	0.55 (0.17)	0.90			
Atherogenic index	3.72 (1.14)	3.39 (1.08)	**0.004**	0.14	–	–
Cholesterol efflux capacity, %	16.9 (10.4)	8.1 (4.2)	**0.004**	**<0.001**	−8 (−10, −7)	**<0.001**

Data represent mean (S.D.) or median (interquartile range) when data were not normally distributed. Model #1: Adjusted for age, sex, BMI, systolic pressure, hypertension, and statins. Model #2: Adjusted for model #1 + rest of lipid molecules (with a P-value < 0.20 in the univariate analysis) other than the one that is compared. Because of collinearity, LDL cholesterol, LDL:HDL ratio, non-HDL cholesterol, apoB:apoA and atherogenic index were excluded from the multivariable analyses in Model 2. HDL: high-density lipoprotein; LDL: low-density lipoprotein.

carotid plaque. CEC was found to be downregulated in patients with carotid plaque [8.67% (4.68) *vs* 6.96% (2.84), $P = 0.002$]. When these relations were adjusted for cardiovascular risk factors (Multivariable model in Table 4), the relationships of triglycerides and lipoprotein A to CEC were lost, but the association of CEC with carotid plaque maintained significance [odds ratio (OR) 0.87 (95% CI: 0.78, 0.97), $P = 0.014$]. Moreover, additional adjustment for triglycerides or lipoprotein A showed the same results, with CEC being independently associated with carotid plaque (data not shown).

Discussion

The present study included the largest series of SLE patients in whom CEC has been assessed. Based on our results, CEC in SLE patients was downregulated independently of traditional cardiovascular risk factors, statin use or other variations in the lipid profile related to the disease. In this respect, disease activity, severity or damage was not found to be associated with CEC in patients with SLE. In addition, we also described, for the first time, that CEC was associated with carotid subclinical atherosclerosis in SLE patients.

Atherosclerotic plaques in the carotid arteries, which may correlate with the presence of similar lesions in the coronary arteries, are found more commonly in patients with SLE than in controls. The prevalence of carotid plaques in our study is in agreement with previous data. For example, in a case–control study that included 197 patients with SLE, carotid artery plaques were found by carotid US in 37% of the patients but in only 15% of the controls [4]. Another longitudinal study on 217 SLE patients followed-up for an average of 4 years revealed that the baseline carotid plaque prevalence increased from 31% to 40% at last follow-up [17]. This is of great relevance, since a study that included 392 women with SLE followed-up for a mean of 8 years demonstrated that the baseline presence of carotid plaques was a strong predictor of any incident cardiovascular event, regardless of traditional cardiovascular risk factors and medication use [18].

CEC has previously been studied in SLE patients. Ronda *et al.* evaluated CEC in 30 SLE patients and 30 healthy controls [19]. Although the disease was under control in most patients, CEC was reduced in SLE patients compared with in controls. Moreover, in keeping with our findings, no correlation between CEC and the activity score SLEDAI was found. This was also the case for differences according to anti-DNA and anti-ENA. In addition, no correlation between CEC and any of the pharmacological treatments given to SLE patients was found [19]. However, unlike our study, that of Ronda *et al.* did not assess association of CEC with subclinical atherosclerosis. In fact, in this study no adjustment was made for other lipid profile molecules, probably due to the sample size ($n = 30$). This was not the case with our study due to the larger number of patients included in the assessment.

The CEC downregulation in SLE patients found in our study, independent of other modifications to the lipid profile, is of potential relevance. With respect to this, CEC in our patients was found to be disrupted in a way unrelated to HDL cholesterol serum concentration. In our study, HDL cholesterol serum concentration did not show differences between patients and controls. This may imply that HDL dynamics are disrupted in SLE patients regardless of serum concentration.

In our study we found that MTX was associated with an upregulated CEC. However, the design of the study

Table 3 Relation of demographics, lipid profile, and disease-related data with efflux capability in SLE patients and controls

	Cholesterol efflux capacity %, beta coefficient (95% CI), *P*	
	Controls	**Patients**
Age, years	−0.09 (−0.35, 0.17), 0.50	−0.04 (−0.09, 0.16), 0.17
Female	0.94 (−2.77, 4.64), 0.62	−0.16 (−2.87, 2.55), 0.91
BMI, kg/m^2	−0.22 (−0.56, 0.12), 0.20	0.04 (−0.07, 0.15), 0.44
Abdominal circumference, cm	−0.12 (−0.24, 0.00), 0.058	0.02 (−0.02, 0.07), 0.33
Systolic blood pressure, mmHg	**−0.12 (−0.23, −0.02), 0.025**	0.01 (−0.02, 0.04), 0.39
Diastolic blood pressure, mmHg	−0.02 (−0.06, 0.02), 0.29	−0.00 (−0.01, 0.01), 0.66
Cardiovascular co-morbidity		
Smoking	1.81 (−2.45, 6.02), 0.40	−0.05 (−1.47, 1.36), 0.94
Diabetes	1.18 (−5.93, 8.30), 0.74	−0.44 (−3.29, 2.41), 0.76
Hypertension	−2.45 (−5.96, 1.06), 0.17	−0.86 (−2.08, 0.37), 0.17
Dyslipidemia	−0.04 (−4.49, 4.41), 0.99	−1.24 (−2.57, 0.09), 0.066
Statins	−2.09 (−7.41, 3.23), 0.44	−0.18 (−1.54, 1.18), 0.79
Laboratory and lipid profile		
CRP, mg/dl	−0.27 (−0.58, 0.04), 0.092	0.01 (−0.04, 0.06), 0.68
Cholesterol, mg/dl	0.04 (−0.010, 0.09), 0.11	−0.01 (−0.03, 0.01), 0.19
triglycerides, mg/dl	−0.02 (−0.06, 0.01), 0.19	−0.01 (−0.01, 0.00), 0.18
HDL cholesterol, mg/dl	0.08 (0.01, 0.18), 0.092	−0.01 (−0.04, 0.02), 0.36
LDL cholesterol, mg/dl	0.03 (−0.03, 0.08), 0.32	−0.00 (−0.02, 0.02), 0.75
LDL:HDL cholesterol ratio	−0.46 (−2.48, 1.55), 0.65	−0.05 (−0.86, 0.75), 0.90
Non-HDL cholesterol, mg/dl	0.01 (−0.04, 0.06), 0.63	−0.01 (−0.03, 0.01), 0.36
Lipoprotein A, mg/dl	0.05 (−0.00, 0.10), 0.059	0.00 (−0.01, 0.01), 0.89
Apolipoprotein A1, mg/dl	0.04 (−0.01, 0.09), 0.089	−0.01 (−0.03, 0.01), 0.27
Apolipoprotein B, mg/dl	0.01 (−0.07, 0.09), 0.77	**−0.03 (−0.05, 0.00), 0.029**
Apo B:Apo A1 ratio	−5.76 (−16.89, 5.37), 0.31	−2.52 (−6.11, 1.08), 0.17
Atherogenic index	−0.72 (−2.42, 0.99), 0.41	−0.09 (−0.65, 0.47), 0.75
SLE-related data		
Disease duration, years		−0.05 (−0.11, 0.02), 0.15
SLICC		0.09 (−0.21, 0.38), 0.57
SLICC $\geq$ 1		0.18 (−1.25, 1.60), 0.81
Katz index		0.18 (−0.15, 0.50), 0.28
Katz index $\geq$ 3		1.05 (−0.21, 2.30), 0.10
SLEDAI		−0.06 (−0.19, 0.08), 0.40
SLEDAI activity categories		
No activity		–
Mild		**2.32 (1.14, 4.68), 0.019**
Moderate		0.94 (0.37, 2.43), 0.91
High and very high		1.21 (0.34, 4.34), 0.77
Moderate, high, and very high		−0.43 (−1.24, 0.37), 0.29
Auto-antibody profile		
Anti-DNA positive		−0.83 (−2.31, 0.64), 0.27
ENA positive		−0.12 (−2.38, 2.14), 0.92
Anti-Ro		0.00 (−1.43, 1.43), 0.99
Anti-La		−0.86 (−2.64, 0.92), 0.34
Anti-RNP		−0.58 (−2.06, 0.90), 0.44
Anti-Sm		−1.15 (−3.09, 0.79), 0.24
aPL battery		0.53 (−0.88, 1.94), 0.46
Lupus anticoagulant		0.09 (−1.55, 1.73), 0.92
ACA IgM		−0.95 (3-05, 1.14), 0.37
ACA IgG		−1.52 (−3.26, 0.22), 0.086
Anti beta2 glycoprotein IgM		0.12 (−2.43, 2.68), 0.92
Anti beta2 glycoprotein IgG		1.51 (−0.54, 3.56), 0.15
C3, mg/dl		−0.00 (−0.03, 0.02), 0.75
C4, mg/dl		0.04 (−0.05, 0.12), 0.38
Current prednisone, *n* (%)		0.71 (−0.50, 1.92), 0.25
Prednisone, mg/day		−0.11 (−0.38, 0.16), 0.43
DMARDs		0.55 (−0.96, 2.06), 0.47

(continued)

Table 3 Continued

	Cholesterol efflux capacity %, beta coefficient (95% CI), *P*	
	Controls	Patients
HCQ		0.15 (−1.18, 1.47), 0.83
MTX		**2.60 (0.78, 4.42), 0.005**
MMF		0.20 (−2.04, 2.44), 0.86
AZA		1.19 (−0.60, 2.97), 0.19
Rituximab		−1.05 (−4.51, 2.40), 0.55
Belimumab		0.42 (−4.43, 5.28), 0.86
CYC		−5.92 (−16.27, 4.43), 0.26

Data represent mean (S.D.) or median (interquartile range) when data were not normally distributed. SLEDAI categories were defined as: 0, no activity; 1–5 mild; 6–10 moderate; >10 high disease activity. SLICC: Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index. Dyslipidaemia was defined if one of the following was present: total cholesterol > 200 mg/dl, triglycerides > 150 mg/dl, HDL cholesterol < 40 in men or < 50 mg/dl in women, or LDL cholesterol > 130 mg/dl. C3 C4: complement; LDL: low-density lipoprotein; ACA: anticardiolipin; HDL: high-density lipoprotein; ANAs: antinuclear antibodies; ENA: extractible nuclear antibodies.

Table 4 Traditional cardiovascular factors, lipid profile, and cholesterol efflux association with carotid plaque in SLE patients

	Carotid plaque, SLE patients (*n* = 195)				
				Multivariable model	
	no = 129	yes = 66	*P*	OR (95% CI) *P*	
Age, years	47 (10)	57 (10)	**<0.001**		
Female, *n* (%)	124 (96)	61 (92)	0.27		
BMI, kg/m^2	27. 1 (5.7)	27.9 (5.2)	0.31		
Abdominal circumference, cm	91 (14)	94 (12)	0.16		
Systolic blood pressure, mmHg	126 (21)	135 (19)	**0.003**		
Diastolic blood pressure, mmHg	85 (63)	81 (10)	0.63		
Cardiovascular co-morbidity					
Smoking, *n* (%)	26 (20)	20 (30)	0.11		
Diabetes, *n* (%)	3 (2)	6 (9)	0.064		
Hypertension, *n* (%)	39 (30)	38 (58)	**<0.001**		
Dyslipidemia, *n* (%)	88 (68)	50 (76)	0.21		
Statins, *n* (%)	21 (16)	31 (47)	**<0.001**		
Laboratory and lipid profile					
CRP, mg/dl	1.9 (0.9–5.0)	2.1 (0.9–3.8)	0.32		
Cholesterol, mg/dl	200 (38)	199 (38)	0.83		
Triglycerides, mg/dl	116 (55)	150 (111)	**0.021**	1.00 (0.99, 1.01)	0.055
HDL cholesterol, mg/dl	63 (19)	64 (24)	0.94		
LDL cholesterol, mg/dl	113 (29)	105 (28)	0.60		
LDL:HDL cholesterol ratio	1.94 (0.76)	1.84 (0.72)	0.38		
Non-HDL cholesterol, mg/dl	137 (33)	135 (35)	0.78		
Lipoprotein A, mg/dl	32 (9–104)	51 (22–146)	**0.047**	1.00 (0.99, 1.01)	0.31
Apolipoprotein A1, mg/dl	179 (37)	181 (39)	0.63		
Apolipoprotein B, mg/dl	94 (25)	97 (22)	0.39		
Apo B:Apo A ratio	0.55 (0.16)	0.56 (0.17)	0.54		
Atherogenic index	3.36 (1.00)	3.45 (1.12)	0.64		
Cholesterol efflux capacity, %	8.67 (4.68)	6.96 (2.84)	**0.002**	0.87 (0.78, 0.97)	**0.014**

Data represent mean (S.D.) or median (interquartile range) when data were not normally distributed. Dyslipidaemia was defined if one of the following was present: total cholesterol > 200 mg/dl, triglycerides > 150 mg/dl, HDL-cholesterol < 40 in men or < 50 mg/dl in women, or LDL-cholesterol > 130 mg/dl. Multivariable Model: Adjusted for age, abdominal circumference, systolic pressure, smoking status, diabetes, hypertension, and statins. OR: odds ratio; LDL: low-density lipoprotein; HDL: high-density lipoprotein.

and the limited number of SLE patients who were on MTX ($n = 23$, 12%) did not allow us to draw a definitive conclusion on this finding. A *post hoc* analysis of a previous report in RA patients with impaired net CEC at baseline did not show an increase in CEC after 6 months of treatment with MTX [20]. The reduction of the inflammatory burden mediated by MTX in patients with RA was associated with improvement of disease-specific outcomes, including cardiovascular events [21]. However, low-dose MTX use in patients with stable atherosclerosis unrelated to inflammatory diseases did not result in any reduction in IL-1β, IL-6, or CRP levels. In addition, use of MTX use in these individuals did not result in fewer cardiovascular events than that of the placebo [22]. For this reason, our findings on SLE warrant further investigation, specifically regarding the influence of MTX on CEC in patients with inflammatory autoimmune diseases.

The lack of association between traditional cardiovascular risk factors or lipid profile with CEC in both SLE patients and controls noted in our study is in agreement with previous reports. In this sense, traditional risk factors explained only 3% of the variance observed in CEC [10]. Moreover, as previously mentioned, CEC cannot be explained by HDL cholesterol or apolipoprotein A1 levels [23]. Similarly, we did not find any association of statins with CEC in SLE patients or controls. This supports the claim that statins most likely exert therapeutic benefit by means of a mechanism that is different from the promotion of cholesterol efflux [9].

To the best of our knowledge, the relationship between CEC and carotid plaques had not previously been studied in patients with SLE. Nevertheless, our findings were in keeping with previous reports on healthy individuals. With respect to this, in a report in which the primary end point was atherosclerotic CVD, defined as a first non-fatal myocardial infarction, non-fatal stroke, or coronary revascularization or death from cardiovascular causes, there was a strong inverse relationship between CEC and the primary end point (adjusted hazard ratio 0.33, 95% CI: 0.19, 0.55, comparing the highest quartile with the lowest) [10] after a median follow-up period of 9.4 years. Also, in a previous report by our group, CEC was found to be independently associated with carotid plaques in patients with RA [15].

We acknowledge several limitations in our study. First, carotid assessments were not available for healthy control subjects. Although CEC has been widely associated with cIMT and cardiovascular events in the general population, the availability of carotid assessments in control subjects would have allowed us to study a different effect or statistical interaction between these two populations. Second, there are other ways of assessing cholesterol efflux *in vitro*. However, most research done in population-based cohorts has been carried out using the same assay as the one described in our study. Third, controls were not sex- and age-matched. However, identical results have been found irrespective of matching or not matching when multivariable regression analysis is applied in epidemiological cross-sectional or case–control studies [24]. We believe, therefore, that the multivariable analysis performed in our study was capable of handling confounding situations in the analysis regarding age and sex non-matched individuals.

In conclusion, CEC is downregulated in patients with SLE independently of other modifications to the lipid profile that occur in this autoimmune disease. CEC was also independently related to carotid plaque. Our findings may help to shed light on the impact of dyslipidaemia in the development of subclinical atherosclerosis in SLE. According to our results, CEC may underlie the increased risk of CVD in patients with SLE.

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