ORIGINAL RESEARCH

Association of Cholesterol Efflux Capacity With Clinical Features of Metabolic Syndrome: Relevance to Atherosclerosis

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***Background*—**The contribution of high-density lipoprotein to cardiovascular benefit is closely linked to its role in the cellular cholesterol efflux process; however, various clinical and biochemical variables are known to modulate the overall cholesterol efflux process. The aim of this study was to evaluate the extent to which clinical and biological anomalies associated with the establishment of the metabolic syndrome modulate cholesterol efflux capacity and contribute to development of atherosclerosis.

***Methods and Results*—**This study involved patients (n=1202) displaying atherogenic dyslipidemia in primary prevention who were referred to our prevention center. Among these patients, 25% presented at least 3 criteria of the metabolic syndrome, as defined by the National Cholesterol Education Program Adult Treatment Panel III. We measured the capacity of 40-fold diluted serum to mediate cholesterol efflux from cholesterol-loaded human THP-1 macrophages. Cholesterol efflux capacity was reduced progressively by 4% to 11% ($P<0.0001$) as a function of the increasing number of coexisting criteria for the metabolic syndrome from 1 to 5. This observation was primarily related to reductions in scavenger receptor class B member 1 and ATP binding cassette subfamily G member 1–dependent efflux. Multivariate analyses indicate that serum efflux capacity was significantly associated with established metabolic syndrome (odds ratio 0.45; 95% CI 0.28–0.72; $P=0.009$) independent of age, low-density lipoprotein cholesterol, status with regard to lipid-lowering therapy, smoking status, and alcohol consumption.

***Conclusions*—**Our study revealed that individual criteria of metabolic syndrome are closely related synergistically to cholesterol efflux capacity. In addition, established metabolic syndrome and cholesterol efflux capacity were independently associated with clinical features of atherosclerosis. **(*J Am Heart Assoc*. 2016;5:e004808 doi: 10.1161/JAHA.116.004808)**

Key Words: ABC transporter • atherosclerosis • cardiovascular risk • cholesterol efflux • macrophage • metabolic syndrome

Dyslipidemias including hypertriglyceridemia and/or hypercholesterolemia are associated with premature atherosclerosis and cardiovascular disease.[1] Among dyslipidemic patients, those presenting with metabolic syndrome (MetS) display a 2-fold increased risk of cardiovascular disease.[2] MetS is associated with several major cardiovascular risk factors that synergistically contribute to acceleration of atherogenesis and atherothrombosis. In particular, MetS patients are characterized by clinical anomalies such as elevated blood pressure, abdominal obesity, and biological perturbations including elevated fasting glucose, high triglyceride levels, and a low high-density lipoprotein cholesterol (HDL-C) phenotype.[2,3] In addition, it has been demonstrated that MetS is associated with systemic oxidative stress and a subclinical inflammation state as a result of alterations in both quantitative and qualitative features of HDL particles that favor development of atherosclerosis.[4]

Cellular cholesterol efflux toward extracellular acceptors represents a mechanism by which macrophages regulate their cholesterol homeostasis to prevent accumulation of cholesterol within the intracellular compartment.[5] It has been demonstrated that efflux of cholesterol from human macrophages occurs toward lipid-poor or lipid-free apolipoprotein AI (apo AI), so-called prebeta HDL, or mature HDL particles and requires the ATP binding cassette A1 (ABCA1) transporter[6] or the scavenger receptor class B member 1 (SR-BI) and CD36 and LIMPII analogous-1 (Cla-1) receptor.[7] In this context, a strong inverse association has been demonstrated among ABCA1-mediated efflux capacity promoted by apo B–depleted serum and carotid intima–media thickness (IMT), the severity

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Received October 6, 2016; accepted October 24, 2016.

Table 1. Clinical and Biological Characteristics of the Study Population According to Quartiles of Cholesterol Efflux Capacity

	All Patients (n=1202)	Cholesterol Efflux Capacity				P Value
		Quartile 1 (n=300)	Quartile 2 (n=300)	Quartile 3 (n=300)	Quartile 4 (n=302)	
CEC*	0.28–1.92	0.28–0.78	0.78–0.88	0.88–1.00	1.00–1.92	<0.0001
MetS, %	25	33	26	27	17	<0.0001
Men/women, n/n	617/585	193/107	155/145	146/154	108/146	<0.0001
Age, y	56.4±13.4	56.8±13.3	56.3±13.3	56.0±13.2	55.1±13.2	0.1376
BMI, kg/m²	26.4±4.7	27.1±4.5	26.9±4.8	26.1±4.3	25.8±4.8	0.0021
Waist, cm	93.5±13.3	96.7±13.8	95.5±13.2	92.7±12.5	91.1±13.0	<0.0001
Hip, cm	99.5±8.6	100.1±8.5	100.2±8.2	99.1±7.3	98.0±9.1	0.0101
W/H ratio	0.94±0.10	0.96±0.11	0.95±0.10	0.94±0.10	0.93±0.10	0.0002
SBP, mm Hg	124.7±15.6	125.2±14.9	124.0±14.4	125.1±16.1	123.0±14.8	0.1022
DBP, mm Hg	71.2±10.3	71.2±10.0	71.1±9.8	71.7±10.2	70.6±10.1	0.4472
FBG, mmol/L	5.11±0.70	5.21±0.73	5.07±0.69	5.12±0.70	5.02±0.73	0.0031
Cholesterol, g/L						
Total	2.13±0.47	2.01±0.44	2.09±0.46	2.17±0.47	2.26±0.47	<0.0001
LDL	1.35±0.43	1.28±0.38	1.33±0.43	1.38±0.42	1.43±0.45	<0.0001
HDL	0.54±0.18	0.48±0.15	0.52±0.17	0.54±0.18	0.58±0.20	<0.0001
Triglycerides, g/L	1.24±0.90	1.25±0.90	1.27±0.96	1.30±1.02	1.25±0.89	0.9942
Triglycerides, g/L†	0.99 (0.72–1.43)	1.04 (0.74–1.42)	1.02 (0.76–1.57)	1.02 (0.70–1.54)	0.97 (0.70–1.46)	
Apolipoproteins, g/L						
AI	1.51±0.28	1.41±0.23	1.49±0.29	1.53±0.27	1.59±0.30	<0.0001
B	1.04±0.26	1.01±0.25	1.02±0.28	1.05±0.26	1.09±0.27	<0.0005
LTT, %	54	56	53	50	47	0.0454
Alcohol intake, %						
<10 g/day	72	67	69	71	74	0.1528
10–40 g/day	24	27	26	27	20	
>40 g/day	4	6	5	2	6	
Current smokers, %	17	17	19	12	19	0.5769
Carotid IMT, mm						
Right	0.642±0.166	0.651±0.164	0.652±0.68	0.639±0.162	0.619±0.160	0.0352
Left	0.664±0.177	0.670±0.173	0.686±0.189	0.678±0.190	0.628±0.160	0.0082
Femoral IMT, mm						
Right	0.552±0.164	0.559±0.160	0.549±0.154	0.560±0.182	0.531±0.150	0.1053
Left	0.552±0.153	0.558±0.149	0.568±0.151	0.548±0.140	0.521±0.125	0.0193
CCA, %						
No plaque	36	35	39	37	48	0.0101
Early plaque	41	41	44	40	33	
Advanced plaque	23	24	17	23	19	
CFA, %						
No plaque	40	39	45	40	49	0.0387
Early plaque	35	33	33	38	31	
Advanced plaque	25	28	22	22	20	

The study population was composed of white patients. Values are shown as mean±SD except as noted. Presence of atherosclerotic plaque was defined as follows: No plaque indicates normal artery without any plaque; early plaque indicates presence of plaques <2 mm; advanced plaques indicates presence of plaques ≥2 mm. *P* value indicates a significant difference between the lowest quartile (Q1) and the highest quartile (Q4) of cholesterol efflux capacity. BMI indicates body mass index; CCA, common carotid artery; CEC, cholesterol efflux capacity; CFA, common femoral artery; DBP, diastolic blood pressure; FBG, fasting blood glucose; HDL, high-density lipoprotein; IMT, intima–media thickness; LDL, low-density lipoprotein; LTT, lipid-lowering therapy; MetS, metabolic syndrome; SBP, systolic blood pressure; W/H, waist:hip ratio.

*Cholesterol efflux capacity represents cholesterol efflux from cholesterol-loaded human THP-1 macrophages determined after 4 hours at 37°C in the presence of 40-fold diluted serum as cellular cholesterol acceptor and is expressed as relative efflux.

†Indicates median and interquartile range Q1 to Q3.

of angiographic coronary artery disease, and cardiovascular events, highlighting the determinant antiatherogenic role of the cholesterol efflux process.[8,9] In addition to HDL particles, apo B–containing lipoproteins equally represent potential cellular cholesterol acceptors for SR-BI.[10,11] It has been proposed that apo B–containing lipoprotein particles contribute to cholesterol efflux from human macrophages as result of a continuous transfer of cholesterol from HDL to apo B–containing lipoproteins, maintaining efficient HDL-mediated cellular cholesterol efflux.[12,13] In addition, various clinical and biochemical variables, such as sex, plasma cholesteryl ester transfer protein activity, or circulating triglyceride levels, have been shown to represent modulators of the ability of plasma to mediate free cholesterol efflux from human macrophage.[14,15] Taken together, these observations led us to consider the use of whole serum as a cellular cholesterol acceptor rather than apo B–depleted serum, which is more commonly used in cholesterol efflux assays.[8,9,16,17] Nevertheless, it is relevant to note that methods used to remove apo B–containing lipoproteins have recently been shown to affect HDL apolipoprotein content and/or size distribution, leading to potential deleterious impact on their biological functions.[18]

The aim of this study was to evaluate the extent to which clinical and biological anomalies associated with the establishment of MetS modulate the capacity of whole serum to mediate cholesterol efflux from the human THP-1 macrophage. We demonstrate a progressive reduction in cholesterol efflux capacity associated with the appearance of clinical features of MetS. We also demonstrated that cholesterol efflux capacity and established MetS represent 2 independent markers of atherosclerotic lesions that synergistically contribute to development of atherosclerosis.

Materials and Methods

Study Population

We included patients referred to our Prevention Center for Dyslipidemia and Cardiovascular Disease (Pitié-Salpêtrière Hospital, Paris, France) for a personal history of dyslipidemia, including total cholesterol >250 mg/dL, low-density lipoprotein cholesterol (LDL-C) >160 mg/dL, and/or triglycerides >150 mg/dL, and/or who were on lipid-lowering therapy. Patients presenting with diabetes mellitus or with hypothyroidism, severe renal insufficiency, or active liver disease due to viral infection were excluded. The study population was composed of 1202 white patients (617 men, 585 women) aged >18 years (Table 1). Overall, 25% of participants displayed hypercholesterolemia with total cholesterol >250 mg/dL and/or LDL-C >160 mg/dL. Moreover, 23% of participants were hypertriglyceridemic, with plasma triglyceride levels >150 mg/dL, and one-third of the population displayed a low HDL-C phenotype. In addition, 54% of participants were receiving a lipid-lowering therapy, with 85% of treated patients receiving a statin as monotherapy or in combination therapy.

Patients were classified as displaying MetS on the basis of the modified Adult Treatment Panel III criteria,[2] which correspond to the concomitant presence of at least 3 of the following 5 criteria: abdominal obesity characterized by increased waist circumference (≥102 cm for men and ≥88 cm for women), hypertriglyceridemia (triglycerides ≥150 mg/dL), a low HDL-C phenotype (≤40 mg/dL for men and ≤50 mg/dL for women), elevated blood pressure (systolic blood pressure ≥130 mm Hg and/or diastolic blood pressure ≥85 mm Hg or current use of antihypertensive drugs), and elevated fasting blood glucose (≥5.6 mmol/L) (Table 2). Patients were separated into 3 groups according to their alcohol consumption: abstention or low alcohol intake (<10 g/day); regular drinkers, with estimated alcohol consumption between 10 and 40 g/day; and heavy drinkers (>40 g/day).

The study conforms to the principles outlined in the Declaration of Helsinki. The study was approved by the ethics committee of the Pitié-Salpêtrière Hospital, and written informed consent was obtained from each patient.

Blood Samples and Biochemical Measurements

Blood samples were withdrawn by venipuncture after a 12-hour overnight fast and collected in gel-containing Vacutainer tubes (Becton-Dickinson). Serum lipids were analyzed in a central reference laboratory on an autoanalyzer (Konelab 30i; Thermo Fisher Scientific) and by using commercial kits for total cholesterol, triglycerides, and direct HDL-C (Thermo Fisher Scientific). LDL-C was calculated using the Friedewald formula when triglycerides levels were <340 mg/dL. Glucose was analyzed using an enzymatic method on the Modular-P (Roche Diagnostics). Apo AI and apo B were measured by immunonephelometry using anti–apo AI and anti–apo B antisera and a BN II nephelometer analyzer (Behring).

Carotid and Femoral Ultrasonography

High-resolution B-mode ultrasound of the common carotid artery (CCA), the carotid bifurcation, the internal carotid artery, and the common femoral artery (CFA) was performed, as described previously.[19] Patients were examined in supine position. For both vessels, the IMT was determined on the far wall of the artery at sites free of any discrete plaques. The IMT was defined as the distance between the intima–lumina interface and the medial–adventitial interface. We used the mean of the 3 right and left longitudinal measurements of IMT of

Table 2. Clinical and Biological Characteristics of the Study Population According to the Number of MetS Criteria

	Patients Without MetS	Patients With 1 or 2 MetS Criteria	Patients With MetS	*P* Value
Patients (men/women), n (n/n)	286 (114/172)	609 (337/272)	307 (166/141)	0.0005
Age, y	52.5±13.6	56.9±13.2	59.0±12.7	<0.0001
BMI, kg/m^2	23.2±3.1	26.2±4.1	29.7±4.8	<0.0001
Waist circumference, cm	81.8±9.5	93.3±11.3	103.4±11.6	<0.0001
Hip circumference, cm	95.1±7.2	99.6±8.2	102.9±8.7	<0.0001
Waist:hip ratio	0.87±0.09	0.94±0.09	1.01±0.09	<0.0001
Systolic blood pressure, mm Hg	115.9±11.8	126.7±15.2	129.3±15.9	<0.0001
Diastolic blood pressure, mm Hg	66.6±8.4	72.5±10.4	72.5±10.6	<0.0001
Fasting blood glucose, mmol/L	4.69±0.44	5.04±0.59	5.64±0.76	<0.0001
Cholesterol, g/L				
Total	2.22±0.48	2.11±0.46	2.07±0.47	<0.0001
LDL	1.42±0.44	1.34±0.41	1.29±0.43	0.0003
HDL	0.65±0.16	0.54±0.17	0.43±0.14	<0.0001
Triglycerides, g/L	0.77±0.26	1.17±0.70	1.83±1.25	<0.0001
Triglycerides, g/L*	0.73 (0.56–0.95)	1.00 (0.75–1.35)	1.62 (1.08–2.12)	
Apolipoproteins, g/L				
AI	1.62±0.27	1.52±0.28	1.41±0.28	<0.0001
B	1.03±0.25	1.04±0.26	1.06±0.26	0.1080
Lipid-lowering therapy, %	61	52	57	0.2725
Alcohol consumption, %				
Low or abstinent (<10 g/day)	76	69	70	0.0675
Regular (10–40 g/day)	23	26	25	
Heavy (>40 g/day)	2	5	5	
Current smokers, %	12	20	18	0.0374
Carotid IMT, mm				
Right	0.597±0.152	0.653±0.172	0.661±0.168	<0.0001
Left	0.615±0.160	0.674±0.185	0.687±0.166	<0.0001
Femoral IMT, mm				
Right	0.550±0.175	0.550±0.161	0.562±0.158	0.4701
Left	0.527±0.129	0.559±0.163	0.565±0.153	0.0089
Common carotid artery, %				
No plaque	53	32	26	<0.0001
Early plaque	34	43	42	
Advanced plaque	13	25	32	
Common femoral artery, %				
No plaque	54	36	34	<0.0001
Early plaque	33	35	36	
Advanced plaque	13	29	30	

The study population was composed of white patients. Patients were categorized based on the number of coexisting MetS criteria present: Patients without MetS criteria did not have any MetS component; patients with 1 or 2 MetS criteria had 1 or 2 of the 5 MetS component; patients with MetS had at least 3 of the 5 MetS components. Values are shown as mean±SD except as noted. Presence of atherosclerotic plaque was defined as follows: No plaque indicates normal artery without any plaque; early plaque indicates presence of plaques <2 mm; advanced plaques indicates presence of plaques ≥2 mm. *P* value indicates a significant difference between participants without MetS compared with patients with MetS. BMI indicates body mass index; HDL, high-density lipoprotein; IMT, intima–media thickness; LDL, low-density lipoprotein; MetS, metabolic syndrome.
*Indicates Median and interquartile range Q1–Q3.

Table 3. Pearson Correlation and Partial Correlation Coefficients With Cholesterol Efflux Capacity

	r Unadjusted	*r* Adjusted for Age	*r* Adjusted for Sex	*r* Adjusted LLT	*r* Adjusted HT Treatment
BMI	−0.154*	−0.154*	−0.141*	−0.165*	−0.146*
Waist	−0.214*	−0.212*	−0.156*	−0.212*	−0.196*
Hip	−0.129*	−0.133*	−0.139*	−0.132*	−0.121*
Waist:hip ratio	−0.173*	−0.170*	−0.090*	−0.168*	−0.152*
SBP	−0.080*	−0.075*	−0.056	−0.068*	−0.032
DBP	−0.051	−0.053	−0.007	−0.053	−0.024
logTG	−0.088*	−0.088*	−0.030	−0.077*	−0.078
Total cholesterol	0.186*	0.184*	0.151*	0.191*	0.178*
LDL-C	0.122*	0.119*	0.104*	0.123*	0.110*
HDL-C	0.264*	0.275*	0.183*	0.259*	0.258*
apo B	0.086*	0.083*	0.091*	0.087*	0.077*
apo AI	0.256*	0.274*	0.180*	0.255*	0.253*
FBG	−0.122*	−0.118*	−0.090*	−0.114*	−0.099*
LLT	−0.042	−0.040	−0.024	—	−0.044
HT treatment	−0.098*	0.095*	−0.098	−0.098*	—
Alcohol consumption	−0.064*	−0.059	−0.004	−0.057	−0.064*
Smoking	0.011	0.003	0.044	−0.020	0.014

Cholesterol efflux capacity represents cholesterol efflux from cholesterol-loaded human THP-1 macrophages determined after 4 hours at 37°C in the presence of 40-fold diluted serum. Results are given for the overall population. apo indicates apolipoprotein; BMI, body mass index; DBP, diastolic blood pressure; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; HT, hypertension; LDL-C, low-density lipoprotein cholesterol; LTT, lipid-lowering therapy; MetS, metabolic syndrome; SBP, systolic blood pressure; TG, triglycerides.
*$P<0.05$.

the CCA (CCA-IMT) and CFA-IMT in the analysis. Near and far walls of the arterial segments were scanned longitudinally and transversally to assess the presence of plaques. If no lesion was detected the IMT was considered as normal. Plaque was defined as an echogenic structure encroaching the vessel lumen with a distinct area 50% greater than the IMT of neighboring sites. Presence of atherosclerotic plaques included early nonstenotic plaques (<2 mm) and advanced plaques (≥2 mm).

Cholesterol Efflux Measurements

Efflux assays were performed using cholesterol-loaded human THP-1 macrophages and cellular models including Fu5AH, Chinese hamster ovary–K1 (CHO-K1), CHO–human ATP binding cassette subfamily G member 1 (CHO-hABCG1), and CHO-hABCA1, as described previously.[14,15] The ^{3}H-cholesterol–labeled cells were incubated 4 hours at 37°C in the presence of 40-fold diluted serum as a cellular cholesterol acceptor. Fractional cholesterol efflux (expressed as a percentage) was calculated as the amount of the label recovered in the medium divided by the total label in each well (radioactivity in the medium plus radioactivity in the cells) obtained after lipid extraction from cells in a mixture of 3:2 hexane:isopropanol (3:2 vol/vol). The background cholesterol efflux obtained in the absence of any acceptor was subtracted from the efflux obtained with samples. ABCG1-dependent efflux was calculated as the difference between efflux to CHO-hABCG1 and CHO-K1 cells. For CHO-hABCA1, the expression of ABCA1 was induced by tetracycline (1 μg/mL). The ABCA1-dependent efflux was calculated as the difference between efflux to activated CHO-hABCA1 and nonactivated cells. A standard serum was tested in all experiments and was used to calculate relative efflux of each sample. All efflux determinations were performed in triplicate for each sample with intra- and interassay coefficients of variation of 2% and 2.3%, respectively.

Statistical Analyses

Statistical analyses were performed using the R statistical software computer program version 2.13.1 (R Foundation for Statistical Computing). For skewed variables (triglycerides), the raw data were logarithmically transformed prior to analyses. Comparisons between participants without any MetS component and those displaying at least 3 MetS components were performed using an unpaired Student *t* test. The qualitative variables presented as proportions were compared using the chi-square test. Cholesterol efflux

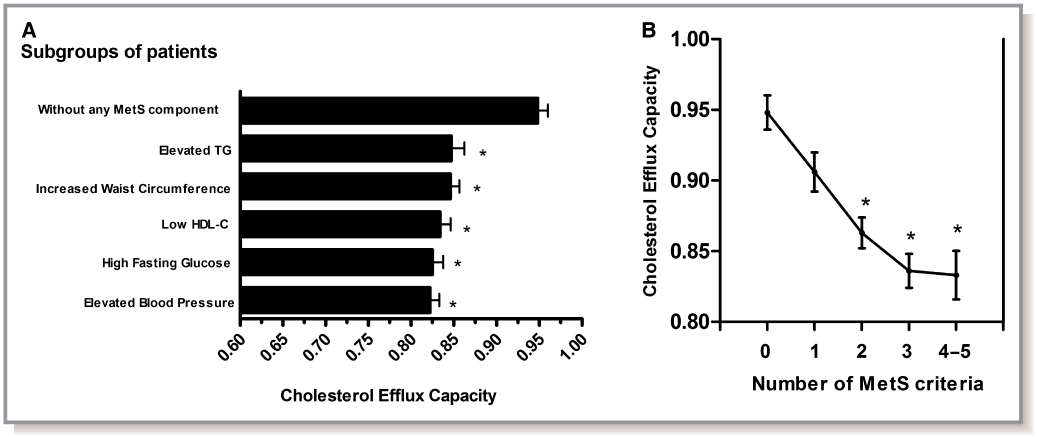

Figure 1. Relationship between cholesterol efflux capacity and MetS criteria. A, Patients with MetS were categorized based on the presence of the indicated specific MetS component. B, Cholesterol efflux capacity as a function of the number of MetS components. MetS0: patients without out any MetS component; MetS 1: patients with 1 MetS component. MetS 2: patients with 2 MetS components; MetS 3: patients with 3 MetS components; MetS 4–5: patients with 4 or 5 criteria of MetS were merged in a single group. Cholesterol efflux capacity represents cholesterol efflux from cholesterol-loaded human THP-1 macrophages determined after 4 hours at 37°C in the presence of 40-fold diluted serum as a cellular cholesterol acceptor and is expressed as relative efflux. *Significantly different from patients without any MetS component. HDL-C indicates high-density lipoprotein cholesterol; MetS, metabolic syndrome; TG, triglycerides.

capacity was modeled either as continuous variables or as a binary variable using the lowest quartiles (Q1, Q2 and Q3) for reference (0=lowest quartiles, 1=highest quartile [Q4]). Each of the 5 MetS components was entered as a dichotomous variable (0=absent, 1=present). Mean IMT of either CCA or CFA was modeled as either continuous or binary variables dichotomized at the median. Univariate and multivariate linear regression analyses were used to determine relationships between numerical or categorical variables and cholesterol efflux capacity. Regression results were expressed as standardized β-regression coefficient corresponding to the impact of 1-SD increase in the independent variable on the variability of the dependent variable. The results were considered to be statistically significant at $P<0.05$.

Results

Relationship Between Serum Efflux Capacity and Cardiometabolic Risk Factors in Patients With MetS

Analysis of the study population according to quartiles of cholesterol efflux capacity revealed that an elevation in the capacity of whole serum to mediate efflux from human THP-1 was associated with reductions in waist circumference and fasting blood glucose and increases in both HDL-C and LDL-C levels (Table 1). Correlation analysis revealed that serum efflux capacity significantly correlated with major clinical and biological features of MetS (Table 3). In particular, a negative correlation was observed between serum efflux capacity and waist circumference, fasting blood glucose levels, and systolic blood pressure. In contrast, serum efflux capacity was positively correlated with HDL-C, Apo AI, total cholesterol, LDL-C, and apo B levels and triglycerides. Most of these associations remained significant following adjustment for age, sex, lipid-lowering therapy, and use of antihypertension treatment. In addition, serum efflux capacity was negatively correlated with antihypertension therapy, reflecting that dyslipidemic patients receiving antihypertension treatment displayed reduced serum efflux capacity.

Among patients included in the study, ≈25% of participants exhibited clinical and biological features of MetS (Table 2). Plasma total cholesterol level was significantly reduced (absolute change −0.15 mg/dL; $P<0.0001$) in patients with MetS compared with those without MetS. This difference results primarily from reductions in both LDL-C (−9%; $P<0.0001$) and HDL-C (−34%; $P<0.0001$) levels in patients with MetS despite no significant difference in the relative proportion of patients receiving a lipid-lowering therapy between the 2 patient groups. It is important to note that the proportions of smokers differed slightly between patients with and without MetS (18% and 12%, respectively; $P=0.037$).

We observed a significant 2.5-fold increase in the relative proportion of participants with advanced atherosclerotic plaques in both carotid and femoral arteries in patients with MetS compared with patients without MetS. We also observed significant increases in both right (+10.7%; $P<0.0001$) and left (+11.7; $P<0.001$) carotid IMT and in left femoral IMT (+7%;

P=0.009) in patients with MetS compared with dyslipidemic patients without any MetS component.

Progressive Increase in the Number of Coexisting MetS Criteria Is Associated With a Reduction in Cholesterol Efflux Capacity

In primary analysis, patients with MetS were classified based on the presence of 1 specific MetS component (Figure 1A). Significant reductions of 8.3% to 11% in cholesterol efflux capacity were observed for each subgroup of MetS patients compared with dyslipidemic patients without any MetS component. Comparison of patient groups based on the number of MetS criteria present revealed a progressive reduction in cholesterol efflux capacity of 4% to 11% (P<0.0001) as a function of the increase in number of coexisting MetS criteria from 1 to 5 (Figure 1B). Univariate regression analyses revealed that a progressive increase in the number of coexisting MetS components was associated with a significant reduction in serum cholesterol efflux capacity from human THP-1 macrophages (β=−0.194; P<0.0001). This reduction remained significant after adjustment for age, LDL-C, lipid-lowering therapy, smoking, and alcohol consumption (β=−0.173; P<0.0001). In addition, logistic regression analyses showed a significant stepwise reduction in the plasma efflux capacity based on an increased number of concomitant MetS criteria (Table 4). Similar conclusions were reached in models adjusted for age, LDL-C, lipid-lowering therapy status, smoking status, and alcohol consumption. In addition, compared with participants without any component of MetS, dyslipidemic patients with MetS were associated with a significant reduction in cholesterol efflux capacity in both unadjusted analyses (odds ratio 0.41; 95% CI 0.27–0.62; P<0.0001) and adjusted analyses (odds ratio 0.45; 95% CI 0.28–0.72; P=0.0009).

Reduction in Cholesterol Efflux Capacity in MetS Is Associated With Reduced SR-BI–Dependent Efflux

To further analyze the mechanism underlying the reduction of serum cholesterol efflux capacity from human THP-1 macrophages in patients with MetS, we used various cellular models, each representative of 1 efflux pathway. As shown in Table 5, clinical and biochemical features of MetS criteria were associated with significantly reduced capacities of serum to mediate cholesterol efflux via both SR-BI (β=−0.242; P<0.0001) and ABCG1 (β=−0.109; P<0.008). Such relationships remained significant following adjustment for age, LDL-C, lipid-lowering therapy, alcohol consumption, and smoking (SR-BI–dependent efflux: β=−0.258, P<0.0001; ABCG1-dependent efflux: β=−0.094, P=0.0267). We observed a significant reduction in the capacity of serum to mediate SR-BI–dependent efflux (−11.4%; P<0.0001) and ABCG1-dependent efflux (−6.4%; P=0.0015) in dyslipidemic patients with MetS compared with those without any MetS component. In contrast, the ABCA1-dependent efflux was not significantly associated with the increased number of MetS criteria in both unadjusted and adjusted analyses. We observed an increase in ABCA1-dependent efflux in patients with MetS compared with those without MetS; however, this difference did not reach statistical significance (P=0.091).

Relationship Between Cholesterol Efflux Capacity and Surrogate Markers of Atherosclerosis

Mean CCA-IMT and CFA-IMT were significantly reduced in patients belonging to the highest quartile of cholesterol efflux capacity (Q4) compared with those in the lowest quartile (Q1; mean CCA-IMT 0.660±0.147 and 0.625±0.149 for Q1 and Q4, respectively [P=0.0116]; mean CFA-IMT 0.567±0.148 and 0.529±0.122 for Q1 and Q4, respectively [P=0.001]). We also observed a significant increase in the relative proportion of patients with atherosclerotic plaques in both carotid and femoral arteries in those with reduced cholesterol efflux capacity (lowest quartile, Q1) compared with those with higher cholesterol efflux capacity (highest quartile, Q4).

Table 4. Influence of the Number of MetS Criteria on Cholesterol Efflux Capacity

Number of MetS Criteria	OR	95% CI	P Value
Unadjusted analysis			
MetS0	1	—	—
MetS1	0.76	0.51–1.12	0.1974
MetS2	0.63	0.43–0.93	0.0183
MetS3	0.40	0.25–0.66	0.0002
MetS4–5	0.43	0.24–0.77	0.0035
Adjusted analysis *			
MetS0	1	—	—
MetS1	0.86	0.55–1.31	0.4783
MetS2	0.63	0.41–0.97	0.0390
MetS3	0.76	0.63–0.91	0.0034
MetS4–5	0.81	0.69–0.95	0.0109

MetS patients were categorized based on the number of coexisting MetS criteria. MetS0: patients without out any MetS component (n=286); MetS1: patients with 1 MetS component (n=288); MetS2: patients with 2 MetS component (n=322); MetS3: patients with 3 MetS component (n=191); MetS4–5: patients displaying 4 or 5 criteria for MetS were merged in a single group (n=116). Cholesterol efflux capacity represents cholesterol efflux from cholesterol-loaded human THP-1 macrophages determined after 4 hours at 37°C in the presence of 40-fold diluted serum as a cellular cholesterol acceptor. Cholesterol efflux capacity was modeled as a binary variable using the lowest quartiles (Q1, Q2, and Q3) for reference (0=lowest quartiles; 1=highest quartile [Q4]). MetS indicates metabolic syndrome; OR, odds ratio.
*Adjustment variables include age, low-density lipoprotein cholesterol, lipid-lowering therapy status, smoking status, and alcohol consumption.

Inversely, participants with elevated cholesterol efflux capacity (Q4, highest quartile) were predominant in the subgroup of patients displaying no atherosclerotic plaque (Table 1).

As shown in Figure 2, unadjusted analysis revealed significant associations between the reduction of cholesterol efflux capacity and surrogate markers of atherosclerosis, namely, mean CCA-IMT and CFA-IMT and the presence of atherosclerotic plaques in both carotid and femoral arteries. Such associations were reduced but remained significant for carotid atherosclerosis after adjustment for traditional risk factors including sex, age, LDL-C, HDL-C, triglycerides, lipid-lowering therapy, smoking, and alcohol consumption.

In multivariate analyses, both cholesterol efflux capacity and MetS were identified as 2 independent determinants of surrogate markers of carotid atherosclerosis, whereas cholesterol efflux capacity represented an independent determinant of mean CFA-IMT, and MetS was independently associated with the presence of atherosclerotic plaque in the femoral artery (Table 6).

Table 5. Influence of the Number of Metabolic Syndrome Criteria on Cholesterol Efflux Capacity via ABCA1, ABCG1, and SR-BI

	Total Population (n=1202) per 1-SD Increase in Number of MetS Criteria		Patients Without MetS (n=286)	Patients With MetS (n=307)
	Standardized β Regression Coefficient	*P* Value	Cholesterol Efflux Capacity	
ABCA1			0.887±0.380	0.966±0.414
Unadjusted analysis	0.095	*0.105*	*0.1890*	
Adjusted analysis	0.103	*0.0787*	*0.0909*	
ABCG1			1.069±0.195	1.000±0.190
Unadjusted analysis	−0.109	*0.0079*	*0.0015*	
Adjusted analysis	−0.094	*0.0267*	*0.0203*	
SR-BI			1.148±0.195	1.017±0.204
Unadjusted analysis	−0.242	*<0.0001*	*<0.0001*	
Adjusted analysis	−0.258	*<0.0001*	*<0.0001*	

Efflux assays were performed using cellular models including Fu5AH, CHO-K1, CHO-hABCG1, and CHO-hABCA1. Cholesterol efflux from cells was determined after 4 hours incubation in the presence of 40-fold diluted serum. Cholesterol efflux capacities expressed in relative efflux are presented in mean±SD. Cholesterol efflux capacity via ABCA1, ABCG1, and SR-BI were modeled as continuous variables. Adjustment variables include age, low-density lipoprotein cholesterol, lipid-lowering therapy status, smoking status, and alcohol consumption. ABCA1 indicates ATP binding cassette A1; ABCG1, ATP binding cassette subfamily G member 1; CHO, Chinese hamster ovary; h, human; MetS, metabolic syndrome; SR-BI, scavenger receptor class B member 1.

Figures 3 and 4 show cholesterol efflux capacity as a function of surrogate markers of atherosclerosis in dyslipidemic patients with or without MetS. The 3-dimensional presentation of cholesterol efflux capacity reveals that the lowest cholesterol efflux capacity was associated with the presence of atherosclerotic plaques in carotid (Figure 3A) and femoral (Figure 4A) arteries and mean IMT of either the CCA (Figure 3C) or CFA (Figure 4C) above the median in patients with MetS. Inversely, the highest serum efflux capacity was observed in dyslipidemic patients with no clinical or biological feature of MetS and who displayed no significant atherosclerotic plaque in both carotid and femoral arteries or a mean IMT of either the CCA or CFA below the median. Patients with MetS and atherosclerotic plaques or elevated IMT in both carotid and femoral arteries were associated with a significant reduction (12–14%; $P<0.0001$) in cholesterol efflux capacity compared with dyslipidemic patients without any clinical or biological features of MetS and no significant clinical feature of atherosclerosis.

We observed that reduced SR-BI–dependent cholesterol efflux capacity was associated with MetS and atherosclerotic plaques (Figures 3B and 4B) or elevated IMT (Figures 3D and 4D) in both carotid and femoral arteries compared with patients without any clinical or biological features of MetS and no significant clinical features of atherosclerosis. Similar conclusions were reached for cholesterol efflux capacity via ABCG1 (Figures 3B and 4D). In contrast, no significant difference was observed in ABCA1-dependent efflux capacity between patients with clinical features of MetS and atherosclerosis compared with those without MetS and no atherosclerosis.

Finally, we observed no significant interaction according to MetS for the association between cholesterol efflux capacity, including macrophage cholesterol efflux (Table 7) and transporter-specific efflux, and surrogate markers of atherosclerosis ($P>0.1$ for interaction in all cases).

Discussion

We report a reduction in cholesterol efflux capacity from human THP-1 macrophages in dyslipidemic patients with clinical features of MetS. Numerous studies have demonstrated that HDL isolated from patients displaying metabolic disorders associated with a low HDL-C phenotype display a panel of functional anomalies including reduced capacity to mediate SR-BI/Cla1 receptor–dependent efflux.[20–23] Such impaired functions have been proposed to account for residual cardiovascular risk in these patients. In agreement with our present observation, Lucero et al[24] recently reported that ABCA1-mediated cholesterol efflux, evaluated using a stably transfected BHK cell line overexpressing ABCA1, is enhanced in patients with MetS as a result of an increase in

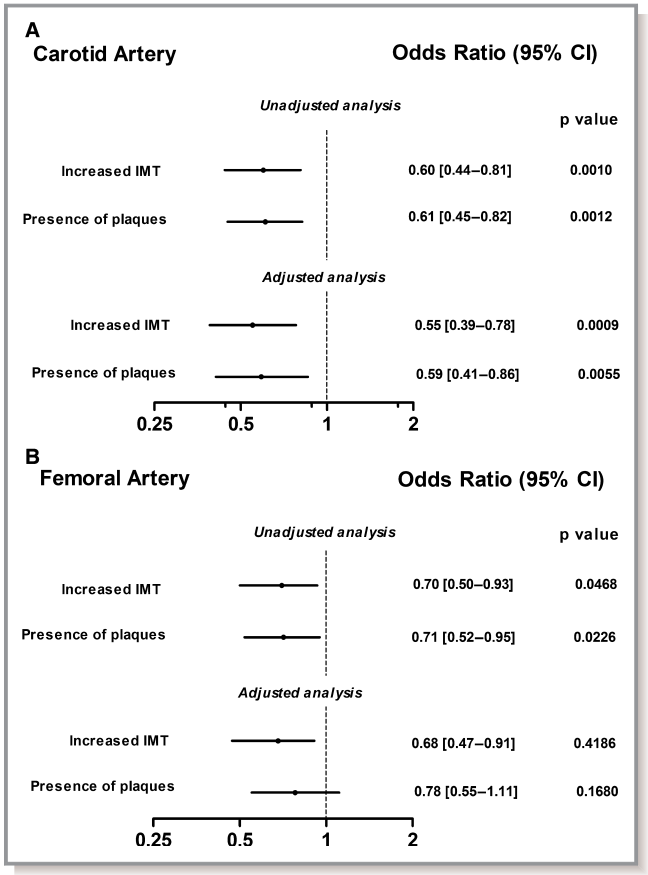

Figure 2. Relationship between cholesterol efflux capacity and surrogate markers of atherosclerosis. Odds ratios for surrogate markers of atherosclerosis on carotid artery (A) and femoral artery (B) according to cholesterol efflux capacity as a dependent variable. Cholesterol efflux capacity represents cholesterol efflux from cholesterol-loaded human THP-1 macrophages determined after 4 hours at 37°C in the presence of 40-fold diluted serum as a cellular cholesterol acceptor. Cholesterol efflux capacity was modeled as a binary variable using lowest quartiles (Q1, Q2, and Q3) for reference (0=lowest quartiles, 1=highest quartile [Q4]). Mean IMT of either the CCA or CFA were modeled as binary variables dichotomized at the median (CCA 0.62 and CFA 0.53). Adjustment variables included age, low-density lipoprotein cholesterol, lipid-lowering therapy status, smoking status, and alcohol consumption. CCA indicates common carotid artery; CFA, common femoral artery; IMT, intima–media thickness.

prebeta HDL levels. However, it is relevant to consider that cholesterol efflux from human macrophages involves both ABCA1 and SR-BI/Cla1 in equivalent contributions. It is well established that metabolic disorders associated with a low HDL-C phenotype, such as MetS, are characterized by a predominance of small dense HDL or prebeta HDL particles and by a reduction in mature HDL particles that result from active intravascular HDL remodeling by the concomitant actions of lipid transfer proteins (ie, cholesteryl ester transfer protein and phospholipid transfer protein) and enzymes such

Table 6. Multiple Regression Analyses for the Association of Cholesterol Efflux Capacity, MetS, and Surrogate Markers of Atherosclerosis

Variables	Standardized β Regression Coefficient per 1-SD Increase in Surrogate Markers of Atherosclerosis	*P* Value
Mean of the CCA		
Cholesterol efflux capacity	−0.137	0.0009
MetS	0.161	0.0001
Age	0.381	<0.0001
LDL-C	0.067	0.1026
Alcohol	0.018	0.6466
Smoking	0.042	0.3445
LLT	−0.025	0.5558
Presence of plaque in carotid artery		
Cholesterol efflux capacity	−0.124	0.0042
MetS	0.143	0.0013
Age	0.474	<0.0001
LDL-C	0.120	0.0053
Alcohol	0.036	0.3911
Smoking	0.077	0.1014
LLT	0.082	0.0625
Mean of the CFA		
Cholesterol efflux capacity	−0.125	0.0223
MetS	0.109	0.0591
Age	0.210	0.0002
LDL-C	0.069	0.2112
Alcohol	0.075	0.1888
Smoking	0.276	<0.0001
LLT	0.022	0.6955
Presence of plaque in femoral artery		
Cholesterol efflux capacity	−0.079	0.0745
MetS	0.110	0.0159
Age	0.381	<0.0001
LDL-C	0.125	0.0048
Alcohol	0.109	0.0113
Smoking	0.134	0.0057
LLT	0.208	<0.0001

Cholesterol efflux capacity represents cholesterol efflux from cholesterol-loaded human THP-1 macrophages determined after 4 hours at 37°C in the presence of 40-fold diluted serum as a cellular cholesterol acceptor. Cholesterol efflux capacity was modeled as a binary variable using the lowest quartiles (Q1, Q2, and Q3) for reference (0=lowest quartiles, 1=highest quartile [Q4]). Mean intima–media thicknesses of CCA and CFA were modeled as continuous variables. CCA indicates common carotid artery; CFA, common femoral artery; LDL-C, low-density lipoprotein cholesterol; LLT, lipid-lowering therapy; MetS, metabolic syndrome.

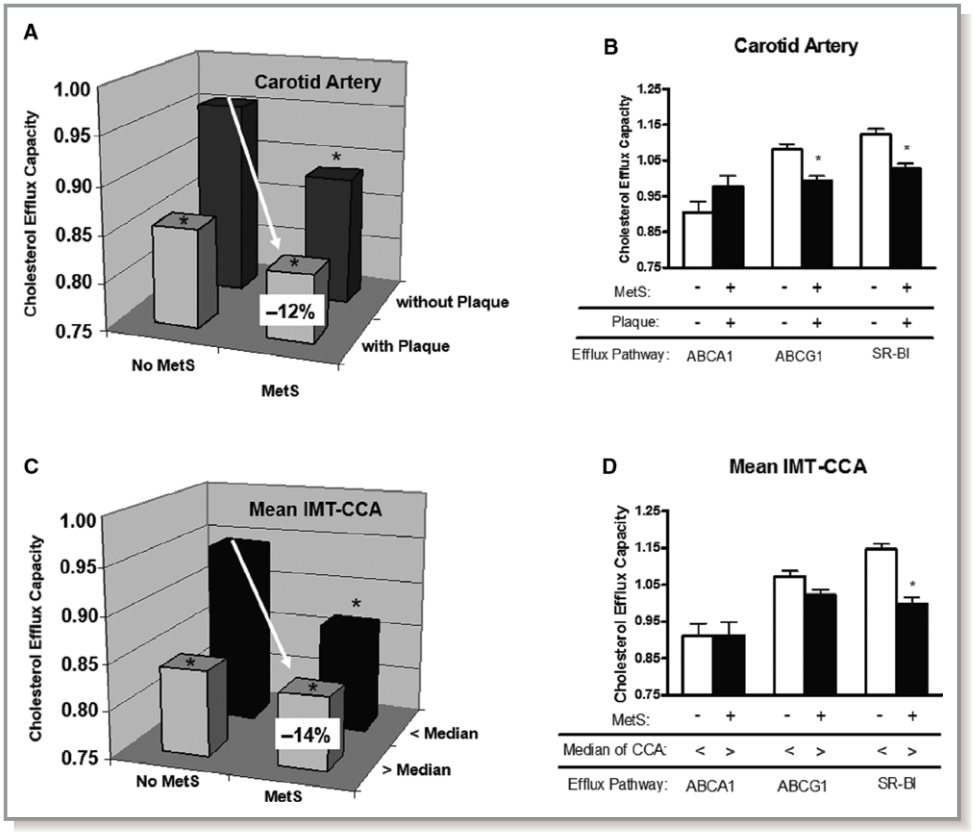

Figure 3. Cholesterol efflux capacity in patients with and without MetS (no MetS) based on surrogate markers of carotid atherosclerosis, namely, presence or absence of atherosclerotic plaques on carotid artery (A and B) and mean IMT of the CCA (IMT-CCA) (C and D). Mean IMT-CCA was modeled as a binary variable dichotomized at the median (median 0.62). A and C, Cholesterol efflux capacity from human THP-1 macrophages. C and D, Cholesterol efflux capacity via ABCA1, ABCG1, and SR-BI. Cholesterol efflux capacity represents cholesterol efflux from cells determined after 4 hours at 37°C in the presence of 40-fold diluted serum as a cellular cholesterol acceptor and is expressed as relative efflux. *Significantly different from patients without MetS and no significant clinical features of atherosclerosis in carotid artery. ABCA1 indicates ATP binding cassette A1; ABCG1, ATP binding cassette subfamily G member 1; CCA, common carotid artery; IMT, intima–media thickness; MetS, metabolic syndrome; SR-BI, scavenger receptor class B member 1.

as lecithin-cholesterol acyltransferase and hepatic lipase.[25] In the context of the present study, it is important to consider that, in addition to ABCA1, the SR-BI/Cla1 receptor equally contributes to human macrophage homeostasis[26] by favoring free cholesterol efflux to mature HDL particles.[27] Taken together, these observations indicate that in patients with MetS, potential acceleration of ABCA1-dependent efflux does not counterbalance defective SR-BI–mediated cholesterol efflux, resulting in the reported observed reduction in plasma efflux capacity from human THP-1 macrophages.

Only a few studies have evaluated the relationship between HDL-mediated cholesterol efflux and blood pressure. It is well established that hypertension and endothelial dysfunction are closely linked.[28] It is thus relevant to note that Vigna et al[29] reported an inverse correlation between ABCA1-mediated cholesterol efflux and pulse wave velocity, a marker of arterial stiffness, indicating a contribution of HDL efflux capacity to vascular endothelial function. In addition, the association between HDL-mediated vasodilatory effect has been related to the capacity of HDL particles to interact with SR-BI and ABCG1, which are both involved in cholesterol efflux toward mature HDL particles.[30] Taken together, these observations strongly suggest that reduction in serum efflux capacity from human THP-1 macrophages presently observed in patients displaying MetS favors an alteration of vascular endothelium function that contributes to acceleration of atherosclerosis development.

In the present study, patients with type 2 diabetes mellitus were excluded from the selected population, which is thus restricted to dyslipidemic patients; however, ≈22% of the study population displayed fasting blood glucose levels between 5.6 and 6.9 mmol/L and 41% had hemoglobin A1c levels between 5.7% and 6.4%. These biological markers represent well-established indicators of prediabetes. By using a lipidomic analytical approach, it has been reported that

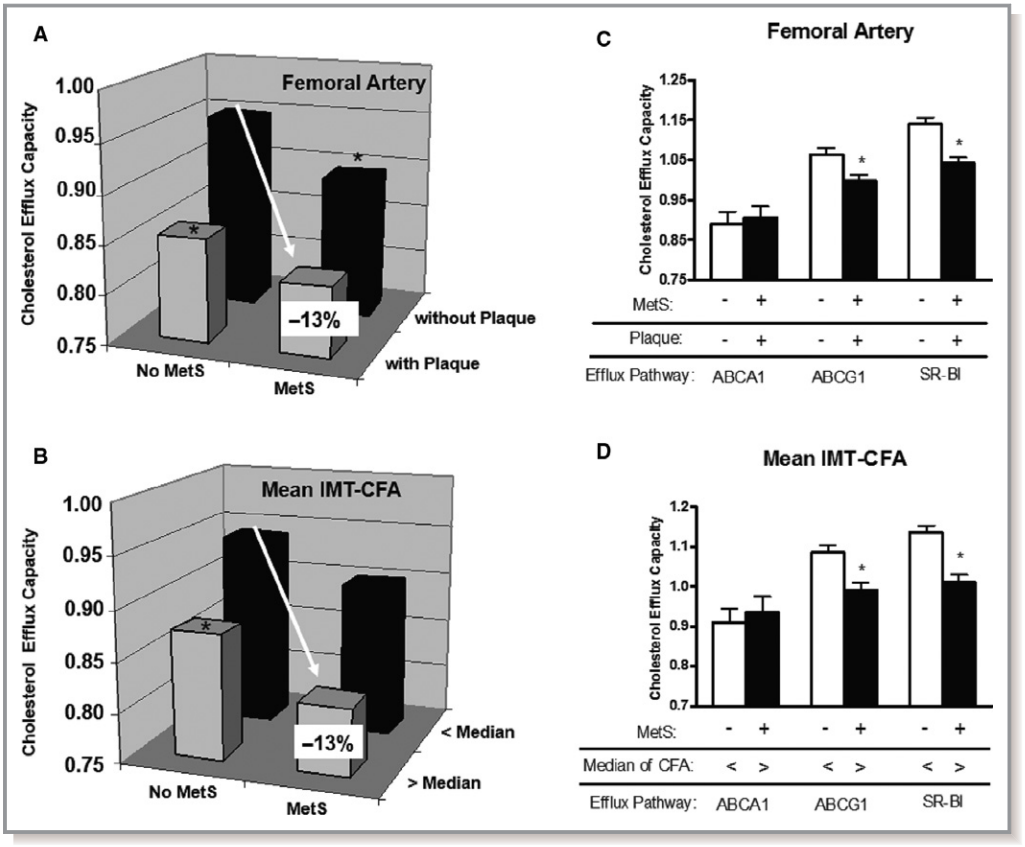

Figure 4. Cholesterol efflux capacity in patients with and without MetS (no MetS) as a function of surrogate markers of femoral atherosclerosis, namely, presence or absence of atherosclerotic plaques on femoral artery (A and B) and mean IMT of the CFA (mean IMT-CFA) (C and D). Mean IMT-CFA was modeled as a binary variable dichotomized at the median (median 0.53). A and C, Cholesterol efflux capacity from human THP-1 macrophages. C and D, Cholesterol efflux capacity via ABCA1, ABCG1, and SR-BI. Cholesterol efflux capacity represents cholesterol efflux from cells determined after 4 hours at 37°C in the presence of 40-fold diluted serum as a cellular cholesterol acceptor and is expressed as relative efflux. *Significantly different from patients without MetS and no significant clinical features of atherosclerosis in femoral artery. ABCA1 indicates ATP binding cassette A1; ABCG1, ATP binding cassette subfamily G member 1; CFA, common femoral artery; IMT, intima–media thickness; MetS, metabolic syndrome; SR-BI, scavenger receptor class B member 1.

abnormal lipid species detected in type 2 diabetes mellitus are equally present in the prediabetes state.[31] This latter study revealed negative associations of various phosphatidylcholine and sphingomyelin species, the 2 major phospholipids in HDL particles, with type 2 diabetes mellitus and prediabetes. Interestingly, HDL phospholipids have been shown to play a determinant role in the cholesterol efflux process, with SR-BI– and ABCG1-mediated cellular cholesterol efflux occurring in a phospholipid-dependent manner. These observations suggest a defective HDL efflux capacity in patients with type 2 diabetes mellitus or prediabetes and are in good agreement with the presently observed reduction in serum efflux capacity in patients with MetS. Conflicting observations, however, have been reported concerning plasma efflux capacity in diabetic patients. Syvanne et al[32] observed reduced SR-BI–dependent plasma efflux capacity, whereas Dullaart et al[33] reported no significant alteration in plasma efflux capacity in diabetic patients compared with nondiabetic participants. In addition, the capacity of HDL-mediated cholesterol efflux from human THP-1 macrophages has been shown to be significantly improved in diabetic patients compared with nondiabetic controls.[16] The degree of hypertriglyceridemia associated with type 2 diabetes mellitus, as an independent modulator of plasma efflux capacity, may account for these apparently conflicting observations. Indeed, elevation in plasma triglyceride levels together with enhanced activities of cholesteryl ester transfer protein, phospholipid transfer protein, and hepatic lipase lead to an increase in circulating levels of small prebeta HDL particles in patients with type 2 diabetes mellitus.[20,34] Although small HDL particles predominate in the plasma of diabetic patients, no significant correlation between small HDL particle levels and cholesterol efflux from human

Table 7. Unadjusted ORs for Cholesterol Efflux Capacity and Surrogate Markers of Atherosclerosis According to MetS

	Patients Without MetS	Patients With MetS	*P* Interaction
	OR (95% CI)	OR (95% CI)	
Carotid artery			
Increased IMT	0.39 (0.19–0.78)	0.59 (0.27–1.30)	0.391
Presence of plaque	0.42 (0.22–0.76)	0.61 (0.27–1.45)	0.437
Femoral artery			
Increased IMT	0.67 (0.0.33–1.34)	0.40 (0.14–1.11)	0.372
Presence of plaque	0.54 (0.29–0.97)	0.78 (0.35–1.85)	0.432

Cholesterol efflux capacity represents cholesterol efflux from cholesterol-loaded human THP-1 macrophages determined after 4 hours at 37°C in the presence of 40-fold diluted serum. Cholesterol efflux capacity was modeled as a binary variable using the lowest quartiles (Q1, Q2, and Q3) for reference (0=lowest quartiles, 1=highest quartile [Q4]). Mean IMT of either the CCA or CFA were modeled as binary variables dichotomized at the median (CCA=0.62 and CFA=0.53). CCA indicates common carotid artery; CFA, common femoral artery; IMT, intima–media thickness; MetS, metabolic syndrome; OR, odds ratio.

THP-1 macrophage was observed, suggesting that type 2 diabetes mellitus is associated with formation and accumulation of dysfunctional HDL subspecies.[34] In this context, in vitro studies have demonstrated that glycation of HDL apo A1 significantly reduces the capacity of HDL particles to mediate cholesterol efflux from human THP-1 macrophages.[35] In addition, an inverse correlation between hemoglobin A1c and cholesterol efflux from human macrophages has been reported previously.[16] Data reported in the present study indicate the appearance of dysfunctional HDL particles in the early phase of diabetes development.

This study demonstrated that serum from dyslipidemic patients displaying clinical features of MetS exhibits a defective ability to mediate cholesterol efflux from human THP-1 macrophage. Our study revealed that individual criteria of MetS synergistically contribute to reduce serum efflux capacity. In addition and in good agreement with earlier reports,[8,17] we observed an inverse relationship between the ability of serum from dyslipidemic patients to mediate cholesterol efflux and surrogate markers of atherosclerosis. These observations highlight and confirm the determinant role of the early step of the reverse cholesterol transport pathway in the prevention of atherosclerosis development. Finally, we identified cholesterol efflux capacity and MetS as 2 independent factors related to atherosclerosis development and revealed that association of clinical and biological features of MetS with cholesterol efflux capacity are closely linked to the development of atherosclerosis. We propose that cholesterol efflux capacity might represent a relevant clinical tool for identification of patients at high risk of premature atherosclerosis and may help define the optimal therapy in personalized medical care.

Acknowledgments

We warmly thank nurses from Department of Endocrinology-Metabolism for her critical assistance in this study. We thank staff from the Department of Metabolic Biochemistry for expert technical assistance for plasma lipid and apolipoprotein levels measurement. We are indebted to the patients for their cooperation.

Sources of Funding

This work was supported by Institut National de la Santé et de la Recherche Medicale (INSERM), by Université Pierre et Marie Curie (UPMC), and by Assistance Publique-Hopitaux de Paris (AP-HP). Gall was recipient of a Research Fellowship from the French Ministry of Research and Technology and from the New French Atherosclerosis Society (NSFA).

Disclosures

None.

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