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Increased plasma activity of metalloproteinase 2 in women with metabolic syndrome

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Abstract

Metalloproteinases (MMPs) play a significant role in vascular remodeling, and they have been suspected to be partly responsible for the pathogenesis of cardiovascular disease. Metalloproteinases have been reported to be increased in atherosclerosis and type 2 diabetes mellitus; however, so far they have not been evaluated in metabolic syndrome (MetS). Plasma activity of MMP-2 and MMP-9, high-sensitivity C-reactive protein concentration, dense low-density lipoprotein, and insulin-resistance markers were measured in 38 nondiabetic women with (n = 19) and without (n = 19) MetS. Women with MetS had significantly higher plasma activity of MMP-2 than controls (median [range], 1.3 [0.4-3.1] vs 0.7 [0.1-1.9]; $P = .001$). MMP-2 activity positively correlated with waist, homeostasis model assessment, and high-sensitivity C-reactive protein ($P < .02$) as well as with apolipoprotein B, dense low-density lipoprotein, triglycerides/high-density lipoprotein cholesterol index ($P < .001$) and negatively with high-density lipoprotein cholesterol ($P < .002$). Our finding of increased plasma activity of MMP-2 in women with MetS is important because they fit in with an early stage of cardiovascular disease; and measurement of soluble molecules may improve the risk assessment, early diagnosis, and prognosis of cardiovascular disease.

1. Introduction

Metabolic syndrome (MetS) represents higher risk of cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM). Type 2 diabetes mellitus, MetS, and prediabetes are characterized by alterations of the arterial vasculature, capillary basal membrane, and extracellular matrix, which are integrally involved with profound cardiovascular and microvascular complications. Extracellular matrix is under the control of matrix metalloproteinases (MMPs) [1]. MMPs, especially MMP-2 and MMP-9, play a significant role in vascular remodeling; and they have been suspected to be partly responsible for the pathogenesis of CVD [1]. Increased MMPs activity has been reported in numerous diseases including atherosclerosis [2], CVD [1,2], and recently in T2DM [3]. These situations are characterized by an increased inflammatory condition, which can be evaluated through the measurement of high-sensitivity C-reactive protein (hs-CRP). Besides, transcriptional regulation and activation of MMPs are controlled by different proinflammatory molecules, which are usually increased in MetS.

By other side, in patients with coronary disease, MMP-9 levels have been reported to be associated with an atherogenic lipoprotein profile [4]; however, no correlation between MMPs and lipoprotein subclasses such as small dense low-density lipoprotein (LDL) has been reported yet.

To our knowledge, only one publication reported MMPs levels in MetS [5], and no correlations were found with the MetS components.

Our objective was to evaluate plasma MMP-2 and MMP-9 activity in a group of women with MetS, excluding T2DM, and their correlation with other characteristics markers of this syndrome, as early indicators of extracellular matrix remodeling.

2. Subjects and methods

For this preliminary study, 60 women were consecutively recruited and clinically evaluated at the Gynecology Division-Clinical Hospital, University of Buenos Aires

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Table 1
Demographic and metabolic characteristics of women with MetS and those without MetS

	MetS (n = 19)	Non-MetS (n = 19)	*P*
Age (y)	55 ± 5	47 ± 15	.060
BMI (kg/m^2)	29.6 ± 3.1	23.8 ± 3.7	.001
Postmenopause (yes/no)[a]	16/3	13/6	.001
Waist (cm)	97.4 ± 7.7	83.2 ± 12.5	.001
Pressure (yes/no)[a]	11/5	2/17	.001
Fasting glucose (mg/dL)	107 ± 13	90 ± 7	.001
Triglycerides (mg/dL)	190 ± 68	95 ± 50	.001
HDL cholesterol (mg/dL)	50 ± 12	65 ± 18	.010
Apolipoprotein B (mg/dL)	140 ± 32	90 ± 29	.001
Triglycerides/HDL cholesterol	4.0 ± 1.9	1.7 ± 1.5	.001
HOMA	3.4 ±1.6	2.2 ± 0.8	.138

Values are expressed as mean ± SD; Student *t* test. HOMA indicates homeostasis model assessment.
[a] χ^2 Test.

(Buenos Aires, Argentina). Individuals younger than 25 or older than 60 years; those with diabetes, CVD, neoplasia, renal disorders; and those with a history of taking antidiabetic, antihypertensive, or lipid-lowering agents as well as hormonal treatment were excluded. Thirty-eight women were finally enrolled for the study: 19 with MetS, according to Adult Treatment Panel III definition [6], and 19 without MetS, who presented up to one component of MetS, as the control group.

Written informed consent was obtained from each subject and was approved by the Ethic Committee of the Faculty of Pharmacy and Biochemistry, University of Buenos Aires.

After 12-hour overnight fast, blood samples were drawn. Cholesterol, triglycerides, and glucose were measured by enzymatic kits, and hs-CRP and apolipoprotein B by immunoturbidimetry (Roche Diagnostics, Mannheim, Germany) in a HITACHI 917 autoanalyzer. High-density lipoprotein (HDL) and LDL cholesterol were determined by precipitation methods. Insulin was evaluated by MEIA (IMX System, Abbott Lab, North Chicago, IL). To estimate insulin resistance, triglycerides/HDL cholesterol and homeostasis model assessment indexes were calculated.

Total LDL (density, 1.019-1.063 g/mL) and small dense LDL (dLDL, 1.048-1.063 g/mL) isolation was simultaneously performed by sequential ultracentrifugation. Cholesterol was assessed in total LDL and in dLDL fractions, and the last one was expressed as a percentage of total LDL [7].

MMPs activity was measured in plasma by zymography [8]. MMP-9 (84 kd [active form]) MMP-2 (72 kd [proform] and 67 kd [active form]) were identified by molecular weight. Conditioned media from the promyelocyte U-937 cell line was used as activity standard. Coefficients of variation were 4.8% (intraassay) and 8.6% (interassay). Band intensities were quantified using Scion-Image J software (Scion Corporation, Frederick, MD), and relative activity was expressed as a ratio to the internal standard.

Differences between groups were tested using the unpaired Student *t* test, χ^2 test, and Mann-Whitney *U* test according to parameter distribution. Correlations between variables were assessed by the Pearson or Spearman correlation tests. Multivariate stepwise regression model was developed to evaluate the effect of age and menopausal status on MMPs activities. Differences were considered significant at *P* less than 5%.

3. Results

Overall characteristics of all the women studied are described in Table 1. Pro-MMP-2 activity, dLDL, and hs-CRP were significantly higher in patients than in controls (Fig. 1). MMP-9 was detected only in 3 patients and 1 control; thus, no statistical analysis was able to be performed. Table 2 illustrates the bivariate assessment of association between pro–MMP-2 and covariates of interest for the whole

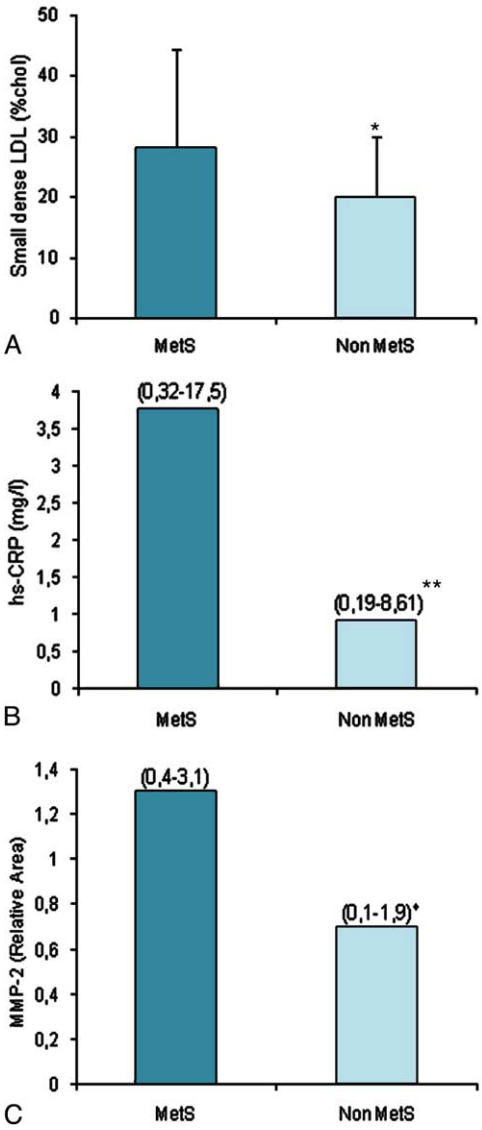

Fig. 1. Small dLDL proportions (A), hs-CRP (B), and MMP-2 (C) in women with MetS and those without MetS. Data are expressed as mean ± SD (A) and median (range) (B and C). *$P < .05$; **$P < .008$; ♦$P < .001$.

Table 2
Bivariate assessments of association between MMP-2 and different MetS characteristics expressed as Spearman correlation

	r (P)
Waist	0.40 (.02)
Triglycerides	0.67 (.001)
LDL cholesterol	0.47 (.005)
Apolipoprotein B	0.47 (.006)
Small dLDL	0.68 (.001)
hs-CRP	0.42 (.01)
Triglycerides /HDL cholesterol	0.68 (.001)
HOMA	0.53 (.02)
HDL cholesterol	−0.51 (.002)

population. When multivariate stepwise regression model was developed to assess the effect of age and menopausal status on MMPs activities, the difference observed in MMP2 activity between women with and without MetS was preserved (F, 6.51; $P = .016$).

4. Discussion

The most novel finding of this preliminary study is that women with MetS presented higher plasma activity of MMP-2, which correlated with the most common markers of MetS and cardiovascular risk factors. Although MMP-2 activity has been reported to be increased in CVD [9], controversial results have been published in reference to MMP-2 expression under high glucose conditions [3,10,11]. Moreover, to date, no data about circulating MMP-2 activity have been described in women with MetS.

In the vasculature, MMP-2 is synthesized mainly by smooth muscle cells, in the presence of different stimulus [11], such as systemic and local inflammation, oxidative stress, or cytokines that are present in the MetS. The positive correlations observed between MMP-2 with different insulin-resistance and inflammatory markers suggest an effect of MetS, as one, on the synthesis and secretion of this metalloproteinase. It is noteworthy that results recently published [12] showed that CRP enhances the endothelial expression of MMPs confirming the connection between these biomarkers. Furthermore, the positive correlation obtained with dLDL would reflect the impact of this modified lipoprotein on the vascular wall.

Although the most prominent form of MMP-2 detected in this study was pro–MMP-2, this fact should not be disregarded, given that these forms are stable in circulation, and could be reflecting the increase of its synthesis and activation in the subendothelium.

In our study, the lack of MMP-9 detection could be attributed to the fact that this MMP is reported to be associated mainly to the plaque rupture in advanced lesions [13]. On the other hand, the increased MMP-2 activity would be associated with the first steps of the atherogenic process, mainly related to the vascular smooth muscle cell migration and intimal thickening [2]. The higher MMP-2 activity could be responsible of a greater matrix degradation of type IV collagen within the basement membrane, accelerating the underlying atherosclerotic process within the arterial vessel wall.

Our results differ from those of Cicero et al [5], who reported increased MMP-9 concentration without changes in MMP-2 in patients with MetS, without correlations between MMPs and MetS components. These controversies could be attributed to the fact that they evaluated MMP concentration by immune-assay methods, which does not necessarily reflect its augmented enzymatic activity. Given that activity and concentration should be correlated, further studies are necessary.

Our study has some limitations: the low number of patients, although it must be considered the strict inclusion criteria. Current assays do not allow us to determine the origin of the measured MMPs, so we cannot rule out that inflammation of adipose tissue, characteristic of MetS, produces MMPs [14]. Finally, we have not studied MMPs inhibitors.

Anyway, our finding of increased plasma activity of MMP-2 in women with MetS is important because they fit in with an early stage of CVD; and measurement of soluble molecules may improve the risk assessment, early diagnosis, and prognosis of cardiovascular disease.

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References

[1] Newby AC. Dual role of matrix metalloproteinases (matrixins) in intimal thickening and atherosclerotic plaque rupture. Physiol Rev 2005;85:1-31.
[2] Johnson JL. Matrix metalloproteinases: influence on smooth muscle cells and atherosclerotic plaque stability. Expert Rev CardiovascTher 2007;5:265-82.
[3] Death AK, Fisher EJ, McGrath KC, et al. High glucose alters matrix metalloproteinase expression in two key vascular cells: potential impact on atherosclerosis in diabetes. Atherosclerosis 2003;168: 263-9.
[4] Blankenberg S, Rupprecht HJ, Poirier O, et al. Plasma concentrations and genetic variation of matrix metalloproteinase 9 and prognosis of patients with cardiovascular disease. Circulation 2003; 107:1579-85.
[5] Cicero A, Derosa G, Manca M, et al. Vascular remodeling and prothrombotic markers in subjects affected by familial combined hyperlipidemia and/or metabolic syndrome in primary prevention for cardiovascular disease. Endothelium 2007;14:1-6.
[6] Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. (Adult Treatment Panel III). JAMA 2001;285:2486-97.

[7] Berg G, Muzzio ML, Wikinski R, et al. A new approach to the quantitative measurement of dense LDL subfractions. Nutr Metab Cardiovasc Dis 2004;14:73-80.
[8] Kleiner DE, Stetler-Stevenson WG. Quantitative zymography: detection of picogram quantities of gelatinases. Anal Biochem 1994;218: 325-9.
[9] Chow AK, Cena J, Schulz R. Acute actions and novel targets of matrix metalloproteinases in the heart and vasculature. Br J Pharmacol 2007; 152:189-205.
[10] Tsilibary EC. Microvascular basement membranes in diabetes mellitus. J Pathol 2003;200:537-54.
[11] Hayden MR, Sowers JR, Tyagi SC. The central role of vascular extracellular matrix and basement membrane remodeling in metabolic syndrome and type 2 diabetes: the matrix preloaded. Cardiovasc Diabetol 2005;4:9.
[12] Montero I, Orbe J, Varo N, et al. C-reactive protein induces matrix metalloproteinase-1 and -10 in human endothelial cells: implications for clinical and subclinical atherosclerosis. J Am Coll Cardiol 2006;47: 1369-78.
[13] de Nooijer R, Verkleij CJ, von der Thüsen J, et al. Lesional overexpression of matrix metalloproteinase-9 promotes intraplaque hemorrhage in advanced lesions but not at earlier stages of atherogenesis. Arterioscler Thromb Vasc Biol 2006;26:340-6.
[14] Chavey C, Mari B, Monthouel MN, et al. Matrix metalloproteinases are differentially expressed in adipose tissue during obesity and modulate adipocyte differentiation. J Biol Chem 2003;278:11888-96.