

## Theme Issue Article

# Role of proteolysis in development of murine adipose tissue

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### Summary

Obesity is a common disorder, and related diseases such as diabetes, atherosclerosis, hypertension, cardiovascular disease and cancer are a major cause of mortality and morbidity in Western-type societies. Development of obesity is associated with extensive modifications in adipose tissue involving adipogenesis, angiogenesis and extracellular matrix proteolysis. The fibrinolytic (plasminogen/plasmin) and matrix metalloproteinase (MMP)

systems cooperate in these processes. A nutritionally induced obesity model in transgenic mice has been used extensively to study the role of the fibrinolytic and MMP systems in the development of obesity. These studies support a role of both systems in adipogenesis and obesity, and suggest that modulation of proteolytic activity may affect development of adipose tissue.

### Keywords

Animal models, fibrinolysis inhibitors, gene knock-out, matrix metalloproteinases, obesity

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## Introduction

Over the last decades obesity and its consequences worldwide have become a major health problem. Between 1976 and 2002, the prevalence of overweight (body mass index [BMI] = 25 kg/m<sup>2</sup>) in the United States has increased from 46% to 66% of the population, and that of obesity (BMI ≥ 30 kg/m<sup>2</sup>) from 15% to 31% (1). Excess weight increases the risk of multiple conditions, including hypertension, cardiovascular and cerebrovascular disease, type 2 diabetes, certain types of cancer, gallstones and osteoarthritis. Obesity is frequently associated with metabolic abnormalities such as impaired glucose tolerance, hyperinsulinemia, dyslipidemia with elevated triglyceride level, decreased high-density lipoprotein cholesterol concentration and increased proportion of small dense lipoparticles. This cluster of metabolic disturbances is called the metabolic syndrome, and represents known risk factors for cardiovascular disease. In addition, obesity negatively affects physical functioning, vitality, and general quality of life (2, 3).

Formation of adipose tissue is a complex process requiring designation of mesodermal stem cells to a preadipocyte lineage and differentiation of preadipocytes into adipocytes. These and other changes lead to rounding and formation of intracellular lipid droplets, which lead to a cellular morphogenesis. Adipocytes secrete compounds that are involved in the control of blood flow and angiogenesis and contribute to proliferation and differentiation of adipose precursor cells. Differentiation is also as-

sociated with an increase in the secretion of basement membrane components such as laminin, proteoglycans and type IV collagen. Differentiating adipocytes create cellular extensions, migrate from the stroma, form cell-cell junctions and become organized into three-dimensional multicellular structures, a process that is associated with migration through and remodeling of the original extracellular matrix (ECM) (4, 5).

Development of obesity is associated with extensive modifications in adipose tissue involving adipogenesis, angiogenesis and proteolysis of the ECM (6). Proteolytic systems, such as the plasminogen/plasmin (fibrinolytic) and matrix metalloproteinase (MMP) system, contribute to tissue remodeling by degradation of the ECM and basement membrane components or by activation of latent growth factors. Specific molecular interactions between both proteolytic systems suggest that they may cooperate in achieving ECM degradation. Proteinases of both systems are collectively able to cleave a wide variety of substrates, including ECM components, other proteinases and their inhibitors, and matrix receptors, whereby adipose tissue remodeling may be facilitated. Furthermore, adipocytes are surrounded by a basement membrane, that has to be extensively remodeled in order to allow the hypertrophic development of adipocytes observed in obesity. MMPs and plasmin can also release, activate or degrade several growth factors and cytokines implicated in obesity and play major roles in angiogenesis. Recently, some evidence has emerged that proteins of the ADAM and ADAMTS families may also be implicated.

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## The fibrinolytic system and adipose tissue development

The fibrinolytic system comprises an inactive proenzyme, plasminogen, that can be converted to the active enzyme, plasmin, that degrades fibrin into soluble fibrin-degradation products. Two immunologically distinct plasminogen activators have been identified: tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA). t-PA-mediated plasminogen activation is mainly involved in the dissolution of fibrin in the circulation. u-PA binds to a specific cellular receptor (u-PA-R or CD87) resulting in enhanced activation of cell-bound plasminogen. Inhibition of the fibrinolytic system may occur either at the level of plasmin by  $\alpha_2$ -antiplasmin, or at the level of the plasminogen activators, by plasminogen activator inhibitors (PAI-1 and PAI-2) (7).

### Role of fibrinolytic components in adipose tissue development

Several nutritionally induced obesity models in transgenic mice have been used to study the role of the fibrinolytic system in the development of obesity. t-PA-deficient mice, kept on high-fat diet, had higher body weight and adipose tissue mass than wild-type controls (8). This was associated with an increase in subcutaneous (SC), but not in gonadal (GON) fat depots. The increase was mostly due to hypertrophy and not to hyperplasia of adipocytes. There was also an increase in the number of stroma and endothelial cells, both in the SC and GON fat tissues, suggesting that targeted inactivation of t-PA increases angiogenesis in the adipose tissue, which may promote adipose tissue formation. Deficiency in u-PA, in contrast, had no effect on nutritionally induced obesity (8), although it was previously shown that overexpression of u-PA in the brain resulted in reduced body weight and size (9). Mice deficient in plasminogen, the substrate for both plasminogen activators, showed reduced fat accumulation associated with reduced differentiation of stromal cells (10). Deficiency of  $\alpha_2$ -antiplasmin had no significant effect on adipose tissue development in mice (11).

PAI-1 is expressed in murine and in human adipose tissue (12–14). In human adipose tissue, its expression is positively correlated with BMI (15). Increased levels of PAI-1 are also observed in patients with the insulin resistance syndrome (16), and improving the lipid profile by diet, exercise or antidiabetic drugs results in reduced PAI-1 levels and enhanced fibrinolytic activity (17). In obese premenopausal women, changes in plasma PAI-1 levels during body weight reduction and body weight regain are correlated with changes in the amount of body fat (18). Circulating PAI-1 is increased in obese subjects with the metabolic syndrome, as well as in patients with type 2 diabetes. The more severe the metabolic syndrome, the higher the plasma level of PAI-1 (19).

The role of PAI-1 in adipose tissue development at present still remains controversial. PAI-1-deficient mice kept on a high-fat diet developed between 3 and 8 weeks of the high-fat diet more adipose tissue than their wild-type counterparts, whereas this difference disappeared at later time points (20). A significantly lower number of stroma cells and of endothelial cells was

observed in the SC and GON fat depots of PAI-1-deficient mice, suggesting a lower degree of angiogenesis. To further elucidate the role of PAI-1 in the development of obesity, transgenic mice were generated with overexpression of murine PAI-1 under control of the adipocyte-specific promoter aP2. High circulating PAI-1 levels and reduced fibrinolytic activity in adipose tissue resulted in a reduction of nutritionally induced obesity (21). This finding is in agreement with a study of Eren et al. (22) who showed that transgenic mice overexpressing a stable human PAI-1 variant had virtually no intraperitoneal fat. Significant adipocyte hypotrophy was observed in the SC adipose tissue of PAI-1transgenic mice on high-fat diet, and the ratio of stroma cells versus adipocytes was significantly lower both in SC and GON adipose tissue of transgenic compared with wild-type mice. Analysis of blood vessels did not reveal significant differences. Overexpression of PAI-1 thus seems to modify the cellularity of adipose tissue, however, without significantly affecting angiogenesis. It may enhance expression of anti-differentiation factors such as Pref-1, which may contribute to reduced tissue mass (21).

In contrast to these studies, disruption of the *PAI-1* gene in genetically obese and diabetic *ob/ob* mice reduced adiposity and improved the metabolic profile (23). Another study reported that PAI-1-deficient mice on a high-fat diet developed less obesity than wild-type controls, and downregulation of PAI-1 by an angiotensin type 1 receptor antagonist in wild-type mice ameliorated diet-induced obesity (24). Thus, there is a difference between genetically determined obesity models (mainly *ob/ob* or *db/db* mice lacking leptin or its receptor) and nutritionally induced models in wild-type mice with intact genome. Leptin provides a local angiogenic signal and improves the efficiency of lipid release from fat stores to maintain energy homeostasis (25). The discrepancies between these studies may also be explained in part by the fact that nutritionally induced models depend on the composition and timing of the diet, and on the age and genetic background of the mice used. Recently, the role of PAI-1 was reinvestigated with the use of PAI-1-deficient mice and true littermate wild-type controls in an identical genetic background (26). When kept on high-fat diet for 15 weeks, there was no difference between both genotypes in body weight and in weight of the SC adipose tissue, whereas the GON fat mass was larger in PAI-1-deficient mice. It can not be excluded that modifier genes in a specific genetic background affect the outcome of such obesity studies.

The role of PAI-1 was also investigated on *de novo* adipogenesis *in vivo* (27). PAI-1 inhibition had no effect on *de novo* fat pad formation after subcutaneous injection of 3T3-F442A preadipocytes in the back of NUDE mice, kept on high-fat diet for four weeks. Weight gain, as well as the weights of the *de novo* fat pads, SC and GON adipose tissues were comparable in both groups. Injection of Matrigel together with bFGF resulted in *de novo* formation of fat pads in both wild-type and PAI-1-deficient mice kept on a high-fat diet for four weeks. No differences were observed in fat pad weight, adipocyte size or density. These data thus do not show an effect of PAI-1 on adipogenesis *in vivo*. Liang et al. investigated the effects and underlying mechanisms of PAI-1 on glucose uptake in adipocytes and on adipocyte differentiation, using primary cultured adipocytes from wild-type or

PAI-1-deficient mice, 3T3-L1 preadipocytes treated with a neutralizing anti-PAI-1 antibody or stably transfected with a PAI-1 adenoviral construct. Collectively, the results from these studies indicated that absence of PAI-1 in adipocytes protects against insulin resistance by promoting glucose uptake and adipocyte differentiation via increased PPAR- $\gamma$  expression (28). In contrast, differentiation of 3T3-F442A preadipocytes was not affected by a neutralizing PAI-1 antibody or by overexpression of PAI-1, and differentiation of PAI-1-deficient murine embryonic fibroblasts into mature adipocytes was comparable to wild-type cells (27).

A recent study has demonstrated that PAI-2 is also expressed in murine adipose tissue and that its deficiency results in impaired adipose tissue development in mice fed a high-fat diet. The mechanism appears to be independent of the antifibrinolytic activity of PAI-2 (29).

### Modulation of fibrinolytic activity

Tiplaxtinin, designed as a synthetic inhibitor of PAI-1, reduced body weight in wild-type mice kept on high-fat diet (30). The weights of the isolated SC and GON fat deposits were also significantly reduced, associated with adipocyte hypotrophy. Inhibitor-treated adipose tissues displayed similar blood vessel size, but a higher blood vessel density. Other studies showed that tiplaxtinin, in a model of diet-induced obesity, exhibited a dose-dependent reduction in body weight, epididymal adipose tissue weight, adipocyte volume, and circulating active plasma PAI-1 (31). Plasma glucose, triglycerides and leptin levels were significantly reduced by tiplaxtinin, whereas insulin resistance tests revealed lower glucose levels at the end of the test. These data suggest that pharmacological inhibition of PAI-1 could be beneficial in diseases associated with expansion of adipose tissue mass and could lead to an improvement of the metabolic syndrome. Therefore PAI-1 inhibitors will be needed that target active circulating PAI-1 as well as PAI-1 bound to vitronectin.

## The MMP system and adipose tissue development

The MMPs belong to a family of over 25 neutral endopeptidases that are collectively able to cleave all of the ECM components as well as several non-ECM proteins, such as adhesion molecules, cytokines, protease inhibitors and other (pro) MMPs. Generally, MMPs are expressed at low levels but are rapidly induced at times of active tissue remodeling. Most MMPs are secreted as inactive proenzymes and require proteolytic processing to become active. MMP activity is modulated through interactions with tissue inhibitors of MMPs (TIMPs). Four TIMPs have been characterized that are able to inhibit the activities of all known MMPs. Consequently, the net MMP activity in tissues is locally determined by the balance between the levels of activated MMPs and TIMPs (32).

### Role of MMP system components in adipose tissue development

Several lines of evidence suggest a potential role of MMPs in the development of adipose tissue. Conditioned medium of rat adipocytes contains a MMP-2 (gelatinase A) like gelatinolytic ac-

tivity, that may play a role in their organization into large multicellular clusters (33). High expression of MMP-2 was reported in adipose tissue of mice with nutritionally induced obesity as well as in genetically obese mice (5). MMP-2 and MMP-9 expression and secretion have also been demonstrated in human adipose tissue (34). Furthermore, MMP-2 increases and TIMP-1 decreases during adipocyte differentiation (35).

To gain further insight into the involvement of the MMPs in the development of adipose tissue, the expression of MMPs and TIMPs was monitored in lean and obese mice (36). This revealed upregulation with obesity of mRNA levels of some MMPs (MMP-3, -11, -12, -13, -14) and downregulation of others (MMP-7, -9, -16, -24). Most of these modulations were specific to the GON fat, supporting the concept that the different fat depots (SC and GON) are not identical.

TIMP-1, which is synthesized by most types of connective tissue cells as well as macrophages, acts against all members of the collagenase, stromelysin and gelatinase classes. Analysis of mRNA expression in adipose tissue of lean and obese mice revealed significant upregulation of TIMP-1 with obesity. In contrast TIMP-4 was downregulated with obesity, whereas TIMP-2 and TIMP-3 expression levels were not significantly modulated, at least in GON adipose tissue (36).

Several nutritionally induced obesity models were used to study the role of MMPs and TIMP-1 in the development of adipose tissue. Inactivation of the stromelysin-1 (MMP-3) gene in mice leads to enhanced development of adipose tissue when fed a high-fat diet (37). The higher body weight of MMP-3-deficient mice resulted essentially from a specific increase of their adiposity, characterized by hypertrophic adipocytes in the SC and GON fat pads. A higher blood vessel density was observed in the adipose tissue of MMP-3-deficient mice, suggesting that MMP-3 affects adipose tissue-related angiogenesis. A regulatory role of MMP-3 has also been suggested in adipogenesis during mammary gland involution in mice; mice with MMP-3 deficiency showed accelerated differentiation and hypertrophy of adipocytes (38). These data thus suggest an inhibitory effect of MMP-3 on adipocyte metabolism and differentiation. Similar results were seen with inactivation of the stromelysin-3 (MMP-11) gene. Indeed, MMP-11 deficiency promoted adipose tissue development and resulted in adipocyte hypertrophy (39). We have recently shown that MMP-2-deficient mice when kept on a high-fat diet, but not MMP-9-deficient mice, show significantly reduced obesity associated with adipocyte hypotrophy, without effect on angiogenesis (unpublished results).

TIMP-1-deficient mice on a high-fat diet gained less weight than their wild-type counterparts and developed less adipose tissue (40). Plasma leptin levels were significantly elevated in wild-type as compared to TIMP-1-deficient mice on high fat diet. Leptin acts as a satiety factor and increases energy expenditure, while its secretion is strongly correlated with body fat mass and adipocyte size (41). This suggests an effect of TIMP-1 deficiency on leptin secretion; alternatively, the lower leptin levels may be related to the lower body fat mass in the TIMP-1-deficient mice. To further substantiate a role of TIMP-1 in nutritionally induced obesity, the effect of TIMP-1 overexpression by adenoviral gene transfer in mice was studied on adipogenesis and adipose tissue development (42). Long-term expression of highly

elevated levels of human TIMP-1 was associated with reduced MMP activity in plasma, as well as in adipose tissue. There was no significant effect on body weight or fat pad mass when the mice were kept on high-fat diet. This is somewhat surprising since TIMP-1 deficiency resulted in impaired adipose tissue development. However, it is possible that physiologic TIMP-1 concentrations in mice are sufficient to promote adipogenesis and adipose tissue development, whereas overexpression has no further effect, and deficiency results in impairment.

### Modulation of MMP activity

The effect of relatively gelatinase-specific or broad-spectrum MMP inhibitors was studied on development of adipose tissue. Administration of galardin, a hydroxamate-based broad-spectrum MMP inhibitor, to wild-type mice kept on high fat diet resulted in significant reduction of adipose tissue weight (43). Ro 28-2653, a synthetic MMP inhibitor with enhanced selectivity for MMP-2, MMP-9 and MMP-14, did not affect adipose tissue development significantly (44). In contrast, genetically obese *ob/ob* mice treated with the MMP inhibitor Bay 12-9566 gained somewhat less weight than controls (45). The more specific gelatinase inhibitor Tolymsam also significantly reduced body weight and adipose tissue mass in the nutritionally induced obesity model in mice. This was associated with significant adipocyte hypotrophy (unpublished results). Proteinase inhibitor treatments revealed a specific role of MMP-9 in the differentiation of human adipocytes (46). However, MMP-9 deficiency in mice did not affect adipose tissue development on high-fat diet. Taken together, available data suggest the potential to impair adipose tissue development by using MMP inhibitors. Specific inhibitors, targeting the MMPs that play key roles in adipose tissue development, will be required to further explore the potential to affect obesity.

## ADAM and ADAMTS families and adipose tissue development

The ADAM (A Disintegrin And Metalloproteinase) family comprises proteins containing disintegrin-like and metalloproteinase-like domains (47). The ADAMTS family includes a subset of ADAM proteins that contain a thrombospondin (TSP) motif (48). Several ADAM and ADAMTS family members are ex-

pressed in adipose tissue and during differentiation of preadipocytes. The expression of ADAM-17 (TACE or TNF- $\alpha$  converting enzyme), ADAMTS-1 and ADAMTS-8 was investigated in adipose tissue of lean (on standard-fat diet) and obese (on high-fat diet) mice, and during differentiation of murine preadipocytes. In SC adipose tissue of obese mice, the expression of ADAM-17 was enhanced and that of ADAMTS-1 reduced, whereas in GON adipose tissue expression of ADAMTS-8 was reduced. During differentiation of murine 3T3-F442A preadipocytes, expression of ADAM-17 and ADAMTS-1 remained virtually unaltered, whereas that of ADAMTS-8 decreased as adipocytes matured (49). Aggrecan, a chondroitin sulphate/keratan sulphate proteoglycan, is highly expressed in cartilage, and aggrecan mRNA was also detected in SC and GON adipose tissues of mice (50). ADAMTS-4 and ADAMTS-5 can degrade aggrecan and are also expressed in murine adipose tissue. In mice with nutritionally induced obesity as well as in lean controls, aggrecan mRNA expression was downregulated, whereas ADAMTS-4 and ADAMTS-5 were upregulated with time. In mice with genetically determined obesity (*ob/ob*), ADAMTS-5 mRNA was upregulated in both SC and GON adipose tissues, as compared to wild-type mice. Thus, aggrecan levels were high at the early stages of adipose tissue development in mice, whereas its production decreased and its degradation increased during development of obesity. A functional role of aggrecan in promoting early stages of adipogenesis is supported by the findings that it stimulated the differentiation *in vitro* of 3T3-F442A preadipocytes and the *de novo* accumulation *in vivo* of fat in Matrigel plaques injected into wild-type mice (50). Proteoglycans in the extracellular matrix of adipose tissue, such as aggrecan, may thus contribute to the regulation of lipid uptake and obesity in mice.

## Conclusion

Studies in transgenic mouse models as well as pharmacologic studies support a role of the fibrinolytic, and MMP systems in adipogenesis and obesity. Both systems may act directly on adipogenesis and adipose tissue development by cleaving a wide variety of ECM components and matrix receptors, thereby facilitating adipose tissue remodeling. Furthermore, plasmin can convert several proMMPs into active MMPs, which in turn can activate other proMMPs. The role of specific members of both families, however, remains to be determined.

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