Insulin resistance in systemic lupus erythematosus patients: contributing factors and relationship with subclinical atherosclerosis

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Abstract

Objective

Insulin resistance (IR) plays a role in the increased cardiovascular risk of systemic lupus erythematosus (SLE) patients. This study aimed to determine the potential association of IR with disease activity, drug exposure and subclinical atherosclerosis in patients with SLE.

Methods

This cross-sectional study encompassed 87 non-diabetic SLE patients and 82 sex-matched controls. Insulin and C-peptide serum levels, IR indexes by homeostatic model assessment (HOMA2) (both insulin-based: HOMA2-IR, and with C-peptide: HOMA2-IR-C-peptide) and lipid profiles were assessed in patients and controls. Activity (SLEDAI), severity (Katz) and damage (SLICC) index scores, as well as carotid intima-media thickness (cIMT) and carotid plaques, were determined in SLE patients. A multivariable regression analysis, adjusted for classic IR related factors, was performed to evaluate the differences in IR indexes between patients and controls and how IR is associated with disease-related characteristics, including carotid ultrasound results, in SLE patients.

Results

SLE patients had higher C-peptide serum levels (2.61±1.51 vs. 1.34±0.62 ng/ml, p=0.00) and elevated HOMA2-IR-C-peptide index (1.90±1.12 vs. 0.97±0.45, p=0.00) than controls. These differences remained statistically significant after adjusting for classic cardiovascular risk factors and prednisone intake. Traditional IR-related factors, such as body mass index, waist circumference or hypertension, and prednisone intake were significantly associated with HOMA2-IR and HOMA2-IR-C-peptide in SLE patients. SLICC damage index was independently associated with HOMA2-IR-C-peptide. The presence of carotid plaques and cIMT values were associated with IR indexes in SLE patients only in the univariate analysis.

Conclusion

C-peptide serum levels are independently up-regulated in SLE patients. Although classic IR factors and prednisone are associated with IR, SLE damage over time also contributes to IR in an independent way.

Key words

insulin resistance, systemic lupus erythematosus, C-peptide, carotid intima-media thickness, carotid plaques.

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Introduction

Systemic lupus erythematosus (SLE) is associated with an increased prevalence of atherosclerosis. A systematic review revealed that the risk of cardiovascular disease (which included myocardial infarction, cerebrovascular disease, and peripheral vascular disease) among SLE patients is at least twice that of the general population (1). Traditional risk factors for atherosclerosis (diabetes, hyperlipidaemia, hypertension, family history of coronary heart disease, obesity, sedentary lifestyle, and cigarette smoking) are common among patients with SLE; in some cases due, in part, to the adverse effects of glucocorticoids (2, 3). However, atherosclerosis occurs prematurely in patients with SLE, independently of traditional risk factors for cardiovascular disease (4). This finding supports a role for disease-related factors in SLE patients' atherogenesis (5). In clinical practice, insulin resistance (IR) refers to a state in which a given concentration of insulin is associated with a subnormal glucose response. IR is likely the best predictor of type 2 diabetes (6) and may, at least in part, be related to inflammatory substances secreted by adipocytes including leptin, adiponectin, tumour necrosis factor alpha, and resistin. For this reason, IR can promote the development of atherosclerosis not only through elevated glucose and insulin concentrations, but also via mechanisms that involve dyslipidaemia, hypertension, and inflammation (7). Inflammation may worsen IR and impair pancreatic beta cell function. Moreover, IR has previously been associated with inflammatory diseases such as rheumatoid arthritis and SLE (8-10). The metabolic changes that IR exerts may also contribute to the development of accelerated atherosclerosis and higher incidence of ischaemic heart disease found in patients with inflammatory and autoimmune diseases.
Previous studies on IR in SLE patients have focused on the metabolic syndrome; a feature related to IR, but which encompasses many others risk factors (11). The contributing factors that lead to impaired glucose metabolism and the relationship of IR indexes, as well as insulin and C-peptide levels, with subclinical atherosclerosis have been less commonly studied in SLE patients.
Taking all of these considerations into account, the main purpose of our study was to analyze the impact of disease-related factors on IR development in SLE patients. A secondary objective was to study the relationship of this IR with subclinical atherosclerosis.

Methods

Study participants

This was a cross-sectional study that included 87 patients with SLE and 82 sex-matched controls. All SLE patients were 18 years old or older and were enrolled when they fulfilled ≥4 American College of Rheumatology (ACR) classification criteria for SLE (12). 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 ≥1 year. SLE patients undergoing biologic therapy (belimumab or rituximab) were not excluded from the present study. The control group consisted of patients recruited from the Spanish Camargo Cohort (13, 14). This cohort was set up between February 2006 and February 2011, and individuals included in this cohort have been followed ever since. The aim of this cohort was to evaluate the prevalence and incidence of metabolic bone diseases and mineral metabolism disorders. Controls included in the current study were sex-matched subjects without any known condition or drug treatment that might influence glucose metabolism and who were not taking any glucose-lowering medications. None of the patients was on glucose-lowering drugs or insulin therapy and none of the controls was receiving glucocorticoids. All patients and controls had a glycaemia <7 mmol/l. Since glucocorticoids are often used in the management of SLE, patients taking prednisone, or an equivalent dose ≤10 mg/day, were not excluded. Patients were excluded if they had a history of diabetes, myocardial infarction, angina, stroke, a history of cancer, or any other chronic disease, or evidence of active infection. In contrast

to a former report related to the same issue (15), in our study a glomerular filtration rate <60 ml/min/1.73 m2 was also considered an exclusion criteria due to the relationship of end-stage renal disease with both cardiovascular disease (16) and with the risk of insulin resistance (17). The study protocol was approved by the Institutional Review Committee at Hospital Universitario de Canarias and all subjects provided informed written consent.

Assessments

The homeostatic model assessment (HOMA) method was performed to determine IR. Briefly, the HOMA model is used to yield an estimate of insulin sensitivity (%S) and β-cell function (%B) from fasting plasma insulin, C-peptide, and glucose concentrations. In this study we have used HOMA2: the updated-computer HOMA model (15). This model can be used to assess insulin sensitivity and β-cell function from paired fasting plasma glucose and specific insulin, or C-peptide, concentrations across a range of 1–2,200 pmol/l for insulin and 1–25 mmol/l for glucose. In our study all of the IR HOMA indexes were calculated using both insulin and C-peptide. C-peptide better estimates β-cell function since it is a marker of secretion; and insulin data is preferable when calculating %S since HOMA-%S is derived from glucose disposal as a function of insulin concentration. The computer model gives a value for insulin sensitivity expressed as HOMA2-%S (where 100% is normal). HOMA2-IR (insulin resistance index) is simply the reciprocal of %S. Insulin (Architect Abbott, 2000I) and C-peptide (Immulite 2000, Siemens) were determined by chemiluminescent immunometric assays. Standard techniques were used to measure plasma glucose, C-reactive protein (CRP), the Westergren erythrocyte sedimentation rate (ESR) and serum lipids.

Data collection

Surveys in SLE patients and controls were performed in the same way. Subjects completed a cardiovascular risk factor and medication use questionnaire and underwent a physical examination to determine their anthropometrics and blood pressure. Hypertension was defined as a systolic or a diastolic blood pressure higher than respectively 140 and 90 mmHg. Dyslipidaemia was defined if one of the following was present: total cholesterol >200 mg/dl, triglyceride >150 mg/dl, HDL cholesterol <40 in men or <50 mg/dl in women, or LDL cholesterol >130 mg/dl.

Table I. Demographic data of controls and patients.

	Controls (n=82)	Patients (n=87)	*p*
Female, n (%)	77 (94)	83 (95)	0.66
Age, years	54 ± 10	50 ± 10	**0.01**
BMI, kg/m^2	29 ± 6	28 ± 5	0.11
BMI > 30 kg/m^2	25 (30)	27 (31)	0.07
Waist circumference, cm	91 ± 12	94 ± 12	0.33
Systolic pressure, mmHg	131 ± 14	135 ± 22	0.35
Diastolic pressure, mmHg	81 ± 11	80 ± 11	0.37
Comorbidity			
Hypertension, n (%)	25 (30)	29 (33)	0.65
Dyslipidaemia, n (%)	23 (28)	23 (26)	0.81
Currently smoking, n (%)	15 (16)	16 (18)	0.69
Antihypertensive treatment, n (%)	27 (33)	27 (31)	0.79
Statins, n(%)	6 (7)	19 (22)	**0.01**
Laboratory data			
CRP, mg/l	2.0 (0.9-4.0)	1.9 (1.0-4.4)	0.21
Cholesterol, mg/dl	208 ± 36	195 ± 32	**0.01**
Triglycerides, mg/dl	112 ± 65	117 ± 60	0.61
LDL, mg/dl	131 ± 35	113 ± 27	**0.00**
HDL, mg/dl	56 ± 13	58 ± 16	0.23
Apo lipoprotein A, mg/dl	184 ± 35	170 ± 31	**0.01**
Apo lipoprotein B1, mg/dl	108 ± 26	88 ± 21	**0.00**
Apo B/Apo A index	0.60 ± 0.17	0.54 ± 0.17	**0.04**
Atherogenic index	3.91 ± 0.92	3.58 ± 1.17	0.06
SLE-related data			
Disease duration, years		16 (9-21)	
SLICC		2 (1-3)	
Katz Index		2 (1-4)	
SLEDAI		2 (0-6)	
SLEDAI activity categories, n (%)			
No activity		35 (40)	
Mild		28 (32)	
Moderate		18 (21)	
High		5 (6)	
Very high		1 (1)	
Anti DNA positive, n (%)		58 (67)	
ENA positive, n (%)		62 (71)	
C3, mg/dl		83 ± 23	
C4, mg/dl		16 ± 7	
Leucocytes, cells/mm^3		5,500 ± 2,000	
Hypocomplementaemia, n (%)		25 (29)	
Current prednisone, n (%)		49 (56)	
Prednisone, mg/day		2 ± 3	
Hydroxychloroquine, n (%)		56 (64)	
Mycophenolate mofetil, n (%)		10 (11)	
Azatioprine, n (%)		16 (18)	
Rituximab, n (%)		2 (0)	
Belimumab, n (%)		2 (0)	
Carotid intima media assessment			
Carotid plaque, n (%)		17 (20)	
cIMT, mm		0.646 ± 0.116	

Data represent means±SD or median (IQR) when data were not normally distributed.
BMI: body mass index; C3 C4: complement; CRP: C-reactive protein; LDL: low-density lipoprotein DMARD: disease-modifying anti-rheumatic drug; ESR: erythrocyte sedimentation rate; HDL: high-density lipoprotein; ANA: antinuclear antibodies; ENA: extractible nuclear antibodies; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; SLICC: Systemic Lupus International Collaborating Clinics/American Colleague of Rheumatology Damage Index; cIMT: carotid intima media thickness.

Table II. Multivariate regression analysis of the differences between controls and patients in IR indexes

	Univariate			Adjusted*	
	Controls	SLE	*p*	beta coef. (95%CI)	*p*
Glucose, mg/dl	87 ± 8	87 ± 9	0.81	2 (-5-2)	0.32
Insulin, uU/ml	9.68 ± 9.27	7.90 ± 5.36	0.13	-3.27 (-7.27-0.73)	0.11
C-peptide, ng/ml	1.34 ± 0.62	2.61 ± 1.51	**0.00**	0.74 (0.35-1.13)	**0.00**
HOMA2-IR	1.13 ± 0.70	1.01 ± 0.67	0.28	-0.19 (-0.49-0.12)	0.23
HOMA2-S%	115 ± 57	139 ± 78	0.03	21 (-13-54)	0.23
HOMA2-B%	98 ± 51	100 ± 42	0.81	-3 (-22-17)	0.78
HOMA2-IR-C-peptide	0.97 ± 0.45	1.90 ± 1.12	**0.00**	0.53 (0.25-0.82)	**0.00**
HOMA2-S%-C-peptide	131 ± 91	75 ± 49	**0.00**	-37 (-63--11)	**0.01**
HOMA2-B%-C-peptide	101 ± 36	155 ± 61	**0.00**	35 (18-52)	**0.00**

Beta coefficient (95% confidence interval) refers to the difference between controls and patients adjusted for age, BMI, statins intake, LDL cholesterol serum levels and prednisone intake.
HOMA2-IR: Homeostatic Assessment Model for determining insulin resistance using insulin and glucose serum levels; HOMA2%B-C-peptide: Homeostatic Assessment Model for evaluating beta cell function using C-peptide and glucose serum levels.

Atherogenic index was calculated using total cholesterol/HDL cholesterol ratio. Medical records were reviewed to ascertain specific diagnoses and medications. SLE disease activity and damage were assessed using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI-2K) (16) and the SLICC/ACR Damage Index (SDI) (17), respectively. For the propose of the present study, the SLEDAI index was broken down into none, mild, moderate, high, and very high activity as previously described (18). Disease severity was measured as well, using the Katz Index (19).

Carotid ultrasound assessment

Carotid ultrasound was performed to assess carotid intima-media wall thickness (cIMT) in the common carotid artery and to detect focal plaques in the extracranial carotid tree in patients with RA. A commercially available scanner, Mylab 70, Esaote (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. 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 (20).

Table III. Demographics and disease-related factors vis-à-vis IR indexes.

	beta coefficient (95% CI), p-value			
	HOMA2-IR		HOMA2B%-C-peptide	
	Univariate model	Adjusted model	Univariate model	Adjusted model
Demographics				
Female	-0.20 (-0.89-0.49), 0.56		-50 (-111-11), 0.11	
Age, years	0.01 (-0.00-0.03), 0.12		**1 (0-3), 0.04**	
Waist circumference, cm	**0.02 (0.01-0.04), 0.00**		**1 (0-2), 0.01**	
BMI, kg/m^2	**0.05 (0.02-0.07), 0.00**		**3 (1-6), 0.01**	
Currently smoking	-0.30 (-0.67-0.07), 0.11		-26 (-59-1), 0.13	
Hypertension	**0.46 (0.17-0.75), 0.00**		**45 (18-71), 0.00**	
Dyslipidaemia	0.21 (-0.12-0.53), 0.21		22 (-7-51), 0.14	
Antihypertensive treatment	**0.47 (0.17-0.77), 0.00**		**46 (19-72), 0.00**	
Statins intake	0.34 (0.00-0.68), 0.05		**37 (6-67), 0.02**	
LDL cholesterol, mg/dl	0.00 (-0.01-0.00), 0.34		-0 (0-0), 0.28	
CRP, mg/dl	0.00 (-0.00-0.01), 0.59		**0.4 (0.04-0.84), 0.03**	
SLE duration and analytical data				
Disease duration, years	0.00 (-0.02-0.02), 0.99	0.00 (-0.02-0.02), 0.89	1 (-1.2), 0.51	0.4 (-1.1-1.9), 0.59
Anti-DNA positive, n (%)	0.31 (-0.18-0.80), 0.20	0.21 (-0.27-0.70), 0.38	13 (-26-54), 0.50	9 (-33-51), 0.68
ENA, n (%)	0.10 (-0.23-0.43), 0.54	0.14 (-0.19-0.47), 0.84	7 (-23-37), 0.64	10 (-21-40), 0.53
Hypocomplementaemia	0.11 (-0.25-0.47), 0.56	0.08 (-0.29-0.44), 0.67	9 (-25-42), 0.61	-10 (-45-24), 0.55
SLE related treatments				
Current prednisone	0.25 (-0.04-0.53), 0.09	0.22 (-0.06-0.50), 0.12	**29 (4-55), 0.02**	**47 (31-63), 0.00**
Prednisone, mg/day	0.02 (-0.01-0.05), 0.11	0.02 (-0.01-0.05), 0.14	**3 (0-5), 0.03**	**2 (0-5), 0.03**
Current any DMARDs	0.05 (-0.33-0.42), 0.81	0.15 (-0.26-0.56), 0.75	-3 (-37-30), 0.84	11 (-67-207), 0.31
Hydroxychloroquine	0.21 (-0.09-0.10), 0.17	0.21 (-0.10-0.52), 0.18	2 (-25-29), 0.87	7 (-23-36), 0.65
Azathioprine	-0.08 (-0.45-0.30), 0.69	0.00 (-0.35-0.36), 0.99	11 (-23-44), 0.53	20 (-12-53), 0.22
Mycophenolate mofetil	-0.04 (-0.49-0.41), 0.86	-0.16 (-0.63-0.31), 0.49	-7 (-47-34), 0.75	-30 (-74-14), 0.18

Adjusted models are adjusted for variables with a p value lower than 0.20 of the previous univariate model.
BMI: body mass index; CRP: C-reactive protein; LDL: low-density lipoprotein; ENA: extractible nuclear antibodies; DMARD: disease-modifying antirheumatic drug; HOMA2-IR: Homeostatic Assessment Model for determining insulin resistance using insulin and glucose serum levels; HOMA2%B-C-peptide: Homeostatic Assessment Model for evaluating beta cell function using C-peptide and glucose serum levels.

Table IV. Multivariate regression analysis of disease activity and damage indexes relationship with insulin resistance parameters and indexes

	beta coef. 95% CI							
	Insulin, uU/ml	C-peptide, ng/ml	HOMA2IR (insulin)			HOMA2IR-C peptide		
			IR	S%	B%	IR	S%	B%
Univariate model								
Katz index	0.0 (-0.6-0.6), 0.98	0.2 (-0.0-0.3), 0.07	0.0 (-0.1-0.1), 0.92	-3 (-12-6), 0.45	-1 (-6-4), 0.62	**0.1 (-0.0-0.2), 0.06**	**-5 (-11-0), 0.06**	4 (-3-11), 0.24
log SLICC index	**2.0 (0.1-3.9), 0.04**	**1.2 (0.7-1.7), 0.00**	**0.3 (0.0-0.5), 0.03**	**-34 (-61--6), 0.02**	10 (-6-25), 0.21	**0.9 (0.5-1.2), 0.00**	**-30 (-47--14), 0.00**	**37 (16-57), 0.00**
SLEDAI activity								
None	-	-	-	-	-	-	-	-
Mild or moderate	1.0 (-1.8-3.9), 0.48	0.3 (-0.5-1.1), 0.43	0.1 (-0.2-0.5), 0.47	2 (-40-44), 0.93	5 (-18-27), 0.68	0.2 (-0.4-0.8), 0.42	7 (-34-19), 0.59	9 (-23-42), 0.57
High or very high	2.3 (-2.3-6.8), 0.33	0.0 (-1.3-1.3), 0.96	0.3 (0.3-0.8), 0.36	-3 (-70-64), 0.93	24 (-11-60), 0.18	0.0 (-1.0-1.0), 0.99	4 (-38-47), 0.84	9 (-43-61), 0.74
Model #1 adjusted for clasical IR factors								
Katz index	-0.1 (-0.7-0.5), 0.65	0.1 (-0.0-0.3), 0.12	-0.0 (-0.1-0.1), 0.72	-3 (-12-6), 0.48	2 (-7-3), 0.37	0.1 (-0.0-0.2), 0.10	**-5 (-11--0), 0.04**	3 (-4-10), 0.40
log SLICC index	1.0 (-1.0-3.1), 0.32	**0.9 (0.3-1.4), 0.00**	0.1 (-0.1-0.4), 0.27	-22 (-52-8), 0.15	4 (-13-20), 0.65	**0.6 (0.3-1.0), 0.00**	**-22 (-40--4), 0.02**	**26 (2-49), 0.03**
SLEDAI activity								
None	-	-	-	-	-	-	-	-
Mild or moderate	2.0 (-0.8-4.7), 0.16	0.6 (-0.2-1.3), 0.12	0.3 (-0.9-0.6), 0.14	-9 (-50-32), 0.66	9 (-13-31), 0.43	0.5 (-0.1-1.0), 0.11	-17 (-42-8), 0.18	15 (-17-47), 0.36
High or very high	3.9 (-1.0-8,7), 0.12	0.6 (-0.7-1.9), 0.37	0.5 (-1.1-1.1), 0.12	-13 (-86-59), 0.72	28 (-10-67), 0.15	0.4 (-0.6-1.4), 0.39	2 (-46-41), 0.92	21 (-36-78), 0.46
Model #2 adjusted for clasical IR factors plus SLE related analytical data and treatments								
Katz index	-0.5 (-1.2-0.2), 0.16	0.0 (-0.1-0.2), 0.73	-0.1 (-0.1-0.0), 0.19	1 (-11-9), 0.82	-4 (-10-1), 0.13	0.0 (-0.1-0.2), 0.67	-3 (-9-3), 0.31	-1 (-8-7), 0.90
log SLICC index	0.4 (-2.0-2.7), 0.76	**0.7 (0.1-1.3), 0.03**	0.1 (-0.2-0.4), 0.70	-19 (-54-15), 0.27	0 (-19-19), 0.97	**0.5 (0.1-1.0), 0.02**	-15 (-35-5), 0.14	19 (-8-45), 0.16
SLEDAI activity								
None	-	-	-	-	-	-	-	-
Mild or moderate	1.5 (-1.4-4.4), 0.30	0.3 (-0.4-1.1), 0.37	0.2 (-0.2-0.6), 0.28	-3 (-46-40), 0.88	7 (-16-30), 0.56	0.3 (-0.3-0.8), 0.35	-9 (-34-17), 0.49	7 (-27-40), 0.70
High or very high	3.7 (-1.2-8.5), 0.14	0.5 (-0.8 1.8), 0.47	0.4 (-0.2-1.1), 0.15	-10 (-83-63), 0.78	27 (-12-66), 0.17	0.3 (-0.6-1.3), 0.49	2 (-41-45), 0.94	17 (-39.-73), 0.55

Model # is adjusted for Table 3 factors asociated with either HOMAIR or HOMA2IR-C-peptide at a p value < 20: age, waist circumpherence, body mass index, smoking, hypertension, dyslipidemia, and antihypertensive treatment. Model #2 is adjusted for Model #1 plus Table 3 SLE related data with a p value < 20: prednisone mg/day.
SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; SLICC: Systemic Lupus International Collaborating Clinics/American Collegue of Rheumatology Damage Index
HOMA2IR: Homeostatic Assessment Model for determining of insulin resistance IR), insulin sensitivity (S%) and β cell function (%B) using insulin and glucose serum levels
HOMA2IR-C peptide: Homeostatic Assessment Model for evaluating insulin resistance (IR), insulin sensitivity (%S) and beta cell function (%B) using C peptide and glucose serum levels.

Statistical analysis

Demographic and clinical characteristics shown in Table I were compared between SLE patients and controls using χ^2 tests for categorical variables or a Student *t*-tests for continuous variables (data expressed as mean ± standard deviation- SD). For non-continuous variables, either a Mann-Whitney U-test was performed or a logarithmic transformation was made, and data are expressed as a median and interquartile range (IQR). To investigate the differences in IR indexes and glucose metabolism molecules serum levels between SLE patients and controls, we constructed two models: an unadjusted model for the univariate differences, and an adjusted model using those variables with a *p*-value lower than 0.20 previously identified in Table I (age, BMI, statins intake, LDL cholesterol serum levels and prednisone intake). Demographic and disease-related data associations with IR indexes are shown in Table III using multivariate linear regression. In this table, adjusted regression models are controlled by confounders defined as those variables associated with both HOMA2-IR and HOMA2-C-peptide indexes at a significant *p*-level lower than 0.20 in the univariate model. The study of the relation of disease activity with IR indexes and insulin and C-peptide serum levels (shown in Table IV) was assessed through 3 multivariate models. For this analysis, SLEDAI was divided in three levels: a reference category of non-activity, and 2 categories of mild or moderate, and high or very high activity, respectively. In this table, model 1 represents the univariate relationship of SLE indexes with HOMA IR indexes and insulin and C-peptide serum levels; model 2 was adjusted for the demographics variables found to have a *p*-value less than 0.20 in Table III (age, body mass index, waist circumference, smoking, dyslipidaemia, hypertension and the presence of treatment for hypertension); model 3 was adjusted for model 2 plus those variables, related to the disease, found to have an association with IR indexes of $p<0.20$ in Table III. In this case, because both binary and continuous prednisone was found to have a '*p*' value less than 0.20, prednisone was included as a continuous variable since it appeared to contribute more information to the model. *All sets* linear regression models with the higher r2 correlation coefficient were selected to illustrate the variability of IR indexes using the minimum number of predictors. All the analyses used a 5% two-sided significance level and were performed using SPSS software, v. 21 (IBM, Chicago, IL, USA), and STATA software, v. 13/SE (Stata Corp., College Station, TX, USA). A *p*-value <0.05 was considered statistically significant.

Results

Demographic, analytical and disease-related data

A total of 169 sex-matched participants, 87 patients with SLE and 82 controls, with a mean ± SD age of 50±10 years and 54±10 years, respectively, were included in this study. Demographic and disease-related characteristics of the participants are shown in Table I. There were no differences between patients and controls with regard to body mass index (BMI), waist circumference, and the presence of hypertension or dyslipidaemia. However, statins intake was more frequently found in SLE patients than in controls.

The median SLE disease duration was 16 years (interquartile range-IQR-9–21) and SLICC and Katz indexes were, respectively, 2 (IQR 1–3) and 2 (IQR 1–4). Forty percent of the patients were categorized as having no activity based on the SLEDAI index, while 32%, 21%, 6% and 1% were classified, respectively, in the mild, moderate, high and very high groups of disease activity. More than a half of them (56%) were taking prednisone (2 ± 3 mg/day). Fifty-eight (67%) patients were found to be positive for anti-DNA, and 62 (71%) expressed some of the extractible nuclear antibodies (ENA) at the time of the study. Fifty-six (64%) patients were on hydroxychloroquine, while mycophenolate mofetil, azathio-

prine and rituximab and belimumab were less frequently used (Table I). Regarding carotid intima media assessment, 20% of the patients in our series had carotid plaques. The average cIMT was 0.646±0.116 mm.

Differences in IR indexes between SLE patients and controls

SLE patients showed higher serum levels of C-peptide (1.34±0.62 *vs*. 2.61±1.51 ng/ml, *p*=0.00) when compared to controls. Similarly, HOMA2-IR-C-peptide (beta coef. 0.53 [95% CI - confidence interval - 0.25–0.82], *p*=0.00), HOMA2-S%-C-peptide (beta coef. -37 [95% CI -63 – -11], *p*=0.01), and HOMA2-B%-C-peptide (beta coef. 35 [95% CI 18-52], *p*=0.00) values yielded statistically significant differences between patients and controls even after adjusting for traditional IR-related factors and prednisone intake. HOMA2-IR indexes related to insulin were not different between patients and controls (Table II).

Association of classic IR and disease-related factors with IR in SLE patients

Since both C-peptide and insulin data were available in our study, and because there is a logic for using C-peptide data to calculate β-cell function (the former is a marker of secretion) and insulin data to calculate IR (HOMA-IR is derived from glucose disposal as a function of insulin concentration), these two indexes, HOMA2-IR and HOMA2-B%-C-peptide, were selected to illustrate the relationship between classic factors associated with IR and disease-related characteristics with IR indexes, as shown in Table III.

Waist circumference, BMI, hypertension and anti-hypertensive treatment were all strongly associated with both IR and β-cell function indexes. Additionally, age and the use of statins were related with HOMA2%B-C-peptide but not with HOMA2-IR. In contrast, factors associated with the disease had no association with HOMA2-IR indexes. In this sense, disease duration, the presence of anti-DNA or ENA, hypocomplementaemia, and the use of DMARDs, hydroxychloroquine, azathioprine or mycophenolate mofetil were not related to IR indexes, even though this analysis was adjusted for the traditional factors associated with IR. Only prednisone, when used in binary or continuous (mg/day) fashion, was linked to HOMA2%B-C-peptide even after adjusting for traditional factors associated with IR. A trend for the same association was also found with HOMA2IR, although statistical significance was not reached.

Relationship between disease scores and IR indexes

Table IV shows how disease activity, damage and severity scores were associated with insulin and C-peptide serum levels and HOMA2IR indexes. The Katz severity index showed a marginally significant association in the univariate analysis, with a higher HOMA2-IR-C-peptide and a lower HOMA2-S%-C-peptide. This association was not found with the HOMA2IR indexes constructed with insulin. When this analysis was adjusted for classic IR factors, the association of the Katz index with a lower HOMA2-S%-C-peptide became significant (beta coef. -5 95%CI [-11– -0], *p*=0.04). However, after adjusting for prednisone intake, this association was lost.

The SLICC index was strongly associated with insulin and C-peptide serum levels and with almost all the HOMA2-IR indexes. In this regard, a *log* SLICC increase of one unit was associated with a reduction in HOMA2-S%-C-peptide of 30% (95%CI[-47– -14], *p*=0.00), and an enhanced beta cell function of 37% (95%CI[16–57], *p*=0.00). When this analysis was performed, adjusting for classic IR factors, some of the effects were attenuated. However, *log* SLICC still showed an association with higher levels of insulin serum levels and with higher HOMA2-IR-C-peptide indexes (Table IV). Interestingly, in the third model, when adjusted for prednisone intake, the association of SLICC index with higher C-peptide (beta coef. 0.7 95%CI [0.1–1.3], *p*=0.03) serum levels and HOMA2IR C-peptide (beta coef. 0.5 95%CI [0.1–1.0], *p*=0.02) was maintained.

The SLEDAI index, an index related to clinical manifestations during the previous 10 days, and which accounts for disease activity, had no relation with IR indexes in the 3 models studied.

The proportion of the variation of both HOMA-IR and HOMA2-B%-C-peptide (expressed as r^2 correlation coefficient) explained by the addition of a different set of variables to the model was studied through an *all sets* multivariate descriptive regression analysis. With respect to HOMA2-IR, the combination of waist circumference, hypertension and current oral prednisone (mg/day) was the model with the highest R2 correlation coefficient (r^2 correlation coefficient = 0.27). In contrast, the HOMA2-B%-C-peptide model was better explained by the combination of BMI, smoking, hypertension, current oral prednisone (mg/day), C-reactive protein (mg/dl) and log SLICC index (r^2 correlation coefficient = 0.28) (Table V).

Subclinical atherosclerosis and IR in SLE patients

In the univariate analysis, while HOMA2IR C-peptide was positively related to carotid plaque (OR 3.15 [95%CI 1.17–8.51], *p*=0.02), HOMAIR-S%-C-peptide was negatively associated with cIMT (beta coef. 0.98 [95%CI 0.96–0.99], *p*=0.03). However, after adjusting for classic cardiovascular risk factor, this relationship was lost (Table VI).

Discussion

In the present study we confirm that IR is increased in SLE patients when compared to controls. It is worth noting that according to our results these differences may be the result of beta cell function impairment. Similarly, we found that this augmented IR is independently related to the damage that the disease causes over time and that, although statistical significance was not reached in the multivariable analysis, it may be associated with the accelerated subclinical atherosclerosis observed in SLE patients.

To the best of our knowledge, C-peptide serum levels had never been assessed in SLE. In our study we disclosed that C-peptide is up-regulated in SLE patients and that it is independent of other classic IR risk factors and/or prednisone intake. Interestingly, the

Table V. All subsets regression model for predicting insulin resistance in SLE patients.

	beta coefficient (95% CI), *p*-value	
	HOMA-IR	HOMA2B%-C-peptide
Waist circumference, cm	0.02 (0.00-0.03), 0.01	
BMI, kg/m^2		0.05 (0.01-0.09), 0.01
Currently smoking		-0.42 (-0.95-0.10), 0.11
Hypertension	0.32 (0.02-0.63), 0.04	
Prednisone, mg/day	0.02 (-0.00-0.05), 0.10	0.35 (-0.10-0.79), 0.13
CRP, mg/dl		1.02 (-0.61-2.67), 0.10
log SLICC index		18 (-4-40), 0.11
R2 correlation coefficient	0.27	0.28

BMI: body mass index, kg/m^2; CRP: C-reactive protein; SLICC: Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index.

Table VI. Multivariate analysis of IR indexes vis-à-vis subclinical atherosclerosis in SLE patients.

	Carotid plaque			IMT, mm		
	OR 95%CI	*p*	*p**	beta coef. 95%CI	*p*	*p**
HOMA2IR	1.45 (0.77-2.74)	0.26	0.31	1.09 (0.51-2.34)	0.83	0.82
HOMA2-S%	0.63 (0.33-1.20)	0.16	0.96	0.99 (0.99-1.00)	0.18	0.56
HOMA2-B%	1.06 (0.56-1.99)	0.86	0.66	0.99 (0.99-1.01)	0.96	0.56
HOMA2IR-C-peptide	3.15 (1.17-8.51)	**0.02**	0.54	1.50 (0.96-2.35)	0.08	0.70
HOMA2S%-C-peptide	0.25 (0.08-0.77)	**0.02**	0.85	0.98 (0.96-0.99)	**0.03**	0.75
HOMA2B%-C-peptide	1.86 (0.83-4.18)	0.13	0.60	1.01 (0.99-1.01)	0.15	0.55

HOMA2IR: Homeostatic Assessment Model for determining insulin resistance using insulin and glucose serum levels; HOMA2%B-C-peptide: Homeostatic Assessment Model for evaluating beta cell function using C-peptide and glucose serum levels.
*p** adjusted for age, hypertension, dyslipidaemia and smoking.

use of C-peptide, instead of insulin, yielded higher HOMA2-IR indexes.

Previous studies found that insulin levels and HOMA-IR are significantly increased in SLE patients compared to controls (15, 24). However, in the present study levels of both parameters were not found increased in SLE patients compared to controls. In general, levels of C-peptide and insulin correlate well in blood but, unexpectedly, in our study SLE patients had higher levels of C-peptide and lower levels of insulin than controls. As discussed before, it is possible that IR in SLE patients may be mainly mediated by a beta cell impairment mechanism rather than being the result of the peripheral IR or insulin sensitivity. However, the excess of the beta cell function could be due to the decrease in peripheral sensitivity. With respect to this, El Magadmi *et al*. found that non-diabetic patients with SLE had a significant decrease in sensitivity to insulin which caused the hyperinsulinaemia observed in these patients (15). Additionally, it is possible that a lower degree of inflammatory burden in SLE when compared with that observed in patients with inflammatory rheumatic diseases such as rheumatoid arthritis may explain in part the reason of our findings (11). Moreover, the finding that the SLICC damage index was also associated with HOMA2-IR C-peptide and not with HOMA2-IR assessed with insulin serum levels further reinforces our point of view.

We also observed that traditional cardiovascular risk factors or factors known to be associated with IR account for the variation of IR in SLE patients. In this regard, age, BMI, waist circumference, hypertension and statins use were associated with an increased IR. This finding is in agreement with previous reports that showed an increased prevalence of traditional risk factors for atherosclerosis in patients with SLE. For example, a series of 250 women from the Toronto Lupus Cohort showed higher prevalence of hypertension, diabetes, premature menopause, sedentary lifestyle, and at-risk body habitus than controls (21). However, in our study disease duration or laboratory data, such as positivity for ANA, ENA or hypocomplementaemia, were not associated with IR in the univariate analysis or after adjusting for traditional cardiovascular risk factors.

We could not find any beneficial effects conferred by hydroxychloroquine or by any other anti-rheumatic drugs on IR indexes or insulin and C-peptide serum levels. This finding is in contrast with two recent studies that reported a protective effect of antimalarial agents on the prevalence of metabolic syndrome in SLE patients (22) that occurred early during the disease course (23). However, in these studies neither insulin or C-peptide serum levels nor HOMA-IR indexes were specifically studied.

SLE patients who receive higher doses of glucocorticoids are also more likely to have more active and severe disease. Therefore, it is possible that disease severity may be responsible for some of the metabolic effects attributed to glucocorticoids in SLE. Nevertheless, in our study we disclosed that glucocorticoids were associated with HOMA2-IR-C-peptide after adjusting for covariates. This finding not only implies that they may independently exert a deleterious effect over IR, but also supports the recommendations for using the lowest possible dose of glucocorticoids for the shortest time to control life- or organ-threatening manifestations in SLE (24).

Our study also constitutes the first attempt in which the association of IR with damage, activity or severity SLE disease indexes was studied using insulin or C-peptide serum levels or HOMA2 models. With respect to this, we established that SLE damage is independently associated, even after adjusting for prednisone intake, with HOMA2-IR-B%-C-peptide. Our finding is in agreement with previous reports that showed disease activity or damage to correlate with IR. For example, Parker *et al*. (23, 25) reported that metabolic syndrome is a persistent phenotype in a significant proportion of patients with SLE and that active inflammatory dis-

ease and damage are SLE-related factors that promote the development of metabolic syndrome.

To further investigate the impact of several disease-related or classic cardiovascular factors on the development of IR in SLE, we constructed a predictive model to explain IR variability for HOMA2-IR and HOMA2-IR-C-peptide indexes. Interestingly, the SLICC damage score included in this predictive model proved to be one of the most decisive factors. This supports, the aforementioned role of disease damage over IR in SLE patients.

Atherosclerotic plaques in the carotid arteries, which correlate with the presence of severe lesions in the coronary arteries, are found in a higher proportion of patients with SLE than in age- and sex-matched controls (5, 26). In our study, we observed an association between IR and carotid plaques and cIMT. However, this association was lost after adjusting for traditional cardiovascular risk factors. One plausible explanation for this may be that our study was underpowered to examine the association of carotid ultrasound abnormalities with IR, since our primary end point was to address the relationship of disease-related factors with IR rather than the impact of subclinical atherosclerosis on the development of IR. Nevertheless, the univariate analysis suggests a possible relationship between IR and subclinical atherosclerosis in SLE. In any case, further studies are needed to better understand the specific nature of this relationship in SLE.

In conclusion, C-peptide serum levels and IR indexes are up-regulated in SLE patients. Not only traditional IR risk factors and prednisone intake, but also SLE disease damage account for this up-regulation. A better understanding of the interplay between IR and disease activity, therapeutic exposure and traditional cardiovascular risk factors may be useful for improving cardiovascular risk stratification in patients with SLE.

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