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ORIGINAL ARTICLE

Adiponectin predicts MMP-2 activity independently of obesity

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ABSTRACT

Background Matrix metalloproteinases (MMPs), especially MMP-2 and MMP-9, have been identified in atherosclerotic plaques and have been directly associated with plaque remodelling and vulnerability. Cardiovascular disease (CVD) is related to insulin resistance (IR) and obesity, characterized by changes in plasma levels of inflammatory markers, such as adiponectin and C-reactive protein (CRP). Our aim was to evaluate the impact of both proteins on MMP-2 and MMP-9 behaviour in individuals with IR.

Materials and methods Plasma MMP-2 and MMP-9 activity, adiponectin and hs-CRP concentration and lipoprotein profile were determined in 52 patients with metabolic syndrome (MS) and 27 controls.

Results Patients with MS presented significantly higher MMP-2 activity than controls: 0·95 ± 0·12 vs. 0·77 ± 0·15 relative units (RU) ($P < 0·001$), while MMP-9 activity was not detectable. MMP-2 activity decreased across quartiles of adiponectin, being significantly reduced in individuals with the highest levels of adiponectin in compared with the lowest levels (0·75 ± 0·17 vs. 0·93 ± 0·09 RU, $P < 0·005$). This difference persisted significant after adjusting by obesity markers. MMP-2 activity was significantly increased in individuals with the highest levels (G3) compared with those with the lowest levels (G1) of hs-CRP (0·94 ± 0·12 vs. 0·86 ± 0·12, $P = 0·041$)

Conclusion In this study, we observed that adiponectin levels predicted MMP-2 plasma activity independently of obesity. This finding suggests that the inflammatory process, associated with the highest CVD risk, would be involved in MMPs vascular production.

Keywords Adiponectin, MMP-2, obesity.

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Introduction

Matrix metalloproteinases (MMPs) constitute a family of zinc-dependent endopeptidases able to degrade extracellular matrix (ECM) components [1]. These enzymes are synthesized by multiple vascular cell types, including endothelial cells, vascular smooth muscles cells, circulatory monocyte, as well as the local tissue macrophages. MMPs have been extensively studied in the pathogenesis of the atherosclerosis process and cardiovascular disease (CVD) because of their major significance in vascular remodelling. Different MMPs, especially MMP-2 and MMP-9, have been identified in atherosclerotic plaques and in regions of foam cell accumulation and have been directly associated with plaque remodelling as well as plaque vulnerability [2]. Hence, they have been suspected to be partly responsible for the pathogenesis of CVD. Moreover, MMPs circulating levels have emerged as potential biomarkers of CVD [3].

Abdominal obesity is one of the main components of MS and one of the main risk factor of CVD. Expansion of fat cell size would require a pliant ECM, and recent studies have suggested that the absence of such pliant matrix could lead to adipose tissue inflammation, characteristic of insulin resistance (IR) states [4]. In this process, MMPs are involved in two important events of this process, the control of proteolysis and adipogenesis during obesity-mediated fat mass development [5].

In previous studies, we have reported that women with metabolic syndrome (MS) present increased activity of circulating MMP-2 [6]. The MS comprises a cluster of

cardiometabolic risk factors with insulin resistance (IR) and adiposity as central features [7–9]. Moreover, MS is characterized by a pro-inflammatory state, evidenced by the decrease in anti-inflammatory molecules such as adiponectin and the presence of elevated concentration of pro-inflammatory molecules such as C-reactive protein (CRP). Adiponectin is one of the cytokines synthesized in adipose tissue, and it may play a protective role against atherosclerosis suppressing lipid accumulation and inhibiting foam cell formation [10]. Although adiponectin circulates at high concentrations in human plasma, it was found reduced in different IR states and has been regarded as a potential link between adiposity and increased CVD risk [11]. Previous studies have suggested that adiponectin could play a protective role in plaque rupture through selectively increasing tissue inhibitors of MMPs (TIMPs) expression and secretion in human monocyte-derived macrophages [12]. CRP is not a mere marker of inflammation; it also may exert direct pro-atherosclerotic effects being an independent risk factor for cardiovascular events [13]. Cimmino *et al.* [14] showed that CRP induced MMP-9 expression and activity in human smooth muscle cells and patients with acute coronary syndrome presented increased plasma levels of MMP-9 directly associated with CRP. We hypothesized that in IR states, circulating activity of MMP-2 and MMP-9 would depend on the different levels of inflammatory markers such as CRP and the anti-inflammatory cytokine adiponectin independently of obesity. Regarding this hypothesis, our aim was to evaluate the impact of both proteins on MMP-2 and MMP-9 behaviour in individuals with IR.

Subjects and methods

For this study, 52 patients (41 women, 11 men) with metabolic syndrome (MS), according to Adult Treatment Panel III definition [15], were consecutively selected at the Hepatology Unit of Prof. Alejandro Posadas National Hospital (Buenos Aires, Argentina). In parallel, 27 subjects (20 women, seven men) recruited among hospital employee volunteers were selected as controls. The following exclusion criteria were considered for both groups: alcohol intake >20 g/day, diabetes, CVD, neoplasia, hypothyroidism, recent history of acute illness, renal disorders and seropositive hepatitis B or C. None of the subjects received corticosteroids, immunosuppressive agents or drugs known to influence lipid metabolism such as statins or fibrates.

The weight, height and blood pressure of each participant were measured, and body mass index (BMI) was calculated to evaluate obesity degree. Waist circumference was taken midway between the lateral lower rib margin and the superior anterior iliac crest in a standing position, always by the same investigator.

Written informed consent was required from all the participants to be included in the study. The study had the approval of the Ethic Committees from the Posadas Hospital and from the Faculty of Pharmacy and Biochemistry, University of Buenos Aires.

Samples

After a 12-h overnight fast, blood samples were drawn. Serum was kept at 4 °C within 48 h for the evaluation of glucose, lipids and lipoproteins, or stored at −70 °C for further determination of adiponectin, TNF-α, insulin, free fatty acids (FFA) and high-sensitivity C-reactive protein (hs-CRP). Plasma was stored at –70 °C for gelatinases activity determination.

Measurements

Total cholesterol, triglycerides (TG) and fasting glucose were measured using commercial enzymatic kits (Roche Diagnostics, Mannheim, Germany) in a Cobas C-501 autoanalyzer, coefficient of variation (CV) intra-assay<1·9%, CV interassay<2·4% and averaging CV values of these parameters. Hgh-density and low-density lipoprotein cholesterol (HDL- and LDL-chol) were determined by standardized selective precipitation methods, using phosphotungstic acid/MgCl2 and polyvinylsulfate as precipitating reagents, respectively, CV intra-assay<2·0% and CV interassay<3·0%. No HDL-cholesterol (No HDL-chol) was calculated as the difference between total cholesterol and HDL-chol. Serum hs-CRP, apolipoproteins A-I (apoA-I) and B-100 (apoB-100) were determined by immunoturbidimetry (Roche Diagnostics, Mannheim, Germany), CV intra-assay<1·9% and CV interassay<2·5% for the three parameters. FFA were determined by a spectrophotometric method (Randox, UK) CV intra-assay <2·6% and CV interassay <3·9%, and insulin was measured with Immulite/Immulite 1000 Insulin (Siemens, USA), CV intra-assay<2·6% and CV interassay<3·9%. To estimate IR, the homeostasis model assessment for insulin resistance (HOMA-IR) index was calculated as fasting insulin (μU/mL)×fasting glucose (mmol/L)/22·5. TG/HDL-chol index was also used as a surrogate marker of IR. Sera levels of adiponectin and TNF-α were determined by monoclonal antibody-based ELISA (R&D Systems, USA).

Gelatinolytic zymography

MMP-2 and MMP-9 activity was measured in plasma by gelatinolytic zymography as previously described [16] in a Mini Protean-3 (Bio-Rad Laboratories). Enzyme activity was detected as colourless bands. MMP-9 (84 kDa) and MMP-2 (67 kDa) were identified by molecular weight. Conditioned media from the promyelocyte U-937 cell line was used as activity standard. Band intensities were quantified using Sion-Image J software (Scion Corporation), and relative activity was expressed as a ratio to the internal standard.

Statistical analysis

Data are presented as mean±SD or median (range) according to the normal or skewed distribution, respectively. Differences between control and MS group were tested using the unpaired Student's t-test, χ^2 test or the Mann–Whitney U-test, according to the data distribution. Each variable was examined for normal distribution, and abnormally distributed variables were log transformed. One-way analysis of variance was used to test differences of gelatinases activity across adiponectin quartiles (Q1 ≤ 4·77; Q2: 4·78–6·84; Q3: 6·85–9·68; Q4 ≥ 9·69 μg/mL) or hs-CRP cutpoints of low risk groups (G1 < 1 mg/L), average risk (G2 1 to 3 mg/L) and high risk (G3 > 3 mg/L) of CVD. Further evaluation was performed using Scheffé multiple comparison test. To verify the difference of MMPs activity between groups, we performed an analysis of covariance (ANCOVA), controlling for necessary confounders such as age and gender. Pearson or Spearman analysis, for parametric or nonparametric variables, was used to determine correlations between parameters. Stepwise and multiple linear regression analyses were used to indentify independent correlations of MMPs. The SPSS 19·0 software package (Chicago, IL, USA) was used for statistical analysis. A $P < 0·05$ was considered significant.

Results

Characteristics of the study population

The clinical and biochemical characteristics of patients and controls are shown in Table 1. Regarding blood pressure, only one individual was hypertensive in control group and 20 in MS group. Patients with MS were older ($P < 0·001$) and presented higher BMI ($P < 0·001$) than controls, and as expected, waist circumference was increased in MS group, in both women and men ($P < 0·001$) (Table 1).

In reference to lipid and lipoprotein profile, the MS group presented higher TG ($P < 0·001$), total cholesterol ($P = 0·020$), LDL-chol ($P < 0·001$), apo-B100 ($P = 0·004$), No HDL-chol ($P < 0·001$) and lower HDL-chol ($P < 0·001$) and apo-AI levels ($P = 0·006$) (Table 1).

As expected, values of glucose, insulin, HOMA-IR, TG/HDL-chol and hs-CRP were higher ($P < 0·001$) and lower ($P < 0·001$) in MS patients compared to controls. No differences in circulating TNF-α level were found between groups (Table 1).

Gelatinases activity in metabolic syndrome

MMP-2 and MMP-9 plasma activity was evaluated in control and MS groups. Patients with MS presented significantly higher MMP-2 activity than controls: 0·95 ± 0·12 vs. 0·77 ± 0·15 relative units (RU) ($P < 0·001$) (Fig. 1), while MMP-9 activity was no detectable. Moreover, MMP-2 activity was positively associated with the number of MS components ($r = 0·357$, $P = 0·002$). In the whole population, there was no difference in MMP-2 activity between men and women: 0·92 ± 0·12 vs. 0·88 ± 0·16 RU ($P = 0·250$), and it was not associated with age ($r = 0·169$; $P = 0·137$).

Even though no associations with age and gender were observed, we performed an ANCOVA analysis including both variables as confounders. The increase of MMP-2 activity in MS group persisted significant after adjusting by age ($F = 23·9$, $P < 0·001$) and gender ($F = 32·6$, $P < 0·001$).

Table 1 Clinical and biochemical characteristics of control and MS group

	Control (n = 27)	MS (n = 52)	*P*
Age (years)	28 (21–60)	50 (25–66)	0·001
Gender (W/M)*	20/7	41/11	0·631
HT (yes/no)*	1/26	20/32	0·001
BMI (kg/m^2)	22·3 (18·2–32·9)	33·1(25·6–54·7)	0·001
WC (cm)			
Women	75·8 ± 12·8	103·5 ± 10·4	0·001
Men	86·3 ± 10·9	107·3 ± 6·3	0·001
TG (mg/dL)	86 (44–261)	173 (92–435)	0·001
Total-chol (mg/dL)	186 ± 39	208 ± 38	0·020
LDL-chol (mg/dL)	108 ± 40	144 ± 35	0·001
HDL-chol (mg/dL)	59 ± 14	42 ± 9	0·001
No HDL-chol (mg/dL)	128 ± 43	167 ± 36	0·001
apoA-I (mg/dL)	168 ± 32	147 ± 28	0·006
apoB-100 (mg/dL)	86 ± 32	106 ± 26	0·004
Glucose (mg/dL)	88 ± 9	102 ± 12	0·001
Insulin (μUI/mL)	5·4 (2·0–11·3)	10·1 (2·0–70·0)	0·001
HOMA-IR	1·2 (0·4–2·9)	2·9 (0·5–21·9)	0·001
TG/ HDL-chol	1·3 (0·5–7·9)	4·0 (1·9–16·1)	0·001
FFA (mmol/L)	0·6 (0·07–1·1)	0·6 (0·3–1·1)	0·431
TNF-α (pg/mL)	1·6 ± 0·2	1·7 ± 0·2	0·392
Adiponectin (μg/mL)	14·2 ± 5·2	5·4 ± 2·1	0·001
hs-CRP (mg/L)	1·2 (0·01–8·6)	3·4(0·3–31·8)	0·001

Data are expressed as mean ± SD or median (range) for skewed distributed data. MS indicates metabolic syndrome; W, women; M, men; HT, hypertensive; BMI, body mass index; WC, waist circumference; TG, triglycerides; Total-chol, total cholesterol; HDL-chol, high-density lipoprotein cholesterol; LDL-chol, low-density lipoprotein cholesterol; apoA-I, apolipoprotein A-I; apoB-100, apolipoprotein B-100; HOMA-IR, homeostasis model assessment for insulin resistance index; TNF-α, tumour necrosis factor- α; hs-CRP, high-sensitivity C-reactive protein.
*χ^2 test.

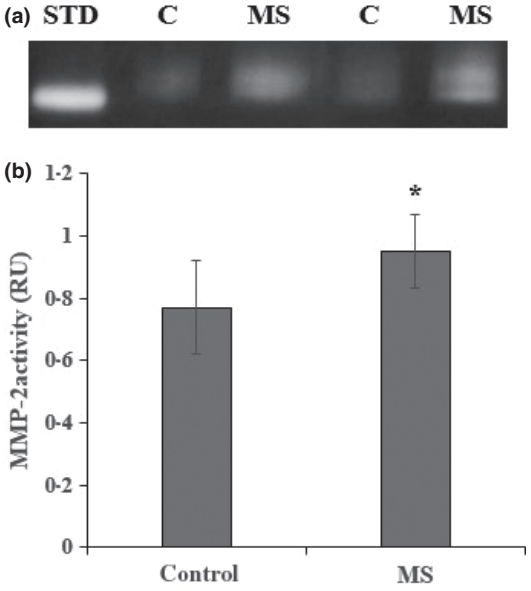

Figure 1 (a) Representative zymography of MMP-2 (b) MMP-2 activity in control (C) and MS group (MS). *< 0·001. RU = relative units.

Association of MMP-2 activity with plasma lipid and lipoprotein concentrations

The MMP-2 activity was positively related to an atherogenic lipoprotein profile. It was directly correlated with TG levels (r = 0·341, P = 0·002), LDL-chol (r = 0·292, P = 0·009), No HDL-chol (r = 0·301, P = 0·007) and inversely associated with HDL-chol (r = −0·328, P = 0·003). After adjusting by BMI, MMP-2 activity remained associated with TG (β = 0·265, P = 0·022), LDL-chol (β = 0·200, P = 0·05) and No HDL-chol (β = 0·207, P = 0·045). MMP-2 activity also showed a tendency to directly correlate with apo B (r = 0·200, P = 0·083).

Association of plasma MMP-2 activity with insulin resistance and inflammatory markers

Direct associations were present between MMP-2 activity and BMI (r = 0·332, P = 0·004) and waist circumference (r = 0·475, P = 0·001), whereas an inverse correlation was observed with adiponectin (r = −0·486, P < 0·001). When gender was considered, MMP-2 persisted associated with adiponectin only in women (r = −0·478, P < 0·001). Surprisingly, no association with CRP levels was found (r = 0·157, P = 0·170), neither with TNF-α (r = 0·292, P = 0·105). To further examine the relationship between MMP-2 and inflammatory markers, we compared MMP-2 activity across quartiles (Q) of adiponectin concentration and according to the hs-CRP reference cut-off values for cardiovascular risk. According to adiponectin, we observed

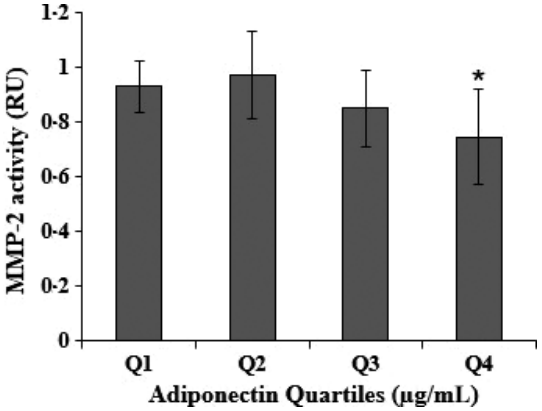

Figure 2 MMP-2 activity across adiponectin quartiles (Q). *Q4 vs. Q1 and Q2, P < 0·005. RU = relative units.

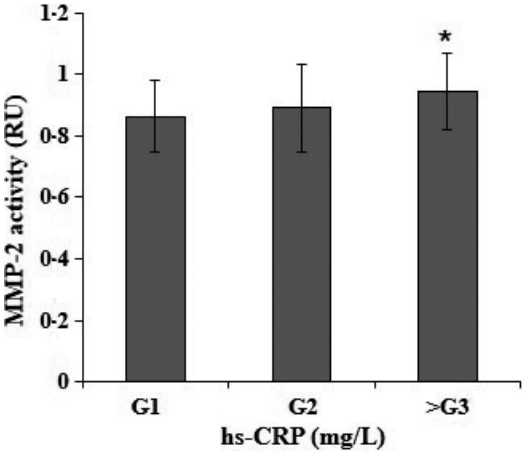

Figure 3 The MMP-2 activity according to hs-CRP cutpoints values for cardiovascular risk. *G3 vs. G1, P = 0·041. RU = relative units.

that MMP-2 activity decreased across quartiles of this cytokine, being significantly reduced in individuals with the highest levels of adiponectin (Q4) in compared to Q1 (0·75 ± 0·17 vs. 0·93 ± 0·09 RU) and Q2 (0·75 ± 0·17 vs. 0·97 ± 0·12) (P < 0·005) (Fig. 2). When MMP-2 was evaluated according to hs-CRP levels, we observed that, although no association between both parameters was found, enzyme activity was significantly increased in individuals with the highest levels (G3) compared with those with the lowest levels (G1) of hs-CRP (0·94 ± 0·12 vs. 0·86 ± 0·12, P = 0·041) (Fig. 3). In addition, MMP-2 activity inversely correlated with adiponectin/ hs-CRP ratio (r = −0·275, P = 0·024).

Table 2 Multivariate regression analyses showing the independent contributions of different variables to MMP-2 activity

Model	Independent variables	β	*P*
Model 1	Adiponectin	−0·351	0·013
	BMI	0·232	0·096
Model 2	Adiponectin	−0·321	0·040
	BMI	0·140	0·577
	WC	0·121	0·663

β is the standardized coefficient; WC, waist circumference; BMI, body mass index. BMI was $\log_{10}$ transformed before analysis.

Effect of obesity on MMP-2 activity and adiponectin

Given that relations between MMP-2 activity and waist circumference and BMI were found, a multivariate regression analysis was performed to distinguish the contribution of both variables to the association of MMP-2 activity and adiponectin levels (Table 2). In the first model, adiponectin and BMI were included; MMP-2 persisted significantly associated with adiponectin ($P = 0{\cdot}013$). After including waist circumference in the model, MMP-2 still persisted associated with this cytokine ($P = 0{\cdot}040$) (model 2), indicating that adiponectin would be a better predictor of plasma MMP-2 behaviour in MS than obesity.

Discussion

In the present study, we have evaluated the impact of adiponectin and CRP on gelatinases behaviour in individuals with IR. We found that MMP-2 plasma activity decreased across quartiles of adiponectin, being significantly reduced in individuals with the highest levels of this cytokine; moreover, MMP-2 activity was also linked to CRP, being increased in subjects with the highest levels of CRP.

Different components of the MS have been identified as possible stimuli for the synthesis and activity of MMPs, such as the inflammatory and pro-oxidant state, hyperglycaemia and dyslipidemia [13]. Previously, in our laboratory, we found higher plasma activity of MMP-2 in women with MS [6], which correlates with other soluble molecules involved in plaque development like vascular cell adhesion molecules (sVCAM) [16]. This increase would be associated with the first steps of the atherogenic process mainly related to the vascular smooth muscle cells migration and intimal thickening [17]. However, other authors reported contradictory results, with no differences in MMP-2 activity and higher levels in MMP-9 activity in MS patients [18], or increase in other MMPs, such as MMP-8 [18,19]. It is possible that gender or methodological differences between studies have affected the results. Meanwhile, in cardiovascular patients, it has been reported increased plasma MMP-2 and MMP-9 activity, which correlated with atherogenic lipoprotein profile and inflammatory markers [16].

Adiponectin belongs to the cytokines secreted by adipose tissue, and it is inversely associated with obesity and inflammation [20]. *In vitro* studies have shown that adiponectin has different effects on MMPs expression. Tong *et al.* [21] showed that adiponectin increased the secretion of MMP-3 in cultured human chondrocytes, whereas this cytokine reduced MMP-2 and MMP-9 protein levels in endometrial cancer cells [22]. So far, little is known about the direct effect of adiponectin on MMPs from adipose tissue. Kumada *et al.* [12] have shown that adiponectin selectively increased TIMP-1 expression in human monocyte-derived macrophages through IL-10 induction, without changes in MMP-9 secretion. Derosa *et al.* [23] found that adiponectin predicted decreased MMP-2 and MMP-9 plasma concentration in patients with combined hyperlipidemia. In this study, we observed that adiponectin levels predicted MMP-2 plasma activity independently of obesity. Furthermore, after adjusting by gender, adiponectin remained associated with MMP-2 only in women. Apparently, the decrease in adiponectin levels would impact stronger in women than in men with IR [24]. Adiponectin would be a main regulator factor of MMP-2 activity beyond other risk factors such as obesity and menopausal status as previously described [25].

The inverse association between MMP-2 plasma activity and adiponectin could suggest a possible contribution of adipose tissue in circulating MMP-2 activity. However, previous studies have demonstrated that there was a lack of association between adipose tissue and plasma levels and activity of gelatinases, suggesting that this tissue is not a major contributor to circulating MMP-2 and -9 [26,27]. Furthermore, *in vitro* studies have shown that leptin, also produced by adipocytes, enhanced MMP-2 activity in cardiac muscle cells mediating myocardial ECM remodelling [28]. It would be useful the measurement of this cytoquine in plasma in further studies to clarify the impact of this cytoquine on MMPs behaviour. Besides, *in vivo* and *in vitro* experiments demonstrated that adiponectin and TNF-α have antagonistic effects, suppressing each other's production and actions in their target tissues. In this study, there were no differences in circulating TNF-α and no association with MMP-2 activity. This finding could be due to the fact that circulating TNF-α levels may not represent its true biological activity, which principally involves the autocrine regulation of IL-6 secretion [29,30].

In reference to CRP, as expected, MS patients presented higher levels of this protein. When MMP-2 activity was evaluated accordingly to the hs-CRP cutpoints values for cardiovascular risk, enzyme activity was significantly increased in individuals with the highest levels compared with those with

the lowest levels of hs-CRP. This finding suggests that the inflammatory process, associated with the highest CVD risk, would be involved in MMPs vascular production.

This study has some limitations, such as the low number of patients included, as well as the number of male studied; however, the strict inclusion criteria must be considered. Similar studies should be performed in larger cohorts before drawing a final conclusion. Moreover, ongoing research should also include the study of TIMPs. Further studies are necessary to elucidate the direct effect of adiponectin and CRP on MMP production and activity in IR.

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