ELSEVIER

Atherosclerosis 199 (2008) 415–423

ATHEROSCLEROSIS

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Alterations in cell adhesion molecules and other biomarkers of cardiovascular disease in patients with metabolic syndrome

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Received 12 September 2007; accepted 7 November 2007

Available online 21 December 2007

Abstract

Metabolic syndrome is considered a hyperinsulinemic and inflammatory state closely associated to endothelial dysfunction causing an increased incidence of ischemic cardiovascular events and high mortality. The main objective of the present study was to determine whether leukocitary and soluble cell adhesion molecules were altered in patients with metabolic syndrome in comparison with control subjects. Cell adhesion molecules, mainly of leukocitary location, have been not previously evaluated in specifically designed cross-sectional studies involving male patients with metabolic syndrome. Moreover, other circulating markers of different candidate atherogenic risk parameters were also studied and the potential existence of a progressive relation between the number of metabolic syndrome components and the above mentioned biomarkers was analyzed. Thirty one male patients with metabolic syndrome (ATPIII definition) and 56 male control subjects were studied. We evaluated different markers of insulin resistance, inflammation and atherosclerosis, as well as protective factors. Patients with metabolic syndrome showed (a) hypoadiponectinemia (4551 ± 2302 ng/ml *vs*. 5865 ± 2548 ng/ml, respectively; $p < 0.05$), (b) an atherogenic lipid and lipoprotein profile, (c) altered HDL chemical composition accompanied by higher cholesteryl ester-triglyceride interchange carried out by CETP, (d) diminished Lp-PLA_2 activity (6.5 ± 1.9 *vs*. 7.3 ± 2.2, $p < 0.05$, respectively), antioxidant enzyme related with LDL oxidation, which was positively associated with QUICKI and negatively with VCAM-1 and lymphocyte CD18, and (e) high soluble (VCAM-1: 17 ± 5 *vs*. 13 ± 4 ng/ml, respectively; $p < 0.0005$) and leukocyte adhesion molecule expression (monocyte CD54: 52 ± 15 *vs*. 45 ± 12 arbitrary units, respectively; $p < 0.0005$; and lymphocyte CD49d: 312 ± 56 *vs*. 284 ± 64 arbitrary units, respectively; $p < 0.05$). The increment in leukocyte and soluble cell adhesion molecules, crucial for leukocyte interaction with the endothelium and migration into the artery wall, in combination with the other disorders described above reinforce the presence of a clinical status with high propensity to type 2 diabetes and atherosclerotic cardiovascular disease.

Keywords: Metabolic syndrome; Atherosclerosis; Inflammation; Biomarkers; Adhesion molecules; Adiponectin

1. Introduction

Metabolic syndrome (MS) has been identified by the Third Report of the National Cholesterol Education Program Expert Panel on detection, evaluation, and treatment of high blood cholesterol in adults (ATPIII) as a multiplex risk factor for cardiovascular disease that deserves more clinical attention [1]. Recognition of the MS is generally based on finding several well-recognized signs in clinical practice:

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doi:10.1016/j.atherosclerosis.2007.11.007

abdominal obesity, elevated triglycerides, reduced high density lipoprotein-cholesterol (HDL-C), raised blood pressure, and elevated fasting plasma glucose.

Operational definitions of MS have been proposed by the World Health Organization (WHO) [2] and the ATPIII [3]. More recently, the International Diabetes Federation (IDF) [4] proposed a global definition that emphasized the importance of central adiposity. These and other definitions reported in the literature include the same core criteria (central obesity, hyperglycemia, dyslipidemia and high blood pressure), but differ in the cut-off points for individual criteria, in specific mandatory requirements (e.g., abdominal obesity or insulin resistance) and in the inclusion of additional factors (e.g., microalbuminuria). Hence, they identify broadly similar, but not identical, groups of individuals with MS [5]. Among all the criteria above mentioned, ATP III is one of the most widely employed [3].

The importance of risk factor clustering with hyperinsulinemia as a predictor of type 2 diabetes and cardiovascular disease has been shown in many prospective studies [6]. However, the physiopathology of the MS has remained unknown, although insulin resistance [7] and visceral obesity [8] have been proposed as underlying causes for this syndrome.

MS is considered a state of chronic inflammation closely associated to endothelial dysfunction causing an increased incidence of ischemic cardiovascular events and high mortality. Low-grade inflammation manifests in patients with increased plasma levels of cell adhesion molecules, e.g., vascular cell adhesion molecule (VCAM)-1, intercellular adhesion molecule (ICAM)-1 and E-selectin, interleukins, oxidative stress, activation and translocation of nuclear factor (NF)-kB, and increased plasma levels of C reactive protein [9].

The main objective of the present study was to determine whether leukocitary and soluble cell adhesion molecules were altered in patients with MS in comparison with control subjects. Cell adhesion molecules, mainly of leukocitary location, have been not previously evaluated in specifically designed cross-sectional studies involving male patients with MS. This work also included the evaluation of inflammatory and atherogenic indicators such as lipid and lipoprotein profile, HDL chemical composition, cholesteryl ester transfer protein (CETP), as well as protective factors like adiponectin, paraoxonase (PON) and lipoprotein-associated phospholipase A_2 (Lp-PLA_2). Moreover, the potential existence of a progressive relation between the number of MS components and the above mentioned biomarkers was further analyzed.

2. Materials and methods

2.1. Subjects

We studied 87 male subjects who were consecutively recruited during a period of about 12 months from Institute of Cardiology and Cardiovascular Surgery of the Favaloro Foundation, Buenos Aires, Argentina. Thirty one patients presented MS according to the ATPIII definition [10], while the other 56 individuals were considered as controls without MS. Subjects were included in the present study when satisfying the following criteria: (1) lack of diabetes evidenced by at least two determinations of fasting glucose levels; (2) normal thyroid function evaluated by plasma levels of thyroid-stimulating hormone and clinical examination of the thyroid gland; (3) normal renal function evaluated by plasma levels of urea, creatinine and proteinuria; (4) normal hepatic function evaluated by biochemical hepatic parameters and absence of hepatomegalia confirmed by clinical examination; and (5) lack of personal history of cardiovascular disease. Special care was taken to avoid including subjects with additional causes of dyslipidemia such as excessive tobacco (>10 cigarettes/day) or ethanol consumption (>30 g/day), therapy with drugs that could affect lipoprotein or carbohydrate metabolism and familial history of diabetes mellitus. Individuals presenting infectious processes or under acute stressing situations were not included. Informed consent was obtained from all participants and the protocol was approved by the Ethical Committees from Hospital de Clínicas José de San Martín, University of Buenos Aires and from Institute of Cardiology and Cardiovascular Surgery of the Favaloro Foundation, Buenos Aires, Argentina.

2.2. Study protocol and samples

After a 12-h overnight fast, venous blood was drawn from the antecubital vein. Aliquots were collected either in clean and $EDTANa_2$-containing tubes. Samples were centrifuged at $1500 \times g$, for 15 min, at 4 °C, and serum was stored at 4 °C and used within 24 h for glucose, lipid profile and lipoprotein isolation by ultracentrifugation. Serum aliquots were also stored at −70 °C for determination of insulin, adiponectin, CETP, PON1, arylesterase (ARE), Lp-PLA_2, VCAM-1, ICAM-1, and E-selectin. Whole blood was stored at 4 °C and employed for leukocyte cell adhesion molecule determination by flow cytometry.

2.3. Analytical procedures

Glucose, triglycerides and total cholesterol were quantified by standardized methods (Roche Diagnostics, Mannheim, Germany) in a Hitachi 727 autoanalyzer. Within-run precision (CV) for triglycerides and total cholesterol were 1.3% and 1.1%, respectively. Between-day precision (CV) were 2.4% and 1.5%, respectively. Laboratory bias was 1.1% and −1.7%, respectively. Low density lipoprotein-cholesterol (LDL-C) level was determined as the difference between total cholesterol and the cholesterol contained in the supernatant obtained after selective precipitation of LDL with 10 g/l polyvinylsulphate in polyetilenglycol (M.W. 600; 2.5% w/v; pH 6.7) [11]. Within-run and between-day pre-

cision (CV) were 4.7% and 5.0%, respectively. HDL was isolated in the supernatant obtained following precipitation of apolipoprotein (apo) B-containing lipoproteins with 0.44 mmol/l phosphotungstic acid in the presence of magnesium ions [12]. Within-run and between-day precision (CV) for HDL-C were 3.2% and 3.8%, respectively. Laboratory bias was −2.0%. Very low density lipoprotein-cholesterol (VLDL-C) was calculated as the difference between the cholesterol contained in the supernatants obtained for LDL and HDL measurements. Quality control was performed by RIQA program (Ireland). In the isolated HDL fraction, triglycerides and cholesterol were measured by the previously mentioned methods, phospholipids by the Bartlett method [13], and proteins by the Lowry method [14]. Total CV for phospholipid determination was 3.1%. Within-run and between-day precision (CV) for protein measurement were 3.0% and 4.2%, respectively. Lipoprotein mass was estimated as the summatory of all components (triglycerides, cholesterol, phospholipids and total proteins) and then the percentage was calculated. Apo B and apo A-I were evaluated by immunoturbidimetry (Roche Diagnostics Mannheim, Germany). Within-run and between-day precision (CV) were 1.2% and 2.1% for apo B, and 1.9% and 2.4% for apo A-I, respectively. The following ratios were calculated: total cholesterol/HDL-C, LDL-C/HDL-C, triglycerides/HDL-C, VLDL-C/triglycerides, and LDL-C/apo B.

2.4. Lipoprotein isolation

HDL (d: 1.063–1.210 g/ml) were isolated by sequential preparative ultracentrifugation [15] in a XL-90 Beckman ultracentrifuge, with a type 90 Ti rotor. The run was performed at 105,000 × *g*, for 18 h, at 4 °C. HDL purity was tested by agarose gel electrophoresis [16] and albumin content tested by inmunoturbidimetry (Roche Diagnostics, Mannheim, Germany). Albumin content was in no case higher than 2% of total protein.

2.5. CETP activity

CETP activity was determined in serum samples following the general procedure previously described with few modifications [17]. Briefly, the ability of serum to promote the transfer of tritiated cholesteryl esters from a tracer amount of biosynthetically labeled HDL_3 (^{3}H-CE-HDL_3) (NEN Life Science Products, Boston, USA) towards serum apo B-containing lipoproteins was evaluated. Samples were incubated with ^{3}H-CE-HDL_3 (50 μmol/l cholesterol) and 1.5 mmol/l iodoacetate for 3 h, at 37 °C. After incubations, lipoproteins were separated by selective precipitation method employing 0.44 mmol/l phosphotungstic acid in the presence of magnesium ions [12]. Radioactivity was measured in the incubation mixture and in the supernatant containing the HDL fraction in a liquid scintillation analyzer (Packard 210TR; Packard Instruments, Meridian, CT). Results were expressed as percentage of ^{3}H-cholesteryl esters transferred from HDL_3 to apo B-containing lipoproteins, per ml, per hour. Measurements were all carried out in duplicate within the same assay. Within-run precision (CV) was 4.9%.

2.6. Paraoxonase/arylesterase activity

The enzyme PON was evaluated employing two different substrates: paraoxon (Sigma Chemical Co.; PON 1 activity) and phenylacetate (Sigma Chemical Co.; ARE activity). Both activities were measured in serum samples following the method of Furlong et al. [18].

PON 1 activity was assessed by adding serum samples (20 μl) to 2 ml Tris/HCl buffer (100 mmol/l, pH 8.0) containing 2 mmol/l $CaCl_2$, 2.6 mmol/l paraoxon (*O*,*O*-diethyl-*O*-*p*-nitrophenylphosphate) and 1.0 mol/l NaCl. The rate of generation of *p*-nitrophenol was determined at 405 nm and 25 °C, in a Hitachi U-1100 spectrophotometer. Increases in the absorbance were recorded at 45 s intervals during 5 min, after 30 s of initial pre-incubation. Enzymatic activity was calculated from the molar extinction coefficient (17,000 $mol^{-1}\,l\,cm^{-1}$) and results were expressed as nmol/ml min. Measurements were all carried out within the same assay. Within-run precision (CV) was 5.5%.

ARE activity was measured by adding serum samples (20 μl of 1/20 dilution in distilled water) to 2 ml Tris/acetate buffer (50 mmol/l, pH 7.8) containing 20 mmol/l $CaCl_2$ and 4.4 mmol/l phenylacetate. The rate of generation of phenol was determined at 270 nm and 25 °C, in a Hitachi U-1100 spectrophotometer. Increases in the absorbance were recorded at 45 s intervals during 5 min, after 30 s of initial pre-incubation. Blanks were included to correct for the spontaneous hydrolysis of phenylacetate. Enzymatic activity was calculated from the molar extinction coefficient (1310 $mol^{-1}\,l\,cm^{-1}$) and results were expressed as μmol/ml min. Measurements were all carried out within the same assay. Within-run precision (CV) was 4.8%.

2.7. Lipoprotein-associated phospholipase A_2

Lp-PLA_2 activity was measured following the radiometric assay described by Blank et al. [19] with few modifications. The separation of the released radiolabeled acetate from the lipid substrate was carried out by phase–phase partitioning and measurement of the radioactivity in the aqueous phase. Briefly, each incubation mixture contained 50 μl of 1/50 diluted serum and 10 μmol/l 1-hexadecyl-2-[3H]acetyl-glicero-3-phosphocholine (specific activity = 25 μCi/μmol) in a total volume of 0.5 ml of phosphate-buffered saline (pH 7.4). The tritiated substrate 1-hexadecyl-2-[3H] acetyl-glicero-3-phosphocholine (13.5 Ci/mmol) was obtained from New England Nucleotides, and the non-tritiated one was obtained from Cayman Chemical. Once the substrates were mixed, the solvents were evaporated under a stream of nitrogen and redisolved in phosphate-buffered saline. There was a sonication step consisting of one cycle of 5 min. Incubation was carried out for 5 min at 37 °C and the enzymatic reaction

was stopped by placing the tubes in an ice bath and by the addition of 1.5 ml of chloroform. Then, 0.5 ml of saturated sodium bicarbonate solution were added. After centrifugation, the aqueous phase was washed twice with 1.5 ml of chloroform. The radioactivity of the aqueous phase of each sample and sample-blanks was measured by liquid scintillation using a Liquid Scintillation Analyzer (Packard 210TR; Packard Instruments, Meridian, CT). Radioactivity of the substrate–buffer was also measured. Results were expressed as μmol/ml h. Measurements were all carried out within the same assay. Within-run precision (CV) for Lp-PLA_2 activity was 5.1%.

2.8. Insulin, adiponectin and soluble cell adhesion molecules

Insulin concentration was measured by microparticle enzyme immunoassay (MEIA) (ABBOTT, Japan). Results were expressed as mU/l. Homeostasis model assessment (HOMA) was calculated by [glucose (mmol l^{-1})·insulin (μU ml^{-1})]/22.5 and quantitative sensitivity check index (QUICKI) by 1/[ln glucose (mmol l^{-1})+ln insulin (mU l^{-1})]. Adiponectin levels were measured by monoclonal antibody-based enzyme-linked immunosorbent assay (ELISA) (R&D Systems, USA). Results were expressed as ng/ml. Levels of VCAM-1, ICAM-1 and E-selectin were determined by monoclonal antibody-based enzyme-linked immunosorbent assay (ELISA) (R&D Systems, USA). Results were expressed as ng/ml.

2.9. Leukocyte cell adhesion molecules

Expression of adhesion molecules on monocytes, polymorphonuclear cells, and lymphocytes was measured by flow cytometry (FACS Sort, Becton-Dickinson, San José, CA) [20]. Blood samples were anticoagulated with EDTA. Staining was performed in whole blood with commercial fluorescein-isothiocyanate (FITC)-conjugated anti-CD54 and anti-CD18, and phycoerytrin (PE)-conjugated anti-CD49d monoclonal antibodies (MAbs, BD Biosciences Pharmingen, Ontario, Canada). Whole blood was incubated with saturating concentrations of conjugated MAbs for 30 min at room temperature. Erythrocytes were lysed with commercially available solution (FACS Lysing Solution, BD Biosciences Pharmingen, Ontario, Canada). The cells were then washed with phosphate-buffered saline and immediately subjected to flow cytometry equipped with a 488 nm argon laser. To analyze monocytes, polymorphonuclear cells, and lymphocytes, a gate was defined by forward (FSC) and right angle light scattering (SSC), using the Cell Quest™ software (Becton-Dickinson, San Jose, CA). Moreover, monocyte identity was confirmed by employing FITC or PE-conjugated anti CD14. The fluorescence intensity of 10,000 events was recorded as the mean channel number over a logarithmic scale of 1–1026 channels. Results were expressed as the mean channel of fluorescence intensity. Fluorescent-conjugated isotype control antibodies were used to detect non-specific binding to cells.

2.10. Data and statistical analysis

Data are presented as the mean ± standard deviation. When data followed the normal distribution, the Student test (*T*-test) was used to compare the different groups, while the Mann–Whitney test (*U*-test) was employed for data that did not follow the normal distribution. The Chi-square test was used to compare proportions of subjects with and without hypertension. Correlations between all variables were carried out by Pearson or Spearman tests. Differences were considered significant at $p < 0.05$ in the bilateral situation.

3. Results

Clinical and general biochemical characteristics of control subjects and patients with MS are shown in Table 1. There were no significant differences in age between both groups of studied subjects. As it was expected, patients presented significantly higher body mass index (BMI), waist circumference, proportion of subjects with hypertension, glucose levels, insulin concentration, and HOMA, while QUICKI was lower than in the control group. In addition, adiponectin levels were significantly reduced in patients with MS in comparison with control subjects (4551 ± 2302 ng/ml *vs.* 5865 ± 2548 ng/ml, respectively; $p < 0.05$).

Lipid and lipoprotein profile is summarized in Table 2. As expected, patients exhibited an atherogenic dyslipidemia typical of the MS, which consisted of moderately high triglyceride levels, due to an increase in VLDL fraction, and a reduction in HDL-C concentration. From the above mentioned parameters, different indexes were calculated. Those indicating atherogenic risk, total cholesterol/HDL-C and LDL-C/HDL-C, as well as triglycerides/HDL-C, an indicator of insulin resistance, were significantly higher in patients than in controls. VLDL-C/triglycerides ratio, an estimator

Table 1
General characteristics from male control subjects and patients with metabolic syndrome

	Control subjects	Patients with MS
N	56	31
Age (years)	43 ± 13	48 ± 9
BMI (Kg/m^2)	26(21–30)	29(23–30)[a]
Waist (cm)	93 ± 7	105 ± 7[a]
Hypertension (% of subjects)	21	77[a]
Glucose (mg/dl)	94 ± 8	101 ± 10[b]
Insulin (mU/l)	7.2(2.6–17.5)	11.8(5.3–23.2)[c]
HOMA	1.9 ± 1.0	3.0 ± 1.2[a]
QUICKI	0.28 ± 0.04	0.24 ± 0.03[c]

MS, metabolic syndrome; BMI, body mass index; HOMA, homeostasis model assessment; QUICKI, quantitative sensitivity check index. Results are expressed as mean ± S.D., except for BMI and insulin which were expressed as median(range). [a]$p < 0.0001$; [b]$p < 0.001$; [c]$p < 0.0005$ *vs.* control subjects.

Table 2
Lipid and lipoprotein profile from male control subjects and patients with metabolic syndrome (mean ± SD)

	Control subjects ($n=56$)	Patients with MS ($n=31$)
TG (mg/dl)	164 ± 104	260 ± 125[a]
TC (mg/dl)	233 ± 48	228 ± 42
VLDL-C (mg/dl)	28 ± 16	40 ± 19[b]
LDL-C (mg/dl)	159 ± 42	146 ± 48
HDL-C (mg/dl)	45 ± 9	33 ± 7[c]
Apo B (mg/dl)	115 ± 28	113 ± 29
Apo A-I (mg/dl)	132 ± 23	125 ± 16
TC/HDL-C	5.3 ± 1.5	6.7 ± 1.3[c]
LDL-C/HDL-C	3.7 ± 1.2	4.6 ± 1.1[d]
TG/HDL-C	4.2 ± 3.4	9.3 ± 8.6[a]
VLDL-C/TG	0.18 ± 0.05	0.15 ± 0.03[e]
LDL-C/Apo B	1.42 ± 0.19	1.32 ± 0.24[f]

MS, metabolic syndrome; TG, triglycerides; TC, total cholesterol; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; apo, apolipoprotein. [a]$p<0.0005$; [b]$p<0.005$; [c]$p<0.0001$;[d]$p<0.001$; [e]$p<0.001$; [f]$p<0.05$ *vs.* control subjects.

of VLDL composition, was lower in the patient group, thus indicating the presence of larger VLDL particles with higher triglyceride content than controls. We also calculated LDL-C/apo B ratio in order to estimate the proportion of small and dense LDL particles and this ratio was also significantly lower in patients with MS.

Table 3 shows lipoprotein-associated proteins and enzymes in control subjects and patients with MS. The patient group showed significantly higher CETP and lower Lp-PLA$_2$ activities. The latter was positively correlated with QUICKI ($r=0.23$, $p<0.05$) and negatively with VCAM-1 ($r=-0.29$, $p<0.01$) and lymphocyte CD18 ($r=-0.33$, $p<0.005$). No statistically significant differences were detected in PON1 or ARE activities (Table 3). The evaluation of lipoprotein composition evidenced the presence of triglyceride-enriched and phospholipid-depleted HDL particles (Fig. 1).

Cell adhesion molecules were evaluated both in plasma and in circulating leukocytes. Regarding the soluble fractions, only VCAM-1 levels were significantly increased in patients in comparison with controls, being ICAM-1 and E-selectin similar in both groups (Fig. 2). Measurement of leukocyte cell adhesion molecules showed an elevation in monocyte CD54 and in lymphocyte CD49d (Fig. 3).

Table 4 lists correlations obtained between the number of MS components present in the studied subjects and different clinical and biochemical parameters, not including those employed for MS diagnosis. The best positive correlation was noted with HOMA, while the best negative one was observed with QUICKI.

Table 3
Lipoprotein-associated proteins and enzymes in male control subjects and patients with metabolic syndrome (mean ± S.D.)

	Control subjects ($n=56$)	Patients with MS ($n=31$)
CETP (%/ml h)	262 ± 27	279 ± 21[a]
PON1 (nmol/ml min)	322 ± 176	347 ± 216
ARE (μmol/ml min)	133 ± 24	134 ± 28
Lp-PL A$_2$ (μmol/ml h)	7.3 ± 2.2	6.5 ± 1.9[b]

MS, metabolic syndrome; CETP, cholesteryl ester transfer protein; PON1, paraoxoanse1; ARE, arylesterase; Lp-PLA$_2$, lipoprotein-associated phospholipase A$_2$. [a]$p<0.01$; [b]$p<0.05$ *vs.* control subjects.

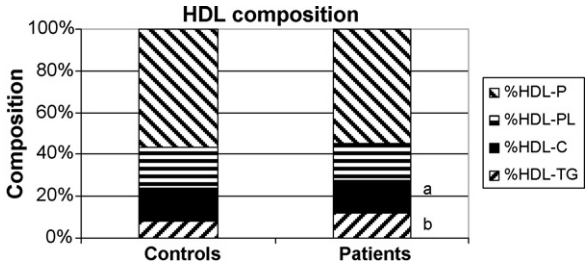

Fig. 1. HDL chemical composition from male control subjects ($n=56$) and patients with metabolic syndrome ($n=31$). HDL, high density lipoprotein; P, proteins; PL, phospholipids; C, cholesterol; TG, triglycerides. [a]$p<0.05$; [b]$p<0.01$, *vs.* control subjects.

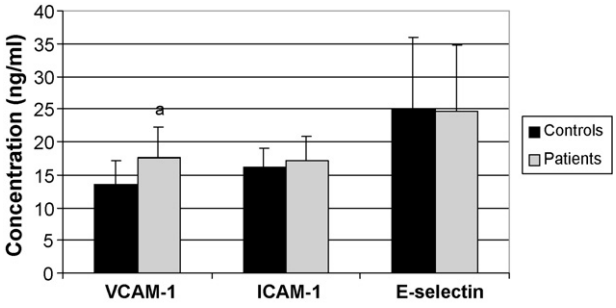

Fig. 2. Plasma soluble cell adhesion molecules (VCAM-1, ICAM-1 and E-selectin) from male control subjects ($n=56$) and patients with metabolic syndrome ($n=31$) (mean ± S.D.). VCAM-1, vascular cell adhesion molecule 1; ICAM-1, intercellular adhesion molecule 1. [a]$p<0.0001$ *vs.* control subjects.

Table 4
Correlations between the number of metabolic syndrome components present in the studied subjects and different clinical and biochemical parameters in male control subjects and patients with metabolic syndrome ($n=87$)

	Number of metabolic syndrome components	
	r	$p<$
BMI	0.52	0.0001
Insulin	0.47	0.0001
HOMA	0.53	0.0001
QUICKI	−0.55	0.0001
Adiponectin	−0.22	0.05
VLDL-C	0.33	0.0001
Apo A-I	−0.30	0.05
CETP	0.34	0.0005
VCAM-1	0.36	0.001
ICAM-1	0.20	0.05

BMI, body mass index; HOMA, homeostasis model assessment; QUICKI, quantitative sensitivity check index; VLDL, very low density lipoprotein; apo, apolipoprotein; CETP, cholesteryl ester transfer protein; VCAM-1, vascular cell adhesion molecule 1; ICAM-1, intercellular adhesion molecule 1.

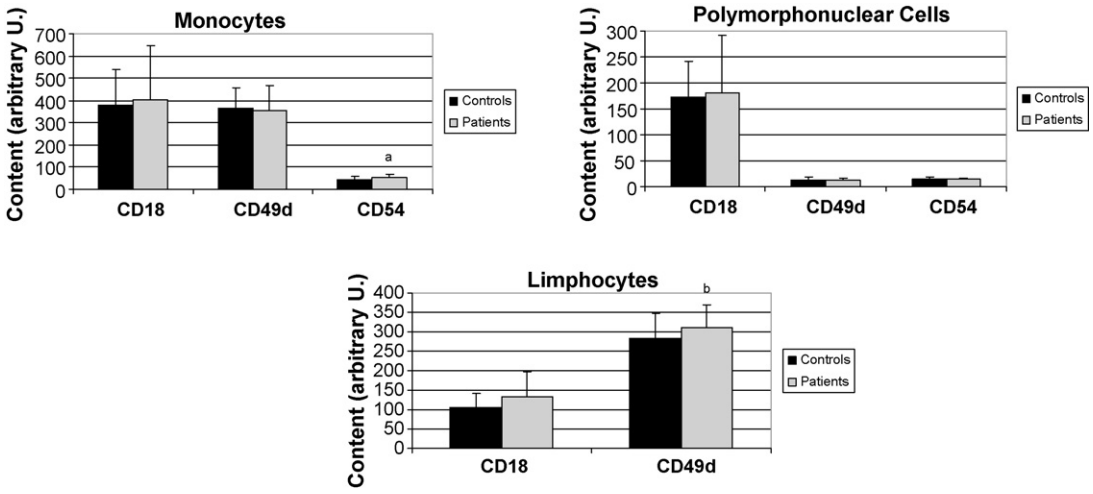

Fig. 3. Cell adhesion molecule content (CD18, CD49d, CD54) in monocytes, polymorphonuclear cells and lymphocytes from male control subjects ($n = 56$) and patients with metabolic syndrome ($n = 31$) measured by flow cytometry (mean ± S.D.). [a] $p < 0.005$; [b] $p < 0.05$ *vs.* control subjects.

4. Discussion

The most relevant finding of the present study is the increment observed in some leukocitary and endothelial cell adhesion molecules in MS patients, which is here described for the first time and evidences a process of cellular activation, crucial for leukocyte interaction with the endothelium and later migration into the artery wall. These inflammatory and atherogenic alterations were associated to: (a) hypoadiponectinemia, (b) an atherogenic lipid and lipoprotein profile, (c) altered HDL chemical composition accompanied by higher cholesteryl ester-triglyceride interchange carried out by CETP, and (d) diminished Lp-PLA_2 activity, antioxidant enzyme related with LDL oxidation. Therefore, patients with MS presented several biochemical disturbances that reflect the involvement of different tissues and metabolic pathways, thus conditioning a state of increased cardiovascular risk.

It is obvious that patients with MS are heterogeneous as regards the presence of individual disorders. Nevertheless, insulin resistance seems to be a common disturbance in all of them, which was here evidenced by altered fasting insulin levels, HOMA, QUICKI and triglycerides/HDL-C ratio. The latter has been proposed as an interesting marker to identify insulin-resistant individuals at high risk of cardiovascular disease [21]. Many investigators believe that insulin resistance plays a key pathogenic role in MS. Moreover, the convergence between insulin resistance and inflammation in atherogenesis has been recognized over the past decade [22,23], but the exact mechanism by which excessive adiposity causes both insulin resistance and vascular dysfunction is not completely understood. Much of the recent work on obesity has highlighted the crucial role of adipose tissue as an endocrine organ secreting a large number of active substances [24]. Among them, adiponectin was pointed out as the only adipocytokine with antiatherogenic and antiinflammatory activities and direct effect on the endothelium and on foam cell formation [25]. In accordance with previous reports carried out in different populations [24,26,27], adiponectin was significantly reduced in patients with MS in comparison with control subjects evaluated in the present study. Furthermore, this constitutes an interesting finding given that it has been suggested that hypoadiponectinemia would be not only a feature of MS, but it could also play a role in the physiopathology of MS [28,29].

As it was expected, patients with MS showed increased triglyceride levels, due to the accumulation of VLDL particles, and reduced HDL-C concentration. As inferred from VLDL-C/triglyceride ratio, VLDL from patients would be larger and triglyceride-enriched than from controls, which is characteristic of the insulin-resistant state [30]. Impaired suppression of circulating free fatty acid during hyperinsulinemia in subjects with MS has been proposed to contribute to enhanced VLDL synthesis in the liver and thus hypertriglyceridemia [31]. Apart from the quantitative alteration which affected HDL fraction form MS patients, these particles were triglyceride-enriched, qualitative modification known to cause an impairment in HDL capacity to promote reverse cholesterol transport and to inhibit LDL oxidation [32,33]. The increase in HDL triglyceride content could be attributed to the enhanced CETP activity observed in our and other studies [34]. Furthermore, patients with MS also showed significantly higher values of atherogenic indexes. Moreover, in the patient group, LDL-C/apo B ratio was significantly reduced, thus suggesting an increment in the proportion of the highly atherogenic small and dense LDL particles [35]. Interestingly, McLaughlin et al. [36] have recently suggested that the above mentioned triglycerides/HDL-C ratio could be employed not only as a predictor of insulin resistance, but also of the proportion of small and dense LDL particles. Accordingly, several studies proposed the increase in this LDL subpopulation as part of the atherogenic phenotype typical of MS [37].

PON1, an antioxidant enzyme exclusively bound to HDL fraction, was here evaluated by employing two different substrates [38]. In no case was the enzyme activity statistically different between both groups, though different studies showed lower PON1 activity and/or mass in patients with MS than in control subjects [39,40]. Regarding another enzyme with potential antioxidant capacity, Lp-PLA_2 was found to be significantly lower in patients with MS. This constitutes a really controversial topic for different reasons. First, while some authors accept the antioxidant role played by this enzyme, others postulate that Lp-PLA_2 exerts a crucial function in LDL oxidation and foam cell formation [41]. Regarding, the relationship between Lp-PLA_2 and MS, Persson et al. [42] showed significantly higher activity and mass in patients than in controls. Moreover, Rizos et al. [43] also found increased Lp-PLA_2 activity in MS patients, though when they selectively measured the enzyme activity in HDL fraction, this resulted to be reduced in patients in comparison with control subjects. Our results suggest an impairment in Lp-PLA_2 antioxidant capacity in the patient group. This conclusion was reinforced by a direct association detected between Lp-PLA_2 and QUICKI, an indicator of insulin sensibility, and by an inverse correlation found between Lp-PLA_2 and both VCAM-1 plasma levels and lymphocyte CD18 content, known markers of inflammation and atherosclerosis.

Cell adhesion molecules were evaluated both as the soluble form, which mainly derives from the endothelium, and associated to circulating leukocytes. Patients with MS presented significantly higher VCAM-1 plasma levels, CD49d in lymphocytes and CD54 in monocytes. Among the wide variety of cell–cell interactions which characterize the chronic inflammatory process underlying atherosclerosis, endothelial cells may interact with leukocytes through the contact of VCAM-1 with the integrin VLA-4 made up of CD29/CD49d, both of which were increased in MS. On the other hand, leukocytes may interact among them through the contact of CD54 with the integrin Mac-1 made up of CD11b/CD18. The increase in cell adhesion molecules coming from the endothelium and those located on leukocyte surface would reflect the activated state of both cell types. The association of adhesion molecules with MS is logical because these molecules have close interaction with proinflammatory cytokines known to be increased in MS, such as interleukin-1ß, tumor necrosis factor (TNF)-α, and C reactive protein produced in the liver in response to interleukin-6 [44]. Moreover, it has been shown that adiponectin inhibits the expression of VCAM-1, ICAM-1 and E-selectin [45], thus hypoadiponectinemia, as found in the present study, could be related to higher cell adhesion molecule expression.

Several issues have been published evaluating the relationship of soluble cell adhesion molecule levels with the presence of cardiovascular disease or with different isolated measures of MS and obesity [46]. Moreover, soluble cell adhesion molecules have been also studied in different populations with type 2 diabetes and control subjects. Nevertheless, to our knowledge, very scarce studies have compared soluble cell adhesion molecule concentrations between MS patients without diabetes or cardiovascular disease and male control subjects in a previously designed cross-sectional study. In fact, Marques-Vidal et al. [47] showed higher VCAM-1 and ICAM-1 levels in men with insulin resistance syndrome defined by HOMA, but the authors did not classify their patients employing any of the available criteria for MS diagnoses. In another study carried out in men and women with symptomatic coronary artery disease and MS [48], the authors were not able to find an independent association between cell adhesion molecule levels and MS. Salmenniemi et al. [49] studied a group of offprints of diabetic probands, applied factor analysis to stratify MS carriers and found higher soluble cell adhesion molecule levels. Finally, Sjögren et al. [50] evaluated a group of elderly men (62–64 years old) and the authors reported an increased endothelial activation score only when comparing MS patients with subjects who presented none MS component.

Interestingly, Arteaga et al. [51] evaluated the presence of vesicular fragments of the endothelial cell membrane released during activation or apoptosis, which form conjugates with circulating leukocytes, and detected that some of them were increased in patients with MS. The authors also reported higher CD11b content in leukocytes from the patient group. To our knowledge, the latter and the present study are unique in evaluating cell adhesion molecules on leukocyte surface from patients with MS, though in both studies different CDs were measured. In diabetes, different results have been reported. Monocyte and neutrophil CD11b from type 2 diabetic patients were found either increased, conserved or reduced in comparison with control subjects [52,53]. In type 1 diabetic patients, CD11b in polymorphonuclear cells was found to be increased [54]. Regarding CD18, its expression in monocytes and polymorphonuclear cells was elevated in most studies performed in diabetic subjects [53,55]. On the other hand, CD54 was unchanged in monocytes and neutrophils from type 2 diabetic patients [56].

We also aimed to determine whether there was a progressive relation between the number of MS components present in the studied subjects and the degree of abnormality of classic and emergent risk factors for cardiovascular disease. Positive and significant associations were detected with BMI, insulin, HOMA, VLDL-C, CETP, VCAM-1, and ICAM-1. On the other hand, negative and significant correlations were found with QUICKI, adiponectin, and apo A-I. Among them, the best positive correlation was noted with HOMA, while the best negative one was observed with QUICKI. Similar results were reported by Solymoss et al. [57], thus confirming that as the number of features of the MS increases, so does the severity of atherogenic markers. Regarding the relationship with cell adhesion molecules, Salmenniemi et al. [49] also found a positive association between the MS score and ICAM-1, but not with VCAM-1.

In conclusion, our findings add worthy information for the understanding of metabolic abnormalities in the MS and the

interactions among them, which clearly define a clinical status with high propensity to type 2 diabetes and atherosclerotic cardiovascular disease.

Acknowledgements

Leonardo Gómez Rosso and María Belén Benítez are research fellows from Ministerio de Salud de la Nación (Ramón Carrillo-Arturo Oñativia Fellowship) and Agencia Nacional de Promoción Científica y Tecnológica, respectively. This work was supported in part by grants from Agencia Nacional de Promoción Científica y Tecnológica (PICT 14299), CONICET (PIP 6111) and University of Buenos Aires (UBACYT B069).

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