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Evaluation of plasmatic MMP-8, MMP-9, TIMP-1 and MPO levels in obese and lean women

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ABSTRACT

Objectives: To compare the plasma concentrations of matrix metalloproteinase (MMP)-9, tissue inhibitor of MMP (TIMP)-1, MMP-8, and myeloperoxidase (MPO) for obese and lean women.

Design and methods: We recruited 30 lean and 36 obese women without comorbidities. The MMP-9, TIMP-1, and MMP-8 levels were measured using enzyme-linked immunosorbent assay (ELISA). MPO activity was assessed by a colorimetric assay.

Results: Obese women had higher MMP-9 levels and MMP-9:TIMP-1 ratios than lean women. Conversely, the MMP-8 levels and MMP-8:TIMP-1 ratios in the obese women were significantly lower than those in the lean women despite neutrophil activation, which was assessed by MPO activity.

Conclusion: We observed that MMP-9 and MMP-8 had distinct profiles, which suggested that these 2 enzymes play different roles in obesity.

Introduction

In recent years, obesity has become an epidemiologic problem in several countries worldwide, mainly because it is a major predisposing factor for the development of cardiovascular diseases [1,2]. Adipose tissue releases a large number of active mediators that influence body weight homeostasis and may modulate blood pressure levels, the coagulation cascade, and inflammatory mechanisms that can alter several physiological processes [3].

The expansion of adipose tissue and the enlargement of fat cells are accompanied by remodeling of the stromal matrix that is effected mainly by matrix metalloproteinases (MMPs) and their endogenous tissue inhibitors (TIMPs) [4]. MMP-9 seems to play an important role in this process in obese individuals, as evident by its elevated plasma concentrations in these subjects [5,10,11]. In addition, clinical studies have shown that alteration in the expression or activity of MMPs and/or TIMPs is an important mechanism underlying the pathophysiology of some cardiovascular diseases [5–8] and contributes to ventricular dysfunction [6] and atherosclerotic lesions [9]. Studies evaluating circulating levels of MMP-8 (neutrophil collagenase) in obese women without associated comorbidities have not yet been evaluated, although some studies have shown higher MMP-8 levels in subjects presenting with cardiovascular disorders [12–15].

Therefore, in the present study, we aimed to compare the plasma concentrations of MMP-9 and MMP-8 in obese and lean women. As suggested by the results of several studies, MMP-9:TIMP-1 and MMP-8:TIMP-1 ratios may be better indexes of net MMP-9 and MMP-8 activity, respectively, because TIMP-1 is a major inhibitor of these enzymes [16]. Moreover, because MMP-8 is mainly produced by neutrophils, we quantified plasma myeloperoxidase (MPO) levels as a marker of neutrophil activity [17].

We hypothesized that the circulating levels of important regulators of extracellular matrix remodeling (MMP-9, TIMP-1, and MMP-8) in obese women are significantly different from those in lean women, which may contribute to the development of obesity-associated diseases.

Materials and methods

Subjects

The Institutional Review Board of Santa Casa de Belo Horizonte, Brazil, approved the use of human subjects, and the subjects gave

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informed consent. The procedures followed were in accordance with institutional guidelines.

We recruited 36 obese women aged 25–50 years from the Endocrinology Ambulatory at the Santa Casa de Belo Horizonte. Obesity was defined according to the guidelines of the World Health Organization. The height was measured to the nearest 0.1 cm by using a wall-mounted stadiometer. The body weight was measured to the nearest 0.1 kg by using a digital scale. The body mass index (BMI) was calculated as the weight in kilograms divided by the squared height in meters. Subjects presenting with BMI $\geq$ 30 kg/m^2 were considered obese. Within the obese group, women with BMIs ranging from $\geq$40 to <50 kg/m^2 were considered morbidly obese, and those with BMI $\geq$ 50 kg/m^2 were considered super obese. The control group consisted of 30 healthy, age-matched, lean female volunteers (BMI, 18.5–24.9 kg/m^2) recruited from the local community.

The exclusion criteria included heart disease, hypertension, diabetes mellitus, thyroid and renal diseases, menopause or climacteric period, obstructive sleep apnea, pregnancy or breast-feeding, cigarette smoking, chronic alcohol consumption, and current use of any medication. The lean and obese women that enrolled in this study underwent clinical and biochemical analyses, and subjects that did not conform to established medical guidelines were excluded.

At the time of clinic attendance, venous blood samples were collected from the recruited subjects before breakfast, after an overnight fast (8–12 h). The samples were collected in standard Vacutainer tubes (Becton-Dickinson, Brazil) containing ethylenediaminetetraacetic acid (EDTA). The tubes were immediately centrifuged at 2000 *g* for 3 min at room temperature, and the plasma samples were stored at −80 °C until the MMP-9, MMP-8, MPO, and TIMP-1 concentrations were measured.

Laboratory analyses

The glucose concentrations and lipid parameters (total cholesterol [TC], triglycerides, and high-density lipoprotein cholesterol [HDL-C]) in plasma and serum, respectively, were measured by routine enzymatic methods with commercial kits (Katal Biotechnology Industry and Trade Ltd, Belo Horizonte, Brazil). The low-density lipoprotein cholesterol (LDL-C) concentration was calculated according to the Friedewald formula [18]. The Castelli index I and II values were determined by the TC/HDL-C and LDL-C/HDL-C ratios, respectively.

Measurement of plasma MMP-9, MMP-8 and TIMP-1 concentrations and MPO activity

The MMP-9, MMP-8, and TIMP-1 plasma concentrations were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA), according to the manufacturer's instructions. MPO activity was measured using a modification of the method reported by Bradley et al. [19].

Statistical analysis

All the results were expressed in terms of mean ± standard deviation (SD). The unpaired Student's *t*-test was used to compare normally distributed clinical and biochemical parameters by using *GraphPad Prism* software. The correlations among the biomarkers and Castelli I and II indexes were analyzed using Pearson or Spearman correlation. A P value of <0.05 was considered statistically significant.

Results

The clinical and laboratory parameters of the 66 subjects enrolled in the present study are shown in Table 1. The ages, triglyceride levels, and fasting glucose levels did not significantly differ between the lean and obese women (P > 0.05 in all cases). The body mass index values of the obese women were higher than those of the lean women (P<0.05; Table 1). The obese women also showed higher LDL-C levels, higher systolic blood pressure, and lower HDL-C levels than the lean women (P<0.05 in all cases; Table 1). In the group containing obese women, 15% was classified as severely obese, 52% as morbidly obese, and 15% as super obese.

Table 1
Clinical and laboratorial characteristics of the study participants.

Parameters	Lean	Obese
N	30	36
Age (years)	36.1 ± 9.6	37.9 ± 10.5
BMI (kg/m^2)	21.2 ± 1.9	42.1 ± 6.2*
Tot chol. (mg/dL)	123.3 ± 19.2	181.7 ± 30.4*
LDL chol. (mg/dL)	89.7 ± 7.6	109.0 ± 31.1*
HDL chol. (mg/dL)	56.8 ± 14.9	45.4 ± 12.7*
SBP (mm Hg)	114.3 ± 10.5	123.6 ± 14.3*
DBP (mm Hg)	73.4 ± 11.9	75.8 ± 9.6
Triglycerides (mg/dL)	92.3 ± 52.7	123.1 ± 17.1
Fasting gluc. (mg/dL)	82.3 ± 17.7	88.4 ± 19.4

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; Tot chol: total cholesterol; LDL chol: LDL cholesterol; HDL chol: HDL cholesterol. Fasting gluc: fasting glucose. Values are the mean ± SD and analyzed using unpaired Student *t* test.
* P<0.05 compared to lean.

The MMP-9 levels in the obese women (496.9 ± 313 ng/mL) were higher than those in the lean women (271.1 ± 145 ng/mL) (P = 0.0006; Fig. 1A), whereas the MMP-8 levels in the obese women (179.7 ± 134 ng/mL) were lower than those in the lean women (329.6 ± 227 ng/mL) (P = 0.0014; Fig. 1D). Despite the fact that the TIMP-1 levels did not differ significantly between the obese and lean women (P > 0.05; Fig. 1B), the MMP-9:TIMP-1 ratios were higher in obese women (1.3 ± 0.8) than in the lean women (0.8 ± 0.4) (P = 0.003; Fig. 1C). Conversely, the MMP-8:TIMP-1 ratios were lower in the obese women (0.4 ± 0.1) than in the lean women (0.9 ± 0.1) (P<0.0001; Fig. 1E). In addition, correlations were not observed among the biomarkers (MMP-9, TIMP-1, and MMP-8) and between the Castelli I and II indexes (P > 0.05 for all cases; data not shown). Because MMP-8 is mainly produced by neutrophils, we assessed the plasma activity of MPO, a marker of neutrophil activation, and observed increased values (approximately 29% higher; Fig. 1F) in the obese women as compared to the lean women. In addition, we compared the concentrations of these biomarkers in the 3 categories of obese women (severely, morbidly, and super obese) and found that these groups did not differ significantly (data not shown) in this regard.

Discussion

The findings reported here confirmed previous findings that showed higher circulating levels of MMP-9 in obese women as compared to lean women [5,10,11]. To our knowledge, we are the first to report a decreased plasma MMP-8 concentration in obese women. These findings suggest that these 2 MMPs are regulated by different mechanisms and probably differ with respect to their involvement in the pathophysiology associated with obesity.

MMP activities are regulated at the level of gene expression, by the activation of proenzymes, and by endogenous inhibitors (TIMPs). Excessive production and release of MMP-9 are observed in adipose tissue; this contributes to tissue remodeling and expansion [20] and may contribute to higher plasma MMP-9 levels in obesity [5,10,11]. Clinically, it is important to note that the increased MMP-9 concentrations reported here may increase the risk of cardiovascular diseases in obese women [5,6]; this observation suggests the potential use of this biomarker as a nontraditional cardiovascular risk factor [21]. The balance among MMPs and TIMPs is an important factor that regulates matrix remodeling, and alteration of this equilibrium may contribute to

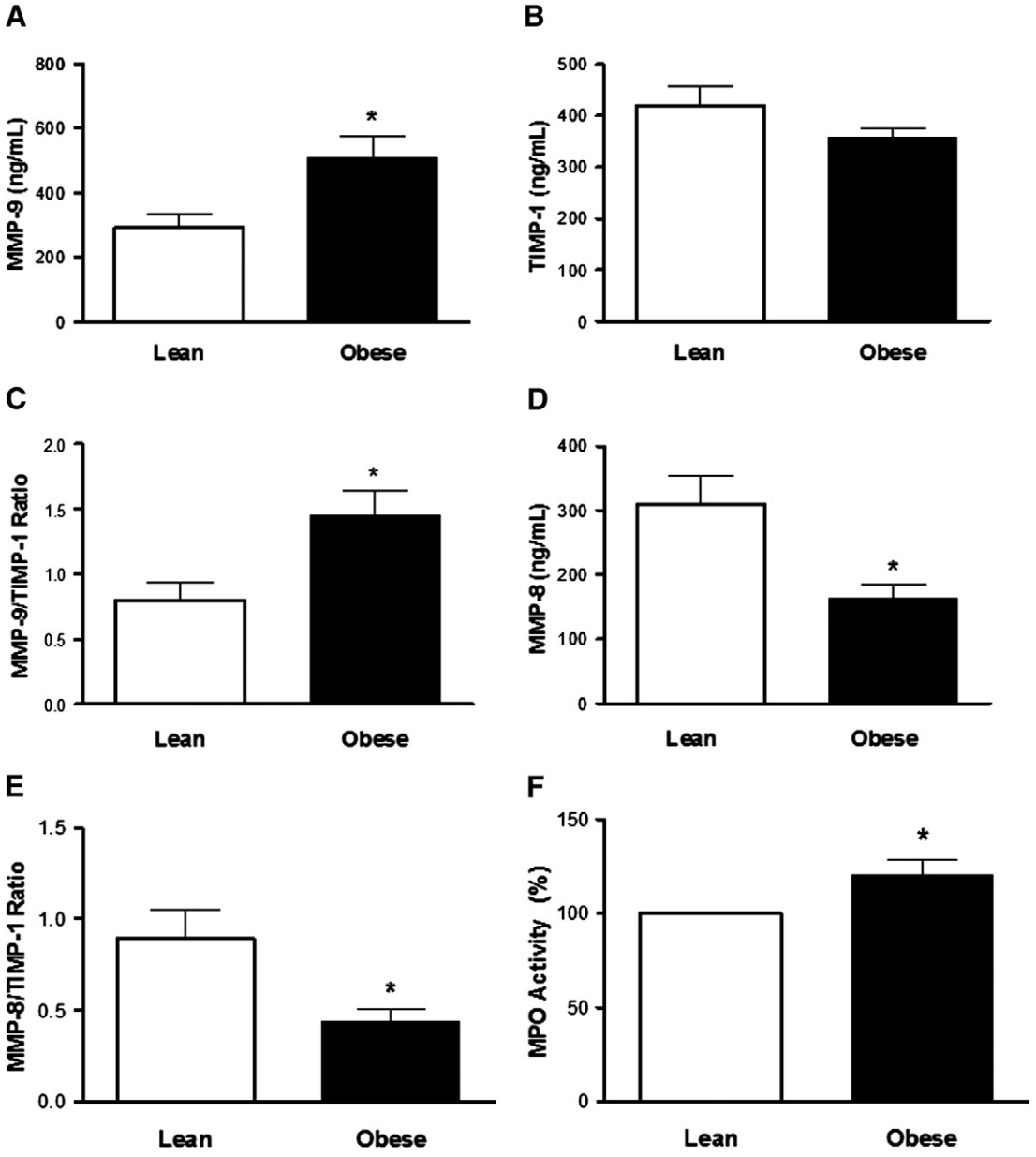

Fig. 1. Plasma MMP-9 levels (A), TIMP-1 levels (B), MMP-9/TIMP-1 ratio (C), MMP-8 levels (D), MMP-8/TIMP-1 ratio (E), and MPO activity (F) concentrations in lean and obese women. The bars show the means value ± SE, and *P<0.05 compared to lean.

many diseases [16]. Interestingly, while an increase in the level of MMP-9 was observed in obese women, a parallel increase was not observed in the level of its natural inhibitor (TIMP-1). A previous study reported total disappearance of the TIMP-1 protein when preadipocytes enter the differentiation stage [20]. These findings suggest that different mechanisms may regulate the expression of TIMP-1.

The most important finding of the current study is that obese women had lower levels of plasma MMP-8 than lean women, even though neutrophil activation was observed in the obese women. Although little is known about the biological effects of MMP-8, some studies have shown elevated levels of MMP-8 in subclinical and clinical atherosclerosis [13], unstable angina [22], metabolic syndrome [12], and childhood obesity [23]. Conversely, reduced levels of MMP-8 were associated with diastolic heart failure [24]. Furthermore, an unexpected anti-inflammatory role of MMP-8 has been reported in acute lung injury in mice [25]. These contradictory findings indicate that distinct pathways of matrix decomposition may be activated in different diseases. In agreement with our results, an interesting study performed on the adipose tissue of mice reported the presence of several members of MMPs and TIMPs, except MMP-8 [26]. A microarray study showed that while various MMP family members were strongly upregulated in adipocytes in a culture-conditioned medium, MMP-8 upregulation was not observed [27]. These data support our findings and suggest that adipose tissue probably does not synthesize MMP-8 in amounts sufficient to interfere with MMP-8 systemic levels.

We observed reduced MMP-8 levels in obese women, which may be explained by the negative regulation of MMP-8 expression by biomolecules such as transforming growth factor-beta 1 (TGF-β1) that are elevated in obesity [28] and that strongly downregulate MMP-8 [29], while stimulating MMP-9 expression [30]. To exclude the possibility of decreased MMP-8 levels due to reduced neutrophil activation, we measured the plasma MPO activity. MPO is a potent enzyme that is stored in the azurophilic granules of neutrophils and is secreted during neutrophil activation. MMP-8 and MMP-9 are stored in specific granules until they are required. In agreement with the results of another study [31], we found increased plasma

MPO levels in obese women, which indicated that neutrophil activation was probably caused by an inflammatory condition associated with obesity. Therefore, these data indicate that despite neutrophil activation, the MMP-8 levels in obese women without comorbidities were lower than in the lean women. It is important to mention that MPO synthesizes radicals and reactive oxidants that contribute to endothelium dysfunction, thereby increasing the risk of cardiovascular disease development in these women [32].

The current study was delineated to exclude clinical conditions and pharmacotherapeutic factors, such as hypertension, diabetes, cardiovascular disease, use of statins, and smoking, that may affect the MMP and TIMP-1 levels [5,8,12,33].

The current study has some possible limitations such as a relatively small number of subjects and the absence of groups with obesity-associated comorbidities (e.g., hypertension, type-2 diabetes mellitus, and dyslipidemia). In addition, we have not explored the relationship between other relevant MMPs and their inhibitors in these subjects, which may contribute to the understanding of matrix remodeling in obesity.

In summary, the present study showed that obese women without comorbidities had higher MMP-9 levels and MMP-9:TIMP-1 ratios than lean women, indicating that obesity alone is sufficient to elevate MMP-9 levels and, consequently, increase the risk of developing cardiovascular diseases. In addition, MMP-8 levels were unexpectedly lower in obese women than in lean women. The biological significance of the distinct MMP-9 and MMP-8 profiles in obese women should be thoroughly evaluated. Taken together, our results add to the existing knowledge regarding the role of MMPs and their endogenous inhibitors in obesity, which may have important implications for therapeutic approaches.

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