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Circulating and adipose tissue matrix metalloproteinases in cardiometabolic risk environments: pathophysiological aspects

Abstract: Matrix metalloproteinases (MMPs) play an important role during physiological tissue remodeling in embryonic development and angiogenesis, as well as in pathophysiological conditions such as obesity and development and vulnerability of atherosclerotic plaque. Moreover, MMP circulating levels have emerged as potential biomarkers of cardiovascular disease. MMP expression and activity are regulated by different factors such as insulin resistance and obesity. Expanded fat tissue has been demonstrated to be an active organ, where MMPs also exert a role in adipogenesis, angiogenesis, and proliferation of extracellular matrix (ECM). However, the lack of association between adipose tissue and plasma levels of some MMPs, specifically MMP-2 and MMP-9, suggests that this tissue is not a major contributor to circulating gelatinases. MMPs are also co-expressed or co-repressed in response to inflammatory adipocytokines, like adiponectin and leptin. Adiponectin may also play a protective role in plaque rupture through selectively increasing the tissue inhibitor of metalloproteinase (TIMP) expression. Leptin induces the expression of MMP-2 activators as well as the expression of MMP-2, MMP-9, and TIMP-1 in different human cells. Furthermore, sex hormones also participate in MMP regulation. In postmenopausal women, hormone replacement therapy produces an increase in MMP activity, leading to a breakdown in ECM homeostasis and accelerated progression of vascular pathologies. Besides, in men, an inverse relationship between testosterone levels and MMP-2 activity has been described. It is still necessary to go forward in the study of MMPs in different metabolic situations to corroborate their role as vulnerable plaque biomarkers.

Keywords: adipose tissue; insulin-resistance; matrix metalloproteinases; sex hormones.

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Introduction

Matrix metalloproteinases (MMPs) are proteolytic enzymes involved in extracellular matrix (ECM) and basement membrane (BM) component degradation. MMPs play an important role during physiological tissue remodeling in embryonic development [1] and angiogenesis [2], as well as in pathophysiological conditions such as atherosclerotic plaque development and vulnerability [3] and obesity [4].

Abdominal obesity is one of the main risk factors for cardiovascular disease (CVD) [5]. The development of adipose tissue is associated with extensive modifications involving adipogenesis, angiogenesis, and ECM remodeling. 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 states [6]. Several lines of evidence suggest a potential role of MMPs in the development of adipose tissue. MMPs are involved in two important events of this process, the control of proteolysis and adipogenesis during obesity-mediated fat mass development [7].

Besides, nowadays, it is well known that vulnerable plaques are mainly responsible for CVD. Atherosclerosis is a chronic disease that evolves with aging, with the decrease in sex hormones being an important factor in this process. The lower levels of sex hormones are also associated with increased abdominal obesity and could influence plaque vulnerability by modifying MMP behavior.

Related to the focus of this review, the role of circulating and adipose tissue MMPs in different cardiometabolic risk situations such as the metabolic syndrome, obesity, and alterations in sex hormone levels will be briefly described.

Matrix metalloproteinases

MMPs are able to degrade ECM components such as collagens, proteoglycans, elastin, laminin, fibronectin, and other glycoproteins [8]. MMPs comprise a family of 25

identified so far related gene products, and, based on sequence homology and substrate specificity, they can be classified into six groups: collagenases, stromelysins, matrilysins, gelatinases, membrane-type metalloproteinases, and zinc- and calcium-dependent endopeptidases [9].

MMPs with collagenase activities share the ability to cleave fibrillar collagen types I, II, and III into smaller fragments, which, in turn, can be degraded by other proteases of the MMP family. The most studied collagenases are MMP-1, MMP-8, and MMP-13. Gelatinases consist of MMP-2 and MMP-9, and they are the main enzymes responsible for the degradation of type IV collagen and denatured collagens (gelatins), elastin, fibronectin, and laminin, among other proteins. Moreover, these enzymes collectively can also cleave several non-ECM proteins, such as adhesion molecules, cytokines, protease inhibitors, and other (pro-) MMPs [10]. MMPs are synthesized by multiple vascular cell types, including endothelial cells, vascular smooth muscle cells (VSMC), fibroblasts, myofibroblasts, and the systemic-circulatory monocyte and macrophages, as well as the local tissue macrophages [11]. Gelatinases in general are highly expressed in fatty streaks and atherosclerotic plaques compared to normal regions of the vessel [12, 13]. ECM degradation by MMPs could reduce fibrous cap thickness and collagen content, which are typical features of vulnerable plaques.

Most MMPs are secreted as inactive, latent proenzymes and require a proteolytic process to become active. Under normal physiological conditions, MMP activities are exactly regulated at the transcription level, at precursor zymogen activation, through interaction with specific ECM components and by inhibition of endogenous inhibitors (TIMPs) [14]. TIMPs are specific inhibitors of MMPs that participate in controlling the local activities of MMPs in tissues [15]. Four TIMPs (TIMP-1, TIMP-2, TIMP-3, and TIMP-4) have been identified and are able to inhibit the activities of all known MMPs. The four members have many similarities and overlapping specificities, but their biochemical properties and local expression patterns exhibit their distinctive features [16]. Consequently, the net resultant of MMP activity in tissues is locally determined by the balance between the levels of activated MMPs and TIMPs. Moderation of MMP activities has generated considerable interest as a possible therapeutic target. As mentioned above, TIMPs are the major naturally occurring proteins that specifically inhibit MMPs. However, TIMPs are probably not suitable for pharmacological applications owing to their short half-lives in vivo. During the last few decades, synthetic MMP inhibitors have been developed in attempts to control MMP enzymatic activities in abnormal bioprocesses, such as obesity.

MMPs in obesity and metabolic syndrome

Abdominal obesity, when accompanied by metabolic derangements, including IR, low high-density lipoprotein cholesterol, elevated triglycerides, and raised blood pressure, significantly increases the predicted CVD risk [17] and constitutes the well-known metabolic syndrome (MS).

Different components of the MS have been identified as possible stimuli for the synthesis and activity of MMPs, like the inflammatory and pro-oxidant state, hyperglycemia, and dyslipidemia. In our laboratory, we found higher plasma activity of MMP-2 in women with MS [18], which correlates with other soluble molecules involved in plaque development like vascular cell adhesion molecules (sVCAM) [19]. However, other authors reported contradictory results, with no differences in MMP-2 activity and higher levels in MMP-9 activity in MS patients (male and female) in comparison to controls [20], or increase in other MMPs, like MMP-8 [20, 21]. There is no clear explanation for these discrepancies. It is possible that gender or methodological differences between studies have affected the results. The different stages of the CVD could also be a main factor in conditioning MMP levels. In the study of Miksztowicz et al. [18], the patients were women with MS but without clinical evidence of unstable plaques. The increased MMP-2 activity was associated with the first steps of the atherogenic process mainly related to the VSMC migration and intimal thickening [22]. The lack of MMP-9 detection could be attributed to the fact that this MMP is reported to be associated mainly with the plaque rupture in advanced lesions [12].

Even though circulating MMPs have emerged as promising biomarkers for human CVD, the question is whether the expanded adipose tissue mass in obesity contributes significantly to the circulating levels of these enzymes. Fat tissue has demonstrated to be an active organ, where MMPs have been shown to exert a role in its expansion. As it is known, the development of obesity is associated with excessive modifications in adipose tissue involving adipogenesis, angiogenesis, and remodeling of ECM. Expansion of adipose tissue can be supported both by neovascularization for adipocyte hyperplasia and by dilation and remodeling of existing capillaries for adipocyte hypertrophy. Furthermore, BMs surrounding adipocytes have to be extensively remodeled to allow the hypertrophic development of adipocytes. MMPs are involved in two important events of this process: the control of proteolysis and the control of adipogenesis during obesity-mediated fat mass development [7]. Observations in in vivo models suggest

that MMPs may contribute to adipose tissue remodeling, by degradation of ECM and BM components or by activation of latent growth factors [23]. To gain further insight into the involvement of MMPs in the development of adipose tissue, Maquoi et al. [24] monitored the expression of MMPs and TIMPs in adipose tissue from lean and obese mice. This study revealed an up-regulation of the mRNA levels of some MMPs (MMP-3, MMP-11, MMP-12, MMP-13, and MMP-14) and a down-regulation of the others (MMP-7, MMP-9, MMP-16, MMP-24, and TIMP-4) in obesity. These modulations differed according to the origin of the adipose tissue (gonadal vs. subcutaneous), supporting the concept that the different localization of fat deposits presents a different metabolic behavior [25].

In a previous study, we evaluated MMP-2 and MMP-9 activity in visceral adipose tissue in an animal model of early IR induced by a sucrose-rich diet [26]. We found that gelatinase activity was decreased in this tissue; however, this was not associated with changes in MMP plasma activity. The lack of association between adipose tissue and plasma activity of the gelatinases suggests that this tissue is not a major contributor to the circulating enzymes. Assuming that our results are applicable to humans, we could suggest that the increase in MMP plasma activity previously observed in MS [18] was not derived from adipose tissue. Consistent with our results, Gummesson et al. [27] studied the plasma concentration and activity of MMP-9 in men and found that circulating levels of insulin, glucose, and C-reactive protein (CRP), as well as blood pressure were related to total and active MMP-9 plasma concentrations. On the contrary, these concentrations were not associated with body mass index or with waist circumference. In parallel, they also studied the gene expression of MMP-9 in adipose tissue in men with and without MS treated with a weight-reducing diet. The authors found 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. In accordance, Van Hul et al. [28] found that MMP-9 does not seem to play a major role in adipose tissue development in murine models of diet-induced obesity, even though the plasma levels of MMP-9 have been found increased in obese patients [29]. Moreover, different MMP knockout models, such as MMP-19-null, MMP-3-null, or MMP-11-null mice, are associated with increase in adipose tissue development when mice are fed a high-fat diet [30–32]. In contrast, in membrane type 1 MMP knockout model, visceral adipose tissue development is aborted, leaving tissues populated by mini-adipocytes that render null mice lipodystrophic [33].

Previous studies have shown the potential of broad-spectrum MMP inhibitors to impair adipose tissue development in mice [34]. The relatively gelatinase-specific inhibitor tolylsam (Shionogi Research Laboratories, Osaka, Japan) has been shown to reduce bodyweight and adipose tissue development, associated with adipocyte hypotrophy, in a nutritionally induced obesity model in mice [35], as well as in leptin-deficient (*ob/ob*) mice [36]. Recently, the effect of ABT-518, an MMP inhibitor with high selectivity and potency against gelatinases, on adipogenesis and adipose tissue development has been studied in mouse models of obesity. This inhibitor had no effect on total bodyweight, but was associated with reduced blood vessel size in fat tissues [37].

In an attempt to elucidate the role of insulin in the molecular mechanism involved in the production of MMPs, Boden et al. [38] observed in rat aorta that free fatty acids and insulin stimulate the production of pro-inflammatory cytokines, which, in turn, promote the activation of MMP-2, MMP-9, and MT1-MMP. However, in the liver, hyperinsulinemia has different effects on MMPs, promoting a decrease in the bioactive isoforms of MMP-2, MMP-9, and MT1-MMP [39], suggesting that insulin does not affect MMPs in the same way in different organs.

Effects of adiponectin and leptin on MMPs

It is well established that expanded adipose tissue is associated with a pro-inflammatory state. This is evidenced by the presence of elevated concentrations of inflammatory molecules including CRP and different cytokines, and a decrease in anti-inflammatory molecules. MMPs are also co-expressed or co-repressed in response to inflammatory cytokines and growth factors. Leptin and adiponectin are the main cytokines synthesized in adipose tissue with different functions and opposing effects on inflammation and the atherosclerotic process [40] (Figure 1).

Leptin

Leptin was the first adipose hormone identified, and, in obesity, its circulating levels are directly associated with adipose tissue mass. Leptin is best known for its central effects including the regulation of food intake and energy expenditure [41]. Leptin receptors are also present in many peripheral tissues, and it has become clear that leptin mediates a wide array of direct peripheral effects,

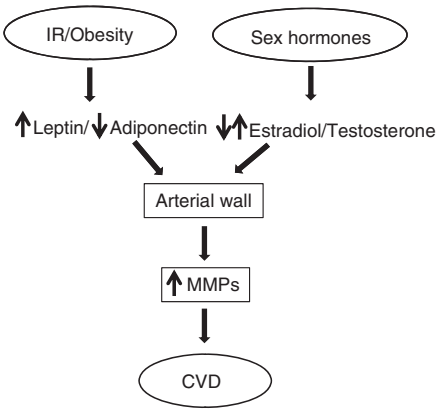

Figure 1 Relationship between adipocytokines, sex hormones, MMPs and cardiovascular disease. IR, insulin resistance; MMPs, metalloproteinases; CVD, cardiovascular disease.

including those specific to the cardiovascular system. The proatherogenic effects of leptin have been described in vitro; these effects include, in part, endothelial and VSMC activation, migration, and proliferation [42, 43]. The overexpression of leptin has a role in the growth of atheromatous plaques. It plays a role in matrix remodeling by regulating the expression of MMPs and TIMPs. Previous studies in vitro have demonstrated the stimulation of MMP proteolytic activity by leptin [44, 45]. It has been reported that exposure of myofibroblasts to leptin significantly increased the expression of MT1-MMP, a known activator of MMP-2, resulting in an increase in MMP-2 activity, without changes in protein levels [46]. Park et al. [42] reported that leptin induces the elevation of MMP-2, MMP-9, and TIMP-1 expression in human umbilical vein endothelial cells and in human coronary artery smooth muscle cells. This effect could be mediated through the generation of intracellular reactive oxidative species (ROS) and could be decreased by metformin treatment [44]. In reference to adipose tissue, in vitro studies have demonstrated that MMP-2 secretion was significantly promoted by leptin treatment in 3T3-L1 preadipocytes [47]. Because plasma leptin concentrations are associated with obesity and type 2 diabetes [48], leptin signaling may represent a therapeutic target for the prevention of obesity and CVD.

Adiponectin

Adiponectin belongs to the cytokines secreted by adipose tissue, and it is inversely associated with obesity and inflammation [49]. Adiponectin has beneficial effects on vascular function and may play a protective role against atherosclerosis. The pathway by which adiponectin affects vascular function has been evaluated through in vitro experiments in endothelial cells from human aortas. Adiponectin increases nitric oxide (NO) production and/or ameliorates oxidized low-density lipoprotein (oxLDL)-induced suppression of endothelial NO synthase activity [50]. Furthermore, adiponectin has been shown to affect atherosclerotic plaque formation and stability [49]. Adiponectin suppresses lipid accumulation and class A scavenger receptor expression in macrophages, resulting in markedly decreased uptake of oxLDL and inhibition of foam cell formation [50]. It also binds to platelet-derived growth factor-BB and subendothelial collagens and suppresses the proliferation and migration of human aortic smooth muscle cell [51]. Adiponectin may also play a protective role in plaque rupture through selectively increasing TIMP expression and secretion in human monocyte-derived macrophages. This effect is mediated via the ability of adiponectin to increase the expression and secretion of interleukine (IL)-10, a TIMP-inducing cytokine [52]. In patients with combined hyperlipidemia, Derosa et al. [53] found that adiponectin predicted decreased MMP-2 and MMP-9 plasma levels. In accordance, in our laboratory, we observed an inverse association between plasma adiponectin levels and circulating activity of MMP-2 in patients with MS (data not published). Moreover, in patients with acute coronary syndrome, a negative relationship between adiponectin and MMP-9/TIMP-1 ratio has been described; this ratio is considered as an independent predictor of atherosclerotic plaque stability and of the severity of coronary atherosclerosis [54]. However, no correlations have been observed between adiponectin and plasma levels of MMP-1 in coronary patients [55]. As mentioned previously, this could be a consequence of MMPs behaving differently in several cardiometabolic risk environments.

In vitro studies have shown that adiponectin has different effects on MMP expressions. Tong et al. [56] 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 [57].

So far, little is known about the direct effect of adiponectin on MMPs from adipose tissue. Kumada et al. [52] have shown that adiponectin selectively increased TIMP-1 expression in human monocyte-derived macrophages through IL-10 induction, without changes in MMP-9 secretion. These results suggest that adiponectin could directly affect the balance of MMP/TIMP expression in macrophages from adipose tissue. Further studies are necessary to elucidate the role of this cytokine in MMP behavior in this tissue.

Effect of sex hormones on MMPs

Estrogen is the predominant sex hormone in women, affecting the development and function of the female reproductive system. Estrogen also promotes the differentiation of other tissues, including adipose tissue, skeletal muscle, and cardiovascular system [58].

Estrogens are produced in the ovary and testes, as well as in adipocytes by the action of aromatase on androgens, and are increased in proportion to total body adiposity [59]. Reduced circulating estrogens, as seen in post-menopausal females, result in the development of increased intra-abdominal adiposity and increased susceptibility to diseases associated with the MS [60]. Estrogens bind to two 'classical' estrogen receptor subtypes, estrogen receptor α (ERα) and estrogen receptor β (ERβ), with similar affinity. Both receptors have been detected in human adipose tissue, suggesting that estrogen has direct effects on this tissue [59].

Epidemiological studies have shown that CVD is less common in premenopausal women (pre-MW) than in men of the same age, suggesting the cardiovascular benefits of estrogen. Also, the risk of CVD is greater in postmenopausal women (post-MW) than in pre-MW and has been related to the decline in plasma levels of estrogen during menopause. In vitro and in vivo studies also suggested the beneficial effects of estrogen on the vasculature. Estrogen modulates vascular tone by targeting endothelial cells, VSMC and ECM [61, 62]. Such beneficial effects include up-regulation of the vasodilatory substances, NO and prostacyclin-2, and down-regulation of the vasoconstrictor molecules endothelin-1 and angiotensin II. The migration of medial VSMCs to the intima region plays a major role in intimal hyperplasia development during vessel remodeling in arterial disorders such as atherosclerosis. Additionally, estrogen-binding receptors have been identified in VSMCs, consistent with the theory that this vascular function may be under direct hormonal control [63]. The decrease in estrogens is associated with higher MMP circulating levels. In a previous study, we observed that circulating MMP-2 activity was higher in overweight and obese women than in normal-weight women; however, this increase was dependent on menopausal status [64]. Moreover, we showed that, in asymptomatic post-MW, the increase in MMP-2 plasma activity correlated with an atherogenic lipoprotein profile, as well as with increased levels of CRP and sVCAM [19].

Davis et al. [59] found that ERα knockdown mice, selectively in intra-abdominal adipose tissue, promotes increased adipose tissue mass, increased adipocyte size, and is associated with increased expression of a marker of macrophages, elevated adipose tissue inflammation and fibrosis in both males and females. Furthermore, in vitro studies have demonstrated that 17β-estradiol regulates the expression of MMPs and TIMPs in human macrophages [65]. There are no studies that evaluate the direct effect of estrogens on MMPs from adipose tissue; however, these findings could suggest that this hormone would have a role in MMP behavior from adipose tissue in postmenopause, and further studies in this topic should be developed.

For several years, hormone replacement therapy (HRT) was considered to reduce CVD risk in post-MW. However, the Women's Health Initiative (WHI) study showed that HRT was not cardioprotective and that its risk/benefit ratio did not favor the use of postmenopausal hormones for prevention of chronic diseases [66]. The use of oral and transdermal estrogen has somewhat different effects on inflammation and coagulation markers. In common, there are reductions in certain inflammation markers (ICAM-1, VCAM-1, MCP-1, E-selection, as well as homocysteine) with divergent effects on CRP and MMPs [67]. In an in vitro study, Grandas et al. [68] showed that estrogens up-regulate MT1-MMP without a corresponding increase in TIMP-2, a known activator and inhibitor of MMP-2, respectively. The downstream effect of hormone stimulation could result in increased MMP enzymatic activity, leading to a breakdown in ECM homeostasis and accelerated progression of vascular pathologies. Moreover, it has been reported that oral estrogen therapy in healthy post-MW produces significant increases in MMP-2 and MMP-9 [67], although other authors observed decreases in MMP-9 [69]. These findings could, in part, explain the lack of beneficial effects of HRT on CVD.

In relation to the analysis of the benefits and risks of HRT in early menopausal women (i.e., ages 50–59 or <10 years postmenopausal), different re-analyses of the WHI indicated the important influences of age and time since the initiation of HRT on benefits and risks [70, 71]. A central issue of discussion in interpreting the findings of the WHI study is the extent to which the effects of HRT are influenced by the timing of its initiation, in terms of either the age of the recipient or the duration of estrogen deficiency (i.e., "time since menopause") [72]. This "timing hypothesis" that early HRT prevents CVD is supported by animal data and by some human studies [73]. The impact of HRT on MMP behavior could be one of the explanations of the paradoxical effects of the therapy.

Besides, MMPs are also relevant in pregnant women, given their role in implantation and placentation. Overactivation of MMPs is clearly associated with gestational diseases such as preeclampsia and premature rupture of membranes. Pustovrh et al. [74, 75] previously found that

NO and ROS can stimulate MMP-9 activity in human-term placentas and in placentas from animal models. In addition, they found that oxidative stress and NO production are increased in term placentas from type 2 diabetic patients, alterations that result in an increase in the formation of peroxynitrites, which can up-regulate MMP-2 and MMP-9 activities in the placenta [76].

In young women, androgen levels can also influence vascular physiology. Polycystic ovarian syndrome (PCOS) and hyperandrogenism are among the most common endocrine disorders in reproductive-age women. Androgens and insulin have a complex relationship: a significant number of hyperandrogenic young women have IR and, conversely, women with diabetes are at greater risk of developing PCOS. MMPs and TIMPs have been implicated also in ovarian physiology and pathophysiology. MMP-2 and MMP-9 and TIMP-1 and TIMP-2 are also expressed in the human ovary [77], and their function is necessary for follicular rupture and oocyte release.

It has been described that women with PCOS have elevated serum concentrations of MMP-2 and MMP-9. Elevated MMP concentrations might be related to increased cardiovascular risk or to abnormalities of ovarian ECM remodeling, multiple cyst formation and chronic anovulation noted in women with PCOS [77].

In reference to men, experimental and epidemiological evidence suggest that androgen deficiency contributes to the onset and progression of CVD [78, 79]. It is known that, in elderly men, low testosterone levels are associated with increased risk of atherosclerosis, independent of age, body mass index, total cholesterol, smoking status, or diabetes [80]. Androgen deficiency is associated with endothelial dysfunction, adverse lipid profiles, inflammatory responses, altered smooth muscle, and hypertension. In vitro and animal studies have demonstrated an inverse association between serum testosterone concentration and intimal hyperplasia attributed to the inhibition of VSMC proliferation [81, 82]. In an in vitro study on male VSMCs, Mountain et al. [83] have reported that testosterone levels differentially affected the expression of regulatory isoforms responsible for the activation and inhibition of MMP-2, leading to an inverse relationship between testosterone levels, MMP-2 activity, and VSMC migration.

Conclusions and future perspectives

During the last decade, MMPs have been extensively studied in the pathogenesis of the atherosclerosis process and CVD because of their major significance in vascular remodeling. Moreover, MMP circulating levels have emerged as potential biomarkers of CVD. MMP behavior is affected by multiple factors like IR, obesity, inflammation, and sex hormones, among others. It is still necessary to go forward in the study of MMPs in these situations to verify their role as vulnerable plaque biomarkers. Therefore, additional clinical and epidemiological research is needed to unequivocally determine the effect of different cardiometabolic risk situations on MMPs synthesized in the arterial wall. In addition, a rational study of lifestyle modifications as well as pharmacological therapies that could influence MMPs are necessary to generate the information that physicians will probably need to improve the treatment of patients with cardiovascular risk.

Highlights

- MMPs are enzymes directly involved in the development and vulnerability of atherosclerotic plaque.
- MMPs are expressed in other tissues like adipose tissue, gonads, and placentas.
- MMP synthesis is regulated by different factors like adipocytokines and sex hormones.
- Circulating MMP-2 and MMP-9 activity is increased in different IR situations.

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