CLIMACTERIC 2012;15:602–606

Increase in MMP-2 activity in overweight and obese women is associated with menopausal status

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Key words: METALLOPROTEINASES, OBESITY, ADIPONECTIN, MENOPAUSAL STATUS

ABSTRACT

Background Metalloproteinases (MMPs) are synthesized in the subendothelium and are involved in the atherosclerosis and cardiovascular disease process because of their major significance in vascular remodeling and plaque rupture. MMPs are also synthesized in adipose tissue during angiogenesis; however, the role of these enzymes in obesity and insulin-resistant states is still controversial.

Objective To evaluate MMP-2 activity in the circulation of overweight and obese women and in normal-weight controls, and to associate the levels of these factors with metabolic, adipose tissue and inflammation biomarkers.

Methods Plasma MMP-2 activity, adiponectin and C-reactive protein concentration, lipoprotein profile and HOMA were determined in 39 healthy women (13 normal weight and 26 overweight/obese).

Results Overweight/obese women were older ($p < 0.001$) than normal-weight women; 20/26 of overweight/obese women were postmenopausal compared with 4/13 of normal-weight women. Overweight/obese women had significantly higher plasma activity of MMP-2 than controls (mean relative area: 0.81 (range 0.4–1.92) vs. 1.33 (range 0.4–3.1); $p < 0.005$); this difference was lost after adjusting for menopausal status. MMP-2 activity positively correlated with waist circumference ($p < 0.002$), HOMA ($p < 0.003$), and high-sensitivity C-reactive protein ($p < 0.05$), apolipoprotein B ($p = 0.006$) and triglyceride/high density lipoprotein (HDL) cholesterol index ($p < 0.001$), and negatively with HDL cholesterol ($p < 0.001$), HDL2 cholesterol ($p < 0.008$), HDL3 cholesterol ($p < 0.05$) and adiponectin ($p < 0.05$). The association with HOMA and adiponectin persisted even after adjusting for menopausal status.

Conclusion Our finding of increased plasma activity of MMP-2 in overweight/obese women, associated with menopausal status, is important given that it fits in with an early stage of cardiovascular disease; the association of MMP-2 activity with obesity markers may be a link between adipose tissue and risk for cardiovascular disease.

INTRODUCTION

Metalloproteinases (MMPs) are endopeptidases that degrade most of the protein and proteoglycan-core-protein components of the extracellular matrix[1]. They contribute to maintain the homeostasis of the vascular structure; however, the imbalance or activation of MMP expression is a feature of numerous pathologic conditions, such as tumor metastasis, vascular and cardiac dysfunction and rheumatic conditions. Thus, MMPs play a significant role in vascular remodeling and they are suspected of being partly responsible for the pathogenesis of cardiovascular disease. MMP-2 is highly expressed in the vulnerable regions of atherosclerotic plaques and for this reason it has been suggested as being causally involved in plaque rupture[2].

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ORIGINAL ARTICLE

DOI: 10.3109/13697137.2012.667174

Received 06-12-2011
Revised 13-02-2012
Accepted 13-02-2012

Obesity is the most prevalent nutrition-related disorder in western countries, and the prevalence of overweight and obesity is increasing world-wide at an alarming rate[3,4]. The prevalence of obesity is typically higher in females than in males globally. While the reasons for this sex difference are not clear, fluctuations in female sex hormones at menarche, pregnancy and menopause may play a role in the expansion of adipose tissue. Moreover, weight gain, with an increase in body fat percentage and a concomitant redistribution of fat accumulation from peripheral locations towards increased intra-abdominal depots, is common after menopause[5,6]. This abdominal obesity is characterized by an inflammatory condition, which can be evaluated through the measurement of different cytokines. Transcriptional regulation and activation of MMPs are controlled by different proinflammatory molecules, like C-reactive protein (CRP)[7]. Adiponectin is one of the cytokines secreted by adipose tissue and is inversely associated with obesity and inflammation[8]. Recent data suggest a direct role of adiponectin in atherosclerotic plaque stability through interactions with MMPs and their inhibitors[9]. The insulin-resistant state accompanying abdominal obesity would be associated with higher levels of circulating MMPs[7]. Different features related with insulin resistance have been identified as possible regulators of MMP synthesis[9].

In this study, our aim was to evaluate the activity of MMP-2 in the circulation of overweight and obese women in comparison to normal-weight controls, and then to associate the levels of these factors with other metabolic, adipose tissue and inflammation biomarkers.

SUBJECTS AND METHODS

Subjects

Thirty-nine women were consecutively recruited and clinically evaluated at the Gynecology Division-Clinical Hospital, University of Buenos Aires (Buenos Aires, Argentina). Fifteen were premenopausal, from 24 to 48 years with an intact uterus, and 24 postmenopausal, from 47 to 66 years with at least 1 year of natural menopause (confirmed in all cases by serum levels of follicle stimulating hormone >40 IU/l). Women were excluded if they presented with a history or underlying symptoms of diabetes, cardiovascular disease, hypothyroidism, neoplasia or renal disorder, or if they were receiving hormonal, hypolipidemic or any other drug known to modify lipid metabolism. The waist circumference, weight and height of each participant were measured and body mass index (BMI) was calculated in order to evaluate the degree of obesity. Women were divided into two groups according to their BMI: those with normal weight (BMI 18–24.9 kg/m^2, $n=13$) and those who were overweight or obese (BMI 25–38.5 kg/m^2, $n=26$). Written informed consent was obtained from each subject and the study was approved by the Ethic Committee of the Faculty of Pharmacy and Biochemistry, University of Buenos Aires.

Samples

After a 12-h overnight fast, samples of serum were obtained, for evaluation of fasting glucose, lipid and lipoprotein profile and CRP, and plasma for MMP-2 determination. Levels of cholesterol, triglycerides and glucose were determined by enzymatic colorimetric methods and of CRP by a high-sensitivity immunoturbidimetric method (hs-CRP) (Roche Diagnostics, Mannheim, Germany) in a Cobas C-501 auto-analyzer. High density lipoprotein (HDL) and low density lipoprotein (LDL) cholesterol were determined by standardized selective precipitation methods[10,11]. HDL3 cholesterol was determined by precipitation according to the method of Warnick[12]. HDL2 cholesterol was calculated as the difference between total HDL and HDL3 cholesterol. Apolipoprotein AI (apo AI) and apolipoprotein B (apo B) were determined by immunoturbidimetry (Roche Diagnostics, Mannheim, Germany). In order to estimate insulin resistance, triglyceride/HDL cholesterol and HOMA (homeostasis model assessment) indexes were calculated. Serum levels of adiponectin were determined by monoclonal antibody-based enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN, USA). MMP-2 activity was measured in plasma by gelatinolytic zymography as previously described[13] in a Mini Protean-3 (Bio-Rad Laboratories). Enzyme activity was detected as colorless bands. MMP-2, 72 kDa (pro-form) and 67 kDa (active form) were identified by molecular weight. 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 $\pm$ standard deviation or median (range) according to data distribution. Differences between groups were tested using the unpaired Student's t-test, χ^2 test or the Mann–Whitney U-test. Pearson or Spearman analysis was used to determine correlations between parameters. Partial correlations of MMP-2 with other metabolic variables, adjusting for menopausal status, were assessed. We performed an analysis of covariance (ANCOVA), where MMP-2 was the dependent variable and the BMI classification was the independent variable, controlling for menopausal status. Stepwise multiple regression analysis was undertaken with MMP-2 as the dependent variable and HOMA and adiponectin as the independent variables with menopausal status as covariable. The SPSS 19.0 software package (Chicago, IL, USA) was used for statistical analysis. A value of $p<0.05$ was considered significant.

RESULTS

The general characteristics of both groups can be observed in Table 1. Overweight/obese women were older and presented, as expected, higher waist circumference than normal-weight

Table 1 Clinical and biochemical characteristics of normal-weight women and overweight/obese women. Data are expressed as mean ± standard deviation except for triglycerides which are expressed as median (range) since the distribution is skewed

	Normal weight ($n = 13$)	*Overweight/obese* ($n = 26$)	*p Value*
Age (years)	37 ± 14	47 ± 10	0.001
Postmenopausal/premenopausal*	9/4	20/6	0.017
Waist circumference (cm)	72.0 ± 7.0	98.0 ± 9.0	0.001
Total cholesterol (mmol/l)	4.65 ± 0.60	6.29 ± 1.09	0.001
Triglycerides (mmol/l)	0.93 (0.50–2.74)	1.86 (1.03–4.08)	0.001
HDL cholesterol (mmol/l)	1.69 ± 0.36	1.30 ± 0.31	0.001
LDL cholesterol (mmol/l)	2.42 ± 0.57	4.32 ± 0.99	0.001
Apo AI (g/l)	1.75 ± 0.26	1.68 ± 0.28	NS
Apo B (g/l)	0.71 ± 0.19	1.39 ± 0.28	0.001
HDL2 cholesterol (mmol/l)	0.76 ± 0.42	0.34 ± 0.21	0.005
HDL3 cholesterol (mmol/l)	1.07 ± 0.26	0.96 ± 0.18	NS

*, χ^2 test
HDL, high density lipoprotein; LDL, low density lipoprotein; Apo, apolipoprotein; NS, not significant

women. In the overweight/obese group, 20 were postmenopausal and six premenopausal while, in the normal-weight group, nine were premenopausal and four were postmenopausal. The lipid and lipoprotein profile showed that overweight/obese women presented higher total cholesterol, LDL cholesterol, triglycerides, apoB and lower HDL cholesterol and HDL2 cholesterol than normal-weight women. No differences were observed in apoA and HDL3 cholesterol between the groups.

Table 2 shows that overweight/obese women presented higher glucose, insulin and hs-CRP levels as well as higher HOMA and triglyceride/HDL cholesterol indexes. In addition, adiponectin levels were lower in overweight/obese than in normal-weight women.

Overweight/obese women presented higher levels of MMP-2 than normal-weight women (Figure 1). However, this difference was lost after adjusting by menopausal status. As seen in Table 3, MMP-2 correlated directly with waist circumference. Positive correlations were also observed between MMP-2 and triglycerides, total cholesterol, LDL cholesterol, apoB, insulin, HOMA, triglyceride/HDL cholesterol index and hs-CRP. Negative correlations were observed between MMP-2 and HDL cholesterol, HDL2 cholesterol, HDL3 cholesterol, apoA and adiponectin.

Stepwise multilinear regression analysis was undertaken with MMP-2 as the dependent variable and adiponectin and HOMA as the independent variables; given the association observed between MMP-2 and menopausal status, we also added this confounder into the model. MMP-2 remained significantly associated with adiponectin ($\beta = -0.37$, $p = 0.032$) and HOMA ($\beta = 0.49$, $p = 0.012$), this parameter being the main predictor for MMP-2 increase in overweight/obese women. Both associations remained significant even after adjusting by menopausal status.

Table 2 Insulin resistance and inflammatory markers in normal-weight women and overweight/obese women. Data are expressed as mean ± standard deviation or as median (range) for skewed distributed data

	Normal weight ($n = 13$)	*Overweight/obese* ($n = 26$)	*p Value*
Glucose (mmol/l)	4.79 (3.85–7.7)	5.72 (4.24–9.74)	0.001
Insulin (pmol/l)	49.3 (13.9–116.7)	83.3 (20.1–459.1)	0.05
HOMA-IR	1.6 (0.3–3.4)	3.4 (1.2–21.2)	0.005
hs-CRP (mg/l)	0.5 (0.1–2.4)	4.3 (0.3–17.5)	0.001
Triglyceride/HDL cholesterol index	1.0 (0.5–6.9)	3.3 (1.3–9.9)	0.001
Adiponectin (μg/ml)	15.6 ± 4.2	11.2 ± 6	0.05

HOMA-IR, homeostasis model assessment for insulin resistance index; hs-CRP, high-sensitivity C-reactive protein; HDL, high density lipoprotein

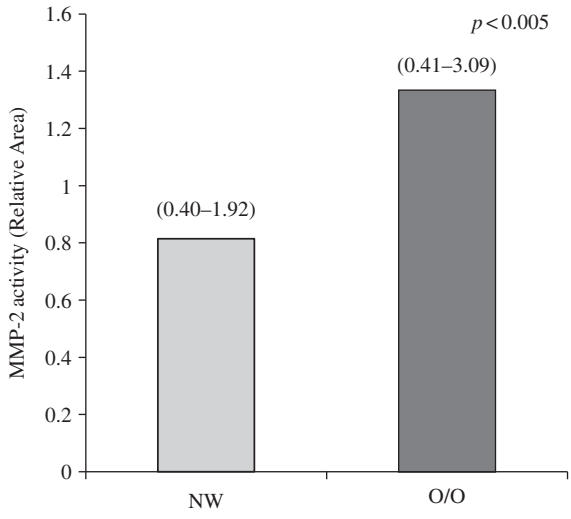

Figure 1 MMP-2 activity in normal-weight (NW) women and overweight/obese (O/O) women

DISCUSSION

In this study we found that the increased activity of circulating MMP-2 observed in overweight and obese women compared to that in normal-weight women is dependent on menopausal status. The higher activity of MMP-2 was associated with surrogate markers of insulin resistance such as HOMA and adiponectin. After adjusting for menopausal status, the difference in MMP-2 levels between groups was lost; however, the associations with the insulin resistance markers remained significant.

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.

Table 3 Spearman correlation coefficients for MMP-2 activity

	r	*p Value*
Waist circumference	0.547	<0.002
Total cholesterol	0.429	0.016
Triglycerides	0.734	0.001
HDL cholesterol	−0.591	<0.001
LDL cholesterol	0.464	0.009
Apo AI	−0.358	0.05
Apo B	0.489	0.006
HDL2 cholesterol	−0.563	<0.008
HDL3 cholesterol	−0.439	0.046
Insulin	0.417	0.043
HOMA-IR	0.572	<0.003
Triglyceride/HDL cholesterol index	0.756	<0.001
hs-CRP	0.373	<0.05
Adiponectin	−0.371	<0.05

HOMA-IR, homeostasis model assessment for insulin resistance index; hs-CRP, high-sensitivity C-reactive protein; HDL, high density lipoprotein; LDL, low density lipoprotein; Apo, apolipoprotein

The high MMP-2 activity might be responsible for a greater matrix degradation and also might activate several growth factors and cytokines, underlying the atherosclerotic process in the arterial vessel wall[9].

Obesity development is accelerated after peri- and postmenopause[14]; factors such as loss of estrogens, the aging process and changes in lifestyle may all be contributors[15,16]. In this study, women were recruited consecutively and the overweight/obese group presented a higher number of postmenopausal women, while the normal-weight group consisted mainly of premenopausal women. Obesity and high accumulation of visceral adipose tissue are highly associated with increased levels of insulin resistance and glucose tolerance[17]. The World Health Organization MONICA Project, monitoring cardiovascular disease in 21 countries, suggested that the relationship between insulin concentration and coronary heart disease was potentially stronger in women than in men[18]. We observed that the increased activity of MMP-2 in the overweight/obese group was lost after adjusting for menopausal status, suggesting the impact of estrogen decrease on MMP-2 activity. Although it is known that estrogen replacement therapy increases MMP-2[19] and MMP-3[20] levels in postmenopausal women, little is known about the behavior of these enzymes after the menopause.

Expanded adipose tissue increases the synthesis of cytokines which contributes to inflammation, as measured by several interleukins, and decreases the synthesis of adiponectin. The positive correlations observed between MMP-2 and waist circumference and different insulin resistance and inflammatory markers suggest an effect of overweight and obesity on the circulating activity of this metalloproteinase. CRP directly correlated with MMP-2 and it is noteworthy that CRP enhances the endothelial expression of MMPs, confirming the connection between these biomarkers[7,21].

Adiponectin, an anti-inflammatory cytokine, is secreted by adipose tissue and is decreased in obesity and inflammation. Decreased adiponectin levels have been observed in coronary artery disease[22] and in postmenopausal women[23]. Adiponectin selectively increases the tissue inhibitors of MMP expression (TIMPs)[24]. In our study, we observed an inverse relationship between MMP-2 activity and adiponectin. Moreover, other surrogate markers like HOMA were also responsible for changes in MMP-2 activity. The insulin-resistant state is a cluster that is very difficult to disaggregate, and both the increase in HOMA and the decrease in adiponectin levels are linked situations that impact on the endothelium, thus increasing cardiovascular disease[25].

High visceral adipose tissue accumulation has been associated with higher triglyceride and lower HDL cholesterol concentrations in pre- and postmenopausal women[14]. In this study, we observed direct correlations between MMP-2 and the atherogenic lipoprotein profile. Even more, we report an inverse correlation between MMP-2 activity and the HDL subfractions.

Current assays do not allow us to determine the origin of the measured MMP-2, so we cannot rule out that the inflamed adipose tissue, characteristic of obesity, produces MMP-2.

However, Gummesson and colleagues[26] studied the expression of MMP-9 in adipose tissue. There was a lack of association between adipose tissue mRNA and plasma levels of MMP-9, suggesting that this tissue is not a major contributor to circulating MMP-9.

This study has some limitations, such as the low number of patients included; however, the strict inclusion criteria must be considered. Ongoing research should include the study of TIMPs, the origin of circulating MMP-2 activity and the behavior of MMPs in reference to changes in female hormones. Given that MMPs have been suspected as being responsible in the pathogenesis of cardiovascular disease and in vulnerable plaques, our finding of increased plasma activity of MMP-2 in women with overweight and obesity is important because this fits in with an early stage of cardiovascular disease; measurement of soluble molecules may improve the risk assessment, early diagnosis, and prognosis of cardiovascular disease.

Conflict of interest The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Source of funding This study was supported by grants from the University of Buenos Aires, B036 and B2103.

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