Nuclear Magnetic Resonance Lipoprotein Subclasses and the APOE Genotype Influence Carotid Atherosclerosis in Patients with Systemic Lupus Erythematosus

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ABSTRACT. ***Objective.*** Patients with systemic lupus erythematosus (SLE) have accelerated atherosclerosis. Since the conventional lipid profile (total plasma cholesterol, triglycerides, low and high density lipoprotein cholesterol) is not consistently altered in SLE, we hypothesized that investigation of lipoprotein subclasses would improve prediction of risk of atherosclerosis in these patients.

Methods. As a quantitative index of atherosclerosis, we measured the carotid intima-media thickness (IMT) in 68 patients with SLE and related the atherosclerosis to a detailed lipoprotein profile generated using nuclear magnetic resonance (NMR). We measured the cholesterol transported by the pool of remnant lipoproteins (RLPc) and evaluated the modulatory effect of the APOE genotype on the lipoprotein subclass profile and atherosclerosis associated with SLE.

Results. Circulating lipoprotein remnant particles [RLPc and intermediate density lipoprotein (IDL)] were positively correlated with IMT, and among them, the indicator that explained 20.2% of the variability in carotid atherosclerosis measured in these patients was IDL, as assessed by NMR. Carriers of the APOE2 allele were at increased risk due to a significant accumulation of IDL particles.

Conclusion. Lipoprotein subclasses are more associated with subclinical atherosclerosis in patients with SLE than the lipid variables that are routinely measured. The IDL fraction, which is significantly modulated by the APOE genotype, is the most strongly, significantly, and positively correlated with IMT. (J Rheumatol First Release August 1 2010; doi:10.3899/jrheum.091175)

Key Indexing Terms:
LUPUS REMNANTS APOE GENOTYPE
INTIMA-MEDIA THICKNESS NUCLEAR MAGNETIC RESONANCE

Systemic lupus erythematosus (SLE) is a systemic inflammatory disease that mainly affects women and that is characterized by the production of autoantibodies of different specificities. Patients with SLE have accelerated atherosclerosis and its sequellae[1]. Women with SLE present with 50-fold increased risk of cardiovascular disease[2]. In patients with SLE, the risk of cardiovascular disease cannot be fully explained by the traditional Framingham risk factors alone and recent studies suggest that a combination of traditional and nontraditional risk factors better characterize SLE[2,3]. These include markers of inflammation, dyslipidemia, enhanced low density lipoprotein (LDL) oxidation, antiphospholipid antibodies, and high levels of homocysteine[4].

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Cardiovascular risk as in SLE is common to other autoimmune diseases such as rheumatoid arthritis or type I diabetes mellitus[5,6]. Dyslipidemia is a well established risk factor in the general population and in these latter diseases[7]. The lipid profile in SLE, described as the "lupus pattern of dyslipoproteinemia," is characterized by decreased high density lipoprotein (HDL), elevated triglycerides, unchanged or only slightly elevated LDL, and raised lipoprotein(a) [Lp(a)]; the underlying mechanisms remaining poorly described[7,8,9]. Routine lipid measurements [total plasma cholesterol and triglycerides, LDL cholesterol (LDLc), and HDLc] may not be sensitive enough to differentiate patients at risk for atherosclerosis[1,10]. This may be explained, at least in part, by additional lipid measures of this atherogenic dyslipidemia, e.g., high triglycerides (TG), low HDL, and small dense LDL particles, accompanied by moderately elevated or normal cholesterol concentrations. Elevated circulating TG are the driving force of increased remodeling of lipoproteins involving cholesteryl ester transfer protein (CETP), which generates a pool of atherogenic smaller lipoprotein particles. These remnant particles are more likely to enter the subendothelial space, are more prone to oxidation, and are more difficult to clear from the circulation, clearance being modulated by the apolipoprotein E genotype[11,12].

More detailed lipid information would improve our ability to predict the risk of atherosclerosis in these patients. We measured carotid intima-media thickness (IMT) in patients with SLE, determined the complete lipoprotein profile using nuclear magnetic resonance (NMR), isolated and measured the cholesterol transported by the pool of remnant lipoproteins (RLPc), and assessed the influence of the APOE genotype on these measures, and on concomitant atherosclerosis.

Our results indicated that lipoprotein subclasses correlate much better with carotid atherosclerosis than traditional routine lipid indicators and, in contrast to observations in the general population, the APOE2 allele, which is linked to the accumulation of remnant lipoproteins, is a significant risk factor for atherosclerosis in patients with SLE.

MATERIALS AND METHODS

Subjects. Sixty-eight patients with lupus erytheromatosus were recruited from the systemic autoimmune diseases unit of the Hospital Universitari de Sant Joan de Reus. Patients fulfilled at least 4 classification criteria of the American College of Rheumatology, as revised in 1997[13]. None had active disease, and all had a SLE Diseases Activity Index (SLEDAI) ≤ 4 points. No diabetes mellitus, nephrotic syndrome, or hypertension had been evident in these patients, and none had had any ischemic or adverse cardiovascular event. Subjects had been prescribed prednisone therapy (< 10 mg/day) but none were receiving hypolipemic agents.

All patients provided informed consent to participate, and the Ethics Committee of the Hospital Universitari de Sant Joan de Reus approved the study.

Biochemical analyses. Fasting venous blood samples were collected in EDTA tubes and centrifuged immediately for 15 min at 4˚C at 1500 g. Samples were then divided into aliquots and stored at –80˚C until the determination of analytical variables.

Standard laboratory methods were used to quantify glucose, HbA1c, total cholesterol, TG, and HDL cholesterol. LDL cholesterol measures were calculated by the Friedewald formula[14]. Measurement of apolipoproteins was by immunoturbidimetry using antisera specific for apoA-1 and apoB (Hoffman-La Roche) and Lp(a) (Incstar Corp., Stillwater, MN, USA). High-sensitivity CRP (hs-CRP) was measured with a high sensitivity near-infrared particle immunoassay (NIPIA) rate method (Beckman Coulter) on a Synchron LXi PRO System automated autoanalyzer (Beckman Coulter).

Carotid intima-media thickness. IMT was measured in the Hospital Universitari Sant Joan de Reus on the same day the blood samples were obtained.

The ultrasound IMT procedure is a noninvasive, relatively inexpensive, safe, and reproducible method for detection of early atherosclerosis. We used a My Lab 50 X-Vision sonograph (Esaote SpA, Barcelona, Spain) with a linear array ultrasound probe small parts broadband transducer (5–12 MHz) to identify and digitally record the far wall of the common carotid artery (1 cm proximal to the bifurcation), the carotid bulb (in the bifurcation), and the internal carotid artery (1 cm distal to the bifurcation) of the left and right carotid arteries. Measurements of IMT were performed at the predefined points using the ThickSoft image processing software[15].

The images were obtained and measured by a single operator to reduce observer variability. We averaged the measurements of 3 static images of left and right carotid arteries to obtain the mean IMT (mIMT). Maximum IMT (maxIMT) was the maximum value of IMT from all measures in each subject[16].

Pathological IMT values were defined as the 75th percentile of the general population mIMT values, banded with respect to age and sex. Thus, in the lupus population (according to tertiles of mean age and based on the Consensus Statement from the American Society of Echocardiography[17]), the age-adjusted pathological mIMT tertile values for the mean-age tertiles of 29.23 years, 43.91 years, and 65.87 years were 0.612 mm, 0.713 mm, and 0.852 mm, respectively. According to our regional reference data, the pathological mIMT values for the same mean-age tertiles were 0.530 mm, 0.580 mm, and 0.820 mm[18].

NMR lipoprotein profile. Total plasma lipids and the distribution of subclasses of lipoproteins were analyzed by NMR spectroscopy (NMR LipoProfile; LipoScience, Raleigh, NC, USA), which simultaneously quantifies subclasses of lipoproteins, lipid content, and average particle size. This technique allows determination of very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), LDL, and HDL. Further, the VLDL fraction is quantified in 3 discrete subclasses, LDL in 4 subclasses, and HDL in 3 subclasses, all according to increasing molecular weight. NMR was performed with EDTA plasma stored at –80˚C and thawed just prior to the analysis.

Separation and quantification of remnant lipoprotein. Remnant lipoprotein cholesterol (RLPc) was measured in plasma using the method described by Nakajima, *et al*, using RLP-Cholesterol Assay Kits (Jimro-II, Japan Immunoresearch Laboratories, Tokyo, Japan)[19].

Remnant lipoprotein particles were separated from plasma by immunoaffinity chromatography with a gel containing monoclonal antibodies raised against epitopes of apoB100 and apoA1. The anti-apoA1 recognized apoA1-containing lipoproteins, whereas the anti-apoB100 recognized all apoB100-containing lipoproteins except the partially lipolyzed apoE-enriched triglyceride-rich remnants[20]. The gel retains HDL, LDL, and the majority of VLDL, while the unbound fraction consists of remnant lipoproteins of intestinal (apoB48) and hepatic origin (apoB100). Briefly, the technique involves plasma EDTA (5 μl) added to 300 μl of gel suspension of anti-human apoA-I and apoB-100 mouse monoclonal antibodies bound to Sepharose®. The suspension is gently mixed for 2 h at room temperature with a vertical magnetic-bead oscillator (RLP Mixer J-100A, Photal; Otsuka Electronics, Osaka, Japan). The mixture is allowed to settle for 30 min, and 200 μl of the supernatant containing unbound fraction is measured by sensitive cholesterol assay on a Cobas Mira centrifugal analyzer (Roche, Laval, Quebec, Canada). All RLP assays were performed with samples stored at –80˚C and thawed just prior to analysis.

APOE genotyping. DNA was isolated from a 10 ml EDTA blood sample following standard procedures. For DNA amplification, we used a 25 μl reaction volume containing 1.25 mM dNTP, 100 nM of each primer, and 1.5 mM MgCl. Polymerase chain reaction amplifications and genotype determinations were conducted as follows: forward: 5'-ACA GAA TTC GCC CCG GCC TGG TAC AC-3'; reverse: 5'-TAA GCT TGG GCA CGG CTG TCC AAG GA-3'. Thermal cycling conditions were denaturation 94˚C for 4 min and 33 cycles of 94˚C for 1 min, 52˚C for 1 min, and 72˚C for 3 min. Digestion was performed using the HhaI restriction enzyme, and the fragments obtained were resolved using 2% agarose gel electrophoresis.

To evaluate the effect of the APOE genotype, patients were categorized into 3 groups: apoE3/3 homozygotes; apoE2/3 heterozygotes or apoE2/2 homozygotes; and apoE3/4 heterozygotes or apoE4/4 homozygotes.

Statistical analysis. Correlations between mIMT and continuous variables were performed with partial correlations adjusted for age, body mass index (BMI), blood pressure (systolic and diastolic), and tobacco consumption. Spearman's correlation coefficient was performed for variables not normally distributed, the variables being adjusted for age, BMI, systolic and diastolic blood pressure, and tobacco use before the test was applied.

Comparisons of means (mIMT tertiles and APOE genotype) were performed with ANCOVA using age, BMI, blood pressure (systolic and diastolic), and tobacco use as covariates, with log-transformed data for variables that were not normally distributed.

Backward linear regression was performed with all the variables included in order to identify the best predictor of high mIMT values. Differences between allele frequencies were evaluated with the chi-square test. Statistical significance was set at $p < 0.05$ level. All statistical analyses were evaluated with SPSS (version 17.0; SPSS, Chicago, IL, USA).

RESULTS

Although conventional lipids are not elevated in SLE, we assessed the contribution of these conventional lipids to the carotid intima-media thickness, focusing as well on NMR subclasses and RLPc. Despite a slightly elevated mean value of LDLc and BMI, the 68 patients diagnosed with SLE had normal glucose and lipid concentrations (Table 1) according to the definitions of the National Cholesterol Education Program, Adult Treatment Panel III and the International Diabetes Federation[21,22]. No patient had disease activity (flare) at the time of the study. Despite their normal lipid profile (Table 1) and their low cardiovascular risk score (score 1%), 25% of our patients had pathological age-adjusted IMT values[17]. This increased to 52.9% when we used regional reference values for women[18].

Correlation between conventional lipids and IMT. None of the routine biochemical indicators correlated significantly with mIMT, except for apoA1 (R = –0.254, p = 0.044). However, the tendencies were as expected, i.e., plasma TG, total cholesterol, and LDLc tended to correlate positively (rho = 0.154, R = 0.015, R = 0.049, respectively) with mIMT, while HDLc was the only measure that correlated inversely with mIMT (R = –0.213). In a model of multiple linear regression analyses, conventionally measured lipids were unable to explain the carotid IMT to any significant extent.

Correlation between NMR lipoprotein subclasses and IMT. Chylomicrons (Qm) and all VLDL subclasses (total, large, medium, small) were positively correlated with mIMT (R = 0.335, R = 0.154, R = 0.243, R = 0.360, respectively) and maxIMT. These correlations were statistically significant for total VLDL and Qm (p = 0.007 for both) and small VLDL particles (p = 0.004).

Table 1. Characteristics of the study population. Data are mean (SD) unless otherwise indicated.

Characteristic	
Female/male, %	91/9
Smokers/ex-smokers/nonsmokers, %	31/3/66
Systolic blood pressure, mmHg	118.94 (17.81)
Diastolic blood pressure, mmHg	74.78 (10.36)
Body mass index, kg/m^2	26.34 (5.70)
Age, yrs	46.59 (16.52)
Age at disease onset, yrs	37.22 (17.18)
Glucose, mg/dl	4.88 (0.55)
HbA1c, %	4.75 (0.60)
Cholesterol, mmol/l	4.92 (1.10)
HDLc, mmol/l	1.64 (0.40)
Friedewald LDLc, mmol/l	2.83 (0.81)
ApoA1, mg/dl	145.31 (13.44)
ApoB100, mg/dl	87.28 (22.71)
Ratio apoB100/A1	0.60 (0.17)
IMT mean, mm	0.67 (0.17)
IMT maximum, mm	1.00 (0.33)
Triglycerides, mmol/l, median (IQR)	0.90 (0.54–1.23)
Lp(a), mg/dl, median (IQR)	13.85 (6.55–37.33)
hsCRP, mg/dl, median (IQR)	1.47 (0.61–3.86)

HbA1c: glycosylated hemoglobin; HDLc: high density lipoprotein cholesterol; LDLc: low density lipoprotein cholesterol; ApoA1: apolipoprotein A1; ApoB100: apolipoprotein B100; IMT: intima-media thickness; IQR: interquartile range; Lp(a): lipoprotein(a); hsCRP: high-sensitivity C-reactive protein.

While total LDL particle concentration tended to correlate positively with mIMT (R = 0.184; nonsignificant), not all LDL NMR subclasses showed the same trend. Of note, while the small (R = 0.247, NS), medium-small (R = 0.184, NS), and very small (R = 0.260, p = 0.039) subclasses were positively correlated with mIMT, the large LDL subclass was inversely correlated (R = –0.193, NS), suggesting distinctly different functional roles of the lipoprotein class (Figure 1).

Similarly, while the largest HDL subclass showed the expected tendency toward inverse correlation with mIMT (R = –0.189, NS) and maxIMT (R = –0.239, NS), the smaller HDL subclasses tended to correlate positively with mIMT (R = 0.103, NS) and maxIMT (R = 0.020, NS).

Lipoprotein particle size clearly indicated that the smaller the lipoproteins, the greater the intima-media thickening, with inverse correlation with the VLDL (R = –0.285, p = 0.024), LDL (R = –0.265, p = 0.036), and HDL (R = –0.190, NS).

Correlation of RLPc and IDL and IMT. Remnants of VLDL (IDL) showed the strongest correlation between NMR subclasses and mIMT (R = 0.360, p = 0.004) and maxIMT (R = 0.430, p < 0.0001). RLPc also correlated positively with mIMT (R = 0.250, p = 0.048).

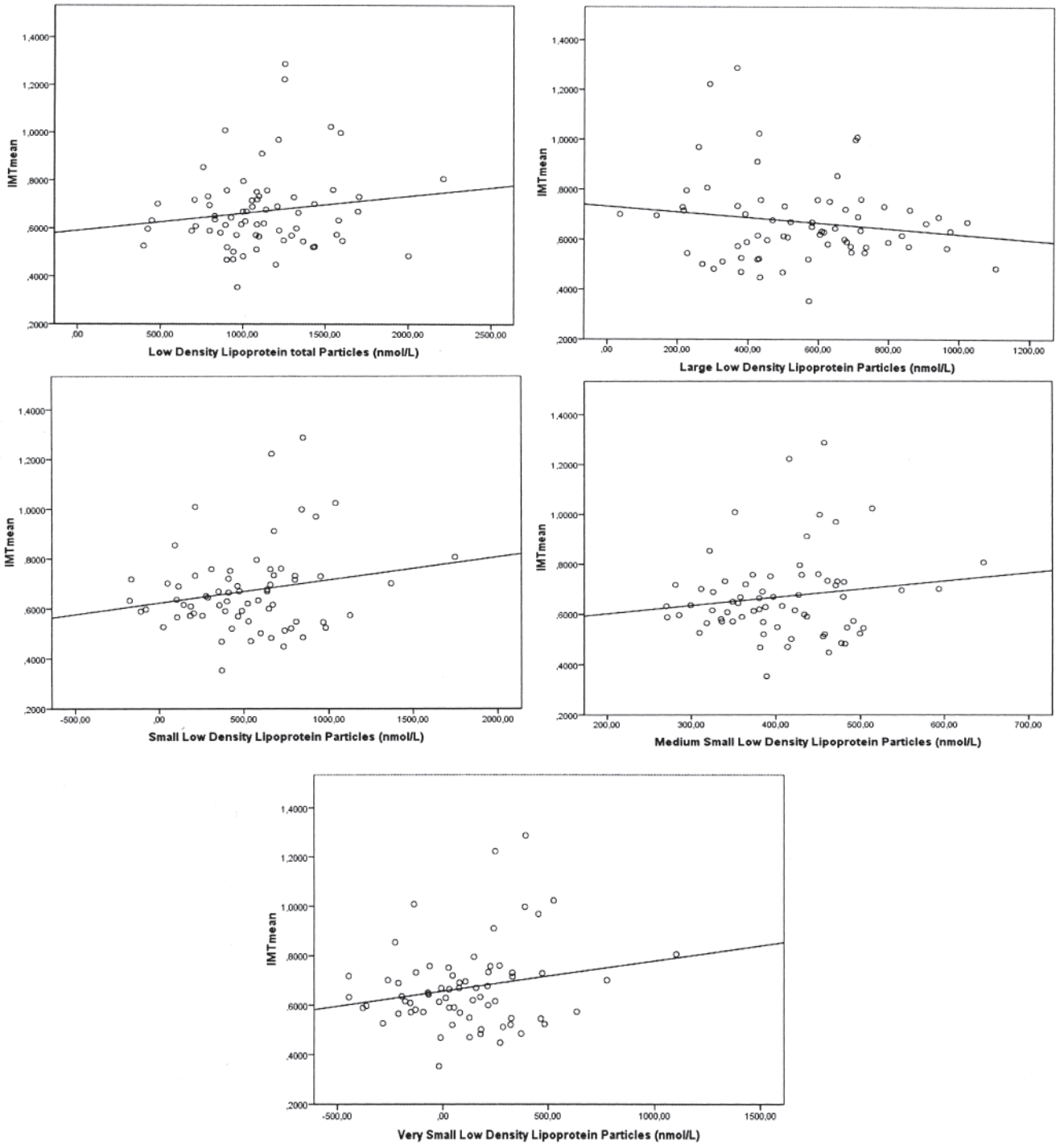

Figure 1. Total LDL particles correlate positively with mean IMT (R = 0.184, NS). For the largest particles (large LDL) the correlation becomes negative (R = –0.193, NS) and, as expected, the smaller subclasses correlate positively with mean IMT (small LDL: R = 0.247, p = 0.051; medium-small LDL: R = 0.184, NS; very small LDL: R = 0.260, p = 0.039). The p values are adjusted for age, body mass index, blood pressure, and tobacco use.

Concentration of conventional lipids, remnant lipoproteins, and NMR lipoprotein profile according to mIMT tertiles. To assess the clinical relevance of the lipid measurements that correlated with IMT, we compared their concentrations according to low, medium, and high mIMT tertiles, that is, 0.52 (0.06) mm, 0.64 (0.06) mm, and 0.84 (0.16) mm ($p < 0.0001$; Table 2).

Among the measures that significantly correlated with mIMT, the small VLDL, IDL, large LDL, small LDL, medium-small LDL, and very small LDL showed significantly

Table 2. Lipid and lipoprotein concentrations by nuclear magnetic resonance, segregated by tertiles of mean intima-media thickness (IMT).

Variable	Mean IMT Tertiles 1	2	3	p
n	22	23	23	
Mean IMT, mm*	0.522 (0.055)	0.639 (0.034)	0.843 (0.167)	< 0.0001
Rank mean IMT, mm	0.353–0.589	0.590–0.696	0.701–1.287	
Lipid concentrations				
Triglycerides, mmol/l†	0.780 (0.498–1.063)	0.820 (0.460–1.100)	1.110 (0.760–1.380)	NS
Chol total, mmol/l*	4.876 (1.306)	4.870 (1.074)	5.005 (0.936)	NS
LDLc, mmol/l*	2.820 (0.935)	2.784 (0.808)	2.895 (0.724)	NS
HDLc, mmol/l*	1.650 (0.509)	1.676 (0.377)	1.598 (0.288)	NS
Apo A1, mg/dl*	144.909 (16.130)	146.783 (13.987)	144.217 (10.117)	NS
Apo B100, mg/dl*	85.636 (24.279)	86.130 (23.137)	90.000 (21.471)	NS
Ratio apoB100/A1*	0.595 (0.172)	0.589 (0.165)	0.629 (0.162)	NS
hsCRP, mg/dl†	1.280 (0.488–5.280)	1.450 (0.580–3.180)	1.490 (0.920–2.060)	NS
Lp(a), mg/dl†	14.250 (8.275–31.100)	14.000 (6.800–50.400)	10.100 (6.100–44.400)	NS
VLDL and chylomicron (Qm) particle concentrations				
VLDL and Qm, nmol/l*	44.645 (38.653)	41.587 (47.086)	61.648 (37.873)	NS
Large VLDL and Qm, nmol/l*	0.977 (2.324)	1.043 (1.541)	1.170 (1.619)	NS
Medium VLDL, nmol/l*	13.923 (19.799)	17.957 (26.592)	22.243 (18.193)	NS
Small VLDL, nmol/l*	29.750 (23.295)	22.574 (20.924)	38.230 (23.447)	0.030
IDL particle concentrations				
IDL, nmol/l*	29.136 (39.723)	19.000 (20.872)	85.522 (80.071)	0.003
LDL particle concentrations				
LDL total, nmol/l*	1052.000 (397.340)	1072.261 (366.892)	1210.652 (399.428)	NS
Large LDL total, nmol/l*	538.091 (269.711)	613.304 (230.839)	510.696 (244.153)	0.029
Small LDL total, nmol/l*	484.636 (416.035)	440.087 (356.970)	617.609 (481.133)	0.022
Medium small LDL, nmol/l*	102.273 (82.319)	95.174 (84.209)	127.174 (96.097)	0.050
Very small LDL, nmol/l*	382.409 (335.349)	344.826 (279.856)	490.435 (385.911)	0.019
HDL particle concentrations				
HDL total, μmol/l*	30.114 (5.463)	31.826 (6.787)	32.909 (4.032)	NS
Large HDL, μmol/l*	9.377 (4.668)	9.913 (3.078)	9.013 (3.204)	NS
Medium HDL, μmol/l*	2.014 (2.268)	2.661 (2.776)	2.770 (2.659)	NS
Small HDL, μmol/l*	18.732 (4.213)	19.248 (6.210)	21.117 (4.817)	NS
Mean particle size				
VLDL, nm*	52.091 (12.986)	55.309 (10.756)	45.787 (7.966)	0.002
LDL, nm*	21.586 (0.855)	21.804 (0.707)	21.370 (0.994)	0.006
HDL, nm*	9.377 (0.596)	9.470 (0.457)	9.213 (0.463)	NS
Remnant lipoprotein cholesterol (RLPc)				
RLPc, mg/dl†	3.515 (3.148–5.893)	4.260 (3.585–5.605)	4.595 (3.585–6.885)	NS

* Values are mean (SD). † Values are median (IQR). IMT: arterial intima-media thickness; chol total: total plasma cholesterol; LDLc: low density lipoprotein cholesterol; HDLc: high density lipoprotein cholesterol; ApoA1: apolipoprotein A1; ApoB100: apolipoprotein B100; hsCRP: high-sensitivity C-reactive protein; Lp(a): lipoprotein(a); VLDL: very low density lipoprotein; LDL: low density lipoprotein; IDL: intermediate density lipoprotein; HDL: high density lipoprotein. NS: nonsignificant. All p values adjusted for age, BMI, blood pressure (systolic and diastolic), and tobacco use.

different concentration distributions in relation to the mIMT tertiles. Of note, when a statistical test for multiple comparisons was applied, the only measure that was significantly different among the 3 mIMT tertiles was IDL particle concentration (1st tertile to 3rd tertile p = 0.004, 2nd tertile to 3rd tertile p < 0.0001).

Cholesterol transported by remnant particles tended to increase with the degree of IMT, but did not reach statistical significance.

Patients in the 3rd mIMT tertile had VLDL and LDL particles of significantly smaller size.

Multiple linear regression models. As noted, multiple linear regression analyses using conventional lipids as variables did not generate a model able to explain a significant amount of variance in the carotid thickening. However, IDL was able to explain 20.2% of the IMT variability (p < 0.0001).

Interaction between lipoproteins and APOE genotype. For the purpose of statistical analysis and because of the limited sample size, the study population was subdivided into 3 APOE genotype subgroups depending on their apoE allele. Thus, ε2 allele group contained the genotypes E2/E2 and E2/E3, ε3 allele group contained E3/E3 genotype, and ε4 allele group contained E3/E4 and E4/E4 genotypes. Carriers of E2/E4, if any, would have been excluded from this analysis.

The allele frequencies of ε2, ε3, and ε4 alleles were 0.10, 0.81, and 0.09, respectively. The ε2 and ε3 alleles tended toward higher and the ε4 toward lower frequencies than normally observed in the general population[23].

The ε2 allele was significantly associated with the indicators previously linked to subclinical atherosclerosis. For example, carriers of the ε2 allele presented significantly higher concentrations of the atherogenic subclasses IDL, small LDL, medium-small LDL, and very small LDL, as well as significantly lower concentrations of the protective particles such as large LDL, together with significantly smaller LDL and HDL particle size (Table 3).

The frequency distribution of the ε2 allele increased with the degree of mIMT. By chi-square test, statistical significance was achieved comparing ε2 and ε4 distribution against the common ε3 allele (ε2 against ε3, p = 0.003; ε4 against ε3, NS; Figure 2).

DISCUSSION

We investigated conventional lipid indicators, NMR lipoprotein subclasses, and RLPc in relation to carotid intima-media thickness in patients with SLE. Our results showed the following: (1) IDL particle concentration, analyzed by NMR, was a more powerful predictor of carotid atherosclerosis than the routinely measured lipids. (2) The ε2 allele was associated with increased mIMT in these patients due to accumulation of IDL resulting, probably, from decreased receptor-mediated uptake and catabolism of the lipoprotein remnant related to this allele. (3) Remnant lipoproteins correlated significantly and positively with

Table 3. Lipid and lipoprotein concentrations by nuclear magnetic resonance, segregated by APOE genotype.

Variable	Genotype E2	E3	E4	p
n (%)	12 (17.65)	44 (64.70)	12 (17.65)	
Mean IMT, mm*	0.774 (0.198)	0.646 (0.149)	0.652 (0.180)	NS
Max IMT, mm*	1.216 (0.473)	0.962 (0.276)	0.923 (0.251)	0.041
Lipid concentrations				
Tryglicerides, mmol/l†	1.040 (0.738–1.638)	0.845 (0.493–1.225)	0.940 (0.630–1.098)	NS
Chol total, mmol/l*	4.625 (0.978)	4.952 (1.120)	5.084 (1.163)	NS
LDLc, mmol/l*	2.583 (0.633)	2.843 (0.824)	3.046 (0.930)	NS
HDLc, mmol/l*	1.511 (0.280)	1.682 (0.436)	1.622 (0.323)	NS
ApoA1, mg/dl*	141.670 (10.500)	146.430 (13.881)	144.830 (14.727)	NS
ApoB100, mg/dl*	82.000 (18.616)	87.550 (23.371)	91.580 (24.678)	NS
Ratio apoB100/A1*	0.580 (0.132)	0.604 (0.177)	0.630 (0.157)	NS
hsCRP, mg/dl†	1.785 (1.193–5.858)	1.370 (0.533–3.885)	1.185 (0.575–3.415)	NS
Lp(a), mg/dl†	6.300 (2.100–40.975)	14.100 (7.300–31.900)	22.000 (10.125–72.225)	NS
VLDL and chylomicron (Qm) particle concentrations				
VLDL and Qm, nmol/l*	64.092 (42.228)	47.148 (44.538)	42.750 (28.292)	NS
Large VLDL and Qm, nmol/l*	1.842 (2.390)	0.871 (1.796)	1.000 (1.010)	NS
Medium VLDL, nmol/l*	25.117 (20.527)	17.409 (24.157)	13.625 (10.747)	NS
Small VLDL, nmol/l*	37.150 (22.910)	28.861 (24.311)	28.108 (19.186)	NS
IDL particle concentrations				
IDL, nmol/l*	93.917 (92.534)	31.800 (39.257)	37.500 (60.089)	0.005
LDL particle concentrations				
LDL total, nmol/l*	1221.583 (295.917)	1080.523 (395.368)	1120.750 (452.542)	NS
Large LDL total, nmol/l*	324.750 (123.302)	597.614 (241.853)	624.833 (249.356)	< 0.0001
Small LDL total, nmol/l*	803.167 (268.524)	451.159 (403.012)	458.333 (516.187)	0.029
Medium small LDL, nmol*	170.167 (59.165)	94.636 (84.334)	96.500 (101.928)	0.023
Very small LDL, nmol/l*	633.083 (211.933)	356.455 (323.084)	361.917 (414.583)	0.033
HDL particle concentrations				
HDL total, μmol/l*	33.958 (4.137)	31.336 (5.765)	30.425 (5.902)	NS
Large HDL, μmol/l*	7.392 (2.962)	9.930 (3.847)	9.667 (3.118)	NS
Medium HDL, μmol/l*	4.458 (2.615)	2.048 (2.560)	2.133 (1.571)	0.009
Small HDL, μmol/l*	22.100 (4.046)	19.359 (5.143)	18.625 (6.014)	NS
Mean particle size				
VLDL, nm*	45.108 (6.113)	52.218 (12.344)	52.692 (9.913)	NS
LDL, nm*	20.733 (0.425)	21.768 (0.823)	21.775 (0.882)	< 0.0001
HDL, nm*	8.900 (0.283)	9.457 (0.500)	9.425 (0.512)	0.002
Remnant lipoprotein cholesterol (RPLc)				
RLPc, mg/dl†	4.059 (3.515–10.219)	4.295 (3.219–5.706)	3.688 (2.543–5.673)	NS

* Values mean (SD). † Values are median (IQR). All p values adjusted for age, BMI, blood pressure (systolic and diastolic), and tobacco use. For definitions see Table 2.

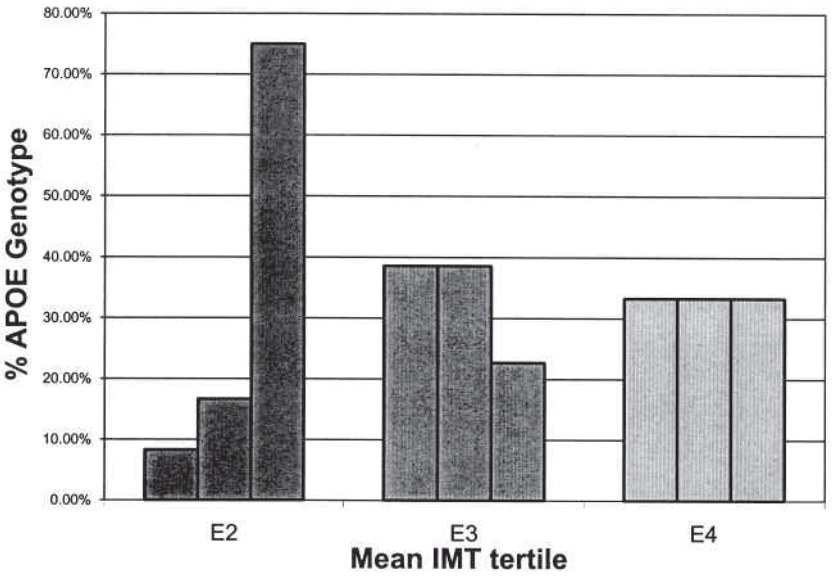

Figure 2. APOE genotype frequency organized by IMT tertiles. APOE2 genotype frequency tended to increase with degree of IMT, and for mean and maximum IMT values, but reaching statistical significance only with mean IMT values; 1st tertile (n = 1): 8.3%, 2nd tertile (n = 2): 16.7%, 3rd tertile (n = 9): 75%. Conversely, subjects carrying the ε3 and ε4 alleles did not show this tendency; 1st tertile (n = 17): 38.6%, 2nd tertile (n = 17): 38.6%, 3rd tertile (n = 10): 22.7%; and 1st tertile (n = 4): 33.3%, 2nd tertile (n = 4): 33.3%, 3rd tertile (n = 4): 33.3%, respectively. Statistical significance was reached (chi-square test) comparing ε2 and ε4 distribution against the common ε3 allele; ε2 vs ε3, p = 0.003; ε4 vs ε3, NS.

mIMT and maxIMT independently of diastolic or systolic blood pressure, smoking habit, age, and BMI.

Our study population was composed mainly of women between the ages of 17 and 81 years, who were normolipidemic. However, despite the normolipidemia, up to 20% of them had pathological values of mIMT adjusted for age and sex.

Conventionally measured lipids showed nonsignificant correlations with mIMT. The correlations were positive for total cholesterol, TG, and LDLc, and negative for HDLc. Of note, the only traditional lipid measure that was almost significantly associated with atherosclerosis in this population was plasma TG, and this finding supports the notion that TG-driven atherogenic dyslipidemia is important in this disease. All VLDL and Qm subclasses correlated positively with IMT (mean as well as maximal values), and this supports the proposition that TG-rich lipoproteins make an important contribution to the atherosclerotic process in these patients.

Lipoproteins that are more difficult to clear from circulation, particularly in the presence of hypertriglyceridemia, are termed "remnants" and have been well documented as being associated with atherosclerosis[24]. We measured TG-rich lipoprotein remnants (IDL) and the cholesterol transported by lipoprotein remnants (RLPc) isolated by immunoaffinity separation. Both measurements were positively and statistically significantly associated with mIMT.

Lipoprotein subclasses of LDL and HDL have different roles in atherosclerosis and were reflected as such in our patient population[25,26,27,28]. While the small, medium-small, and very small subclasses were positively associated with mIMT, the large LDL subclass showed an inverse correlation.

A similar trend was observed for HDL; the large HDL subclass was inversely associated with atherosclerosis while the smaller and denser subclasses tended to correlate positively with mIMT. It has been well documented that small HDL may become proinflammatory and proatherogenic in certain environments[29,30,31].

These results are in good agreement with current understanding of the role of lipoprotein subclasses, in which the importance of these subclasses as a proatherogenic or a protective factor in SLE has been proposed[25,26,27,28]. Overall, our results suggest that the smaller the particles, the more atherogenic they are. This is confirmed by the inverse correlation between VLDL, LDL, and HDL subclasses and IMT — mIMT as well as maxIMT.

To translate these associations into potential clinical value, we divided study patients into tertiles of mIMT values, and compared particle concentration for each subclass. The only indicator that was able to differentiate subjects in each category of mIMT was the IDL subclass, or remnants of the VLDL particles, which reportedly play an important role in the progression of atherosclerotic plaques[32,33]. This

is in agreement with the results of multiple linear regression analyses that showed that the model that best explained IMT progression included IDL particles.

In a similar study, Chung and colleagues concluded that NMR lipoprotein subclasses do not correlate with the degree of coronary atherosclerosis[34]. This is not necessarily in contradiction to our findings. While Chung, *et al* compared these measures between SLE patients and control individuals or between patients with and without coronary atherosclerosis, we compared our NMR findings with the more subtle indicators of atherosclerosis, i.e., IMT of the carotid artery. While most subclasses of one type or another may relate to atherosclerosis in SLE patients and controls, we have shown that the concentrations of IDL particles do differentiate SLE patients with low, medium, or high degree of atherosclerosis.

Since the removal of lipoproteins from the circulation, particularly TG-rich lipoproteins, is mediated by apolipoprotein E, and IDL appears to be the most potent lipoprotein risk factor in atherogenesis in these patients, we hypothesized that the APOE genotype could be a significant modulator of this process. We observed that carriers of the ε2 allele had significantly elevated concentrations of the IDL subclasses, as well as small, medium-small, and very small LDL, and also had significantly lower concentrations of the protective large LDL. This suggests that the ε2 allele may be an atherosclerosis risk factor in these patients. This was confirmed by the increasing frequency of the allele in the IMT tertiles, an aspect that did not occur with the ε3 or ε4 alleles. These findings confirm those of a study by Orlacchio, *et al*, in which the ε2 allele in SLE patients was associated with more rapid development of cardiovascular disease (CVD)[35], as would occur in other conditions of accelerated atherosclerosis linked to accumulation of IDL in the presence of ε2 homozygosity[36]. However, this is contrary to what is found in the general population, i.e., the ε4 allele is associated with increased cholesterol concentrations and CVD risk. Thus, considering that apoE2 isoform has less affinity to LDL receptor (LDLR) family members (LDLR, LDLR-related protein 1, VLDLR) under high remnant concentration conditions, it is feasible that in patients with SLE this isoform is more closely associated with atherosclerosis[37,38].

One limitation of our study was the small patient population, which may account for statistical significance not achieved in some of the correlations. However, the trends observed were internally consistent; their associations with IMT were confirmed in the comparisons within the IMT tertiles and by multiple regression analysis.

Another limitation could be the lack of control individuals. However, the study was designed to investigate the lipid and lipoprotein measures that were more informative in relation to subclinical atherosclerosis, i.e., thickening of carotid artery in this normolipidemic population. Comparing these lipoprotein measures in patients with low and high degree of IMT, the objective consisted of identifying probable mechanisms that promote atherosclerosis in patients with SLE.

We have shown that lipoprotein subclasses are more informative than the routinely measured lipid indicators when assessing atherosclerosis risk in patients with SLE. Remnant lipoproteins correlated significantly and positively with median IMT. The IDL fraction had the highest correlation with IMT and was strongly modulated by the APOE genotype.

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