Deficiency of tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) impairs nutritionally induced obesity in mice

H. Roger Lijnen, Diego Demeulemeester, Berthe Van Hoef, Désiré Collen, Erik Maquoi
Center for Molecular and Vascular Biology, University of Leuven, Leuven, Belgium

Summary
Tissue inhibitor of matrix metalloproteinase-1 deficient (TIMP-1$^{-/-}$) mice and wild-type (TIMP-1$^{+/+}$) controls were kept on a standard (SFD) or a high fat diet (HFD) for 15 weeks. At the time of sacrifice, TIMP-1$^{-/-}$ mice on HFD had a significantly lower body weight (29 ± 1.5 versus 41 ± 1.8 g, p <0.005), and significantly less subcutaneous (0.81 ± 0.19 versus 1.78 ± 0.21 g, p <0.05) and gonadal (0.87 ± 0.17 versus 1.85 ± 0.18 g, p <0.005) fat mass. These differences were much less pronounced for mice on SFD. On HFD but not on SFD, adipocyte diameters were significantly lower in the adipose tissue of TIMP-1$^{-/-}$ mice. Plasma leptin levels in TIMP-1$^{-/-}$ mice on HFD were significantly lower as compared to TIMP-1$^{+/-}$ mice, and strongly correlated with adipose tissue mass for both genotypes. Staining with an endothelial cell specific lectin revealed a significantly higher blood vessel density, larger stained area and vessel size in adipose tissue of TIMP-1$^{-/-}$ mice on HFD. This difference disappeared after normalization to the adipocyte number, suggesting that it does not represent a true enhancement of angiogenesis. Thus, in a murine model of nutritionally induced obesity, TIMP-1 promotes adipose tissue development.

Keywords
Tissue inhibitor of metalloproteinase-1, TIMP-1, adipose tissue, obesity, angiogenesis

Thromb Haemost 2003; 89: 249–55

Introduction

Obesity is a common disorder, and its related diseases such as non-insulin-dependent diabetes mellitus, atherosclerosis and hypertension are a major cause of death and disability in Western societies. Development of obesity is associated with extensive modifications in adipose tissue involving adipogenesis, angiogenesis and extracellular matrix (ECM) proteolysis (1). Proteolytic systems, e.g. the matrix metalloproteinase (MMP) system, contribute to tissue remodeling by degradation of ECM and basement membrane components or activation of latent growth factors (2-4). Gelatinolytic activity was observed in adipose tissue of mice with nutritionally induced obesity as well as in genetically obese mice (5) and also in human adipocytes (6). For rat adipocytes it was previously suggested that a MMP-2 (gelatinase A) like activity, may play a role in their organization into large multicellular clusters (7). In vivo, MMPs are inhibited by endogenous tissue inhibitors (TIMPs), of which 4 different types have been identified (8, 9). 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 (2, 8). Analysis of mRNA expression in adipose tissue of lean and obese mice revealed significant upregulation of TIMP-1 with obesity (10). TIMP-1 was mainly expressed in the stromal-vascular cells and only marginally in adipocytes (10). In order to establish a potential role of TIMP-1 in development of adipose tissue, we have studied the effect of *TIMP-1* gene inactivation in a mouse model of nutritionally induced obesity (11).

Correspondence to:
H.R. Lijnen,
Center for Molecular and Vascular Biology,
University of Leuven, Campus Gasthuisberg,
Herestraat 49, B-3000 Leuven, Belgium
Tel 32-16-345771, Fax 32-16-345990,
E-mail: roger.lijnen @med.kuleuven.ac.be

Received October 3, 2002
Accepted after revision November 30, 2002

Materials and methods

Experimental protocol

TIMP-1-deficient (TIMP-$1^{-/-}$) and wild-type (TIMP-$1^{+/+}$) mice were obtained by courtesy of P. Soloway (Roswell Park Cancer Institute, Buffalo, NY) and characterized as described (12, 13). They were maintained as outcrossed animals arising from F1 (C57Bl/6 × 129 SvJae) founders. Because of the X-linked inheritance pattern, male deficient mice are hemizygous (TIMP-$1^{-/0}$; for convenience we will always refer to TIMP-$1^{-/-}$ as homozygotes). Genotyping of offspring was performed by Southern blotting using tail tip DNA (data not shown).

Five week old male TIMP-$1^{+/+}$ and TIMP-$1^{-/-}$ mice were kept in microisolation cages on a 12 h day-night cycle and fed a standard fat diet (n = 4 or 5) (SFD, KM-04-k12, Muracon, Carfil, containing 13% kcal as fat, with a caloric value of 10.9 kJ per g) or a high fat diet (n = 10 or 11) (HFD, Harlan TD 88137, Zeiss, the Netherlands, containing 42% kcal as fat, and a caloric value of 20.1 kJ/g). Mice were weighed every week and sacrificed after 15 weeks, following overnight fasting, by i.p. injection of 60 mg/kg Nembutal (Abbott Laboratories, North Chicago, IL). Blood was collected from the retroorbital sinus with or without addition of trisodium citrate (final concentration 0.01 mol/L). Plasma and serum were stored at –20° C. Intra-abdominal (gonadal, GON) and inguinal subcutaneous (SC) fat pads were removed and weighed; portions were immediately frozen at –80° C for extraction, other portions were used for histology. Therefore, 15 μm cryosections and 7 μm paraffine sections were prepared.

Food intake was measured for 24 h periods (16 times during the diet), and expressed as g per 24 h per mouse (4 or 5 animals in each group). To evaluate physical activity, mice were placed in a separate cage equipped with a turning wheel linked to a computer to register full turning cycles in 72 h periods at 3 weekly intervals during the diet. Data are reported as number of cycles per 12 h (at night) (8 or 9 measurements with 4 or 5 mice in each group); the data were first averaged per mouse and are given as mean ± SEM of the number of animals studied.

All animal experiments were approved by the local ethical committee and were performed in accordance with the guiding principles of the American Physiological Society and the International Society on Thrombosis and Haemostasis (14).

Assays

The mean size of adipocytes was determined by computer-assisted image analysis in cryosections of adipose tissue stained with haematoxylin-eosin. For each animal, 3 to 5 areas in 4 different sections each were analyzed; the data were first averaged per section and then per animal. Staining of fibrillar collagen with Sirius red was performed on paraffine sections, and quantified by computer-assisted image analysis. Blood vessels were visualized using the biotinylated Bandeiraea (Griffonia) Simplicifolia BSI lectin (Sigma-Aldrich) (15). Nine to twelve sections were analyzed per animal and then averaged.

Extraction of adipose tissue was performed as described (16), and the protein concentration of the supernatants was determined (BCA assay, Pierce). Zymographic analysis of gelatinase activity in the extracts was performed as described (17); the lysis of the substrate gel was determined by computer-assisted image analysis and expressed in arbitrary units (AU) of lysis per mg total protein in the extract (16). In situ zymography on cryosections of adipose tissue using casein- or gelatin-containing gels was performed essentially as described (18). The substrate gel (0.5% agarose) contained 1.0 mg/mL resorufin-labeled casein (Boehringer Mannheim) or pig skin gelatin Oregon Green™ 488 conjugate (Molecular Probes, Leiden, The Netherlands). Overlays were analyzed by computer-assisted image analysis after incubation for 24-48 h in a moist chamber at 37° C and the lysis area was normalized to the total section area.

White blood cells, red blood cells, platelets, haemoglobin and haematocrit levels were determined using standard laboratory assays. PAI-1 antigen levels were measured with a specific home-made ELISA (19), and leptin was measured with a commercially available ELISA (R&D Systems, Abingdon, U.K.). Blood glucose concentrations were determined using Glucocard strips (Menarini Diagnostics), and triglyceride, total and HDL cholesterol levels were evaluated using routine clinical assays.

Statistical analysis

Data are expressed as mean ± SEM. Statistical significance for differences between groups was analyzed by non-parametric t-testing. Correlation analysis was performed using the nonparametric Spearman's rank correlation coefficient test. Values of $p < 0.05$ were considered statistically significant.

Results

Body weight, adipose tissue mass and cellularity

At 5 weeks of age, the body weight of the TIMP-$1^{+/+}$ mice (21 ± 0.34 g) was comparable to that of the TIMP-$1^{-/-}$ mice (20 ± 0.65 g). After 15 weeks on HFD, the body weight of the TIMP-$1^{-/-}$ mice was significantly lower than that of the TIMP-$1^{+/+}$ mice (Table 1 and Fig. 1), whereas after 15 weeks of SFD no difference was observed. The body weight gain on the HFD was significantly lower in TIMP-$1^{-/-}$ mice (11 ± 1.6 versus 23 ± 1.7 g, $p < 0.005$). The total weight of the isolated SC or GON fat depots was also significantly lower in the TIMP-$1^{-/-}$ mice on HFD, whereas on SFD the differences were less pronounced (Table 1). Analysis of the cellularity of the fat depots revealed significantly smaller adipocytes with higher density in the TIMP-$1^{-/-}$ mice on HFD as compared to the TIMP-$1^{+/+}$ mice,

Table 1: Effect of TIMP-1 deficiency in mice on body weight gain and on adipose tissue weight and cellularity after 15 weeks of SFD or HFD.

	SFD		HFD	
	TIMP-1$^{+/+}$	TIMP-1$^{-/-}$	TIMP-1$^{+/+}$	TIMP-1$^{-/-}$
Body weight (g)[a]	29.4±0.6	28.6±0.4	41±1.8	29±1.5***
Total SC fat (g)	0.18±0.009	0.13±0.004*	1.78±0.21	0.81±0.19**
Total GON fat (g)	0.29±0.035	0.20±0.032	1.85±0.18	0.87±0.17***
Adipocyte area (μm^2)				
SC	780±110	660±70	5,860±660	3,220±590**
GON	1,260±48	1,150±66	7,030±680	3,780±540**

[a]Body weight at time of sacrifice, after overnight fasting.

*, $p < 0.05$; **, $p < 0.01$ and ***, $p < 0.005$ versus TIMP-1$^{+/+}$.

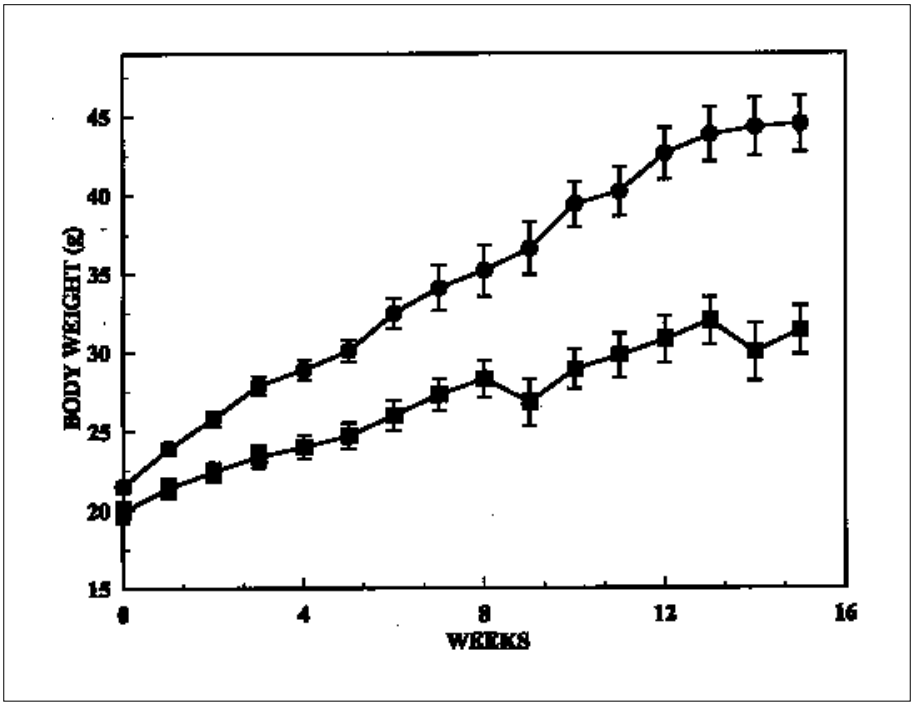

Figure 1: Increase in body weight as a function of time for TIMP-1$^{+/+}$ (●) or TIMP-1$^{-/-}$ (■) mice on a high fat diet. Data are mean ± SEM of 11 or 10 animals, respectively. The increase in body weight is significantly different from week 2 on ($p < 0.05$) and is highly significant at week 15 ($p < 0.001$).

whereas these differences were not observed in mice on SFD (Table 1). The percentage of body fat, calculated on the basis of the weight of the GON adipose tissue (20), was significantly lower in TIMP-1$^{-/-}$ mice on HFD (2.9 ± 0.41 versus 4.4 ± 0.38%, $p = 0.024$).

Glucose, total cholesterol and HDL cholesterol levels were lower in TIMP-1$^{-/-}$ mice on HFD than in the wild-type mice, whereas triglyceride levels were not different (Table 2). Plasma leptin levels were significantly higher in TIMP-1$^{+/+}$ mice on HFD as compared to TIMP-1$^{-/-}$ mice (Table 2). Overall for both genotypes combined, a strong correlation was observed between leptin levels and the weight of the SC or GON adipose tissue ($p < 0.00001$).

Haematologic parameters including white and red blood cells and platelet counts, haemoglobin and haematocrit were also undistinguishable (not shown).

	TIMP-1$^{+/+}$	TIMP-1$^{-/-}$
Glucose (mg/dL)	91 ± 5.3	72 ± 3.5*
Triglycerides (mg/dL)	87 ± 7.6	100 ± 16
Total cholesterol (mg/dL)	200 ± 13	150 ± 9.7*
HDL cholesterol (mg/dL)	150 ± 7.7	120 ± 6.1*
Leptin (ng/mL)	53 ± 8.4	12 ± 4.9**
PAI-1 (ng/mL)	2.8 ± 0.3	2.2 ± 0.2

* and **, $p < 0.05$ and $p < 0.005$ versus TIMP-1$^{+/+}$.

Table 2: Metabolic parameters of TIMP-1$^{+/+}$ and TIMP-1$^{-/-}$ mice after 15 weeks of HFD.

		Lysis/section area (%)	
		Gelatin	Casein
SC	TIMP-1$^{+/+}$	17 ± 1.8	21 ± 2.1
	TIMP-1$^{-/-}$	18 ± 1.9	19 ± 1.9
GON	TIMP-1$^{+/+}$	16 ± 1.8	21 ± 3.3
	TIMP-1$^{-/-}$	19 ± 2.6	16 ± 2.2

Data are mean ± SEM of 6 to 8 determinations.

Table 3: Gelatinolytic and caseinolytic activity in adipose tissue of mice kept on HFD for 15 weeks, as measured by in situ zymography.

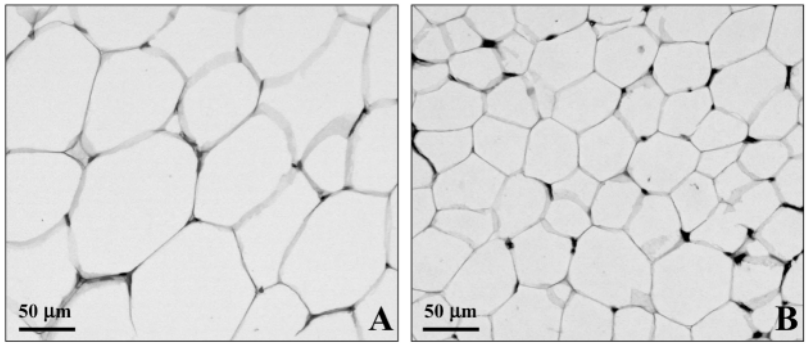

Figure 2: Staining with the Bandeirae Simplicifolia lectin of gonadal adipose tissue derived from TIMP-1$^{+/+}$ (A) or TIMP-1$^{-/-}$ (B) mice kept on HFD for 15 weeks.

Staining of adipose tissue sections with Sirius red revealed the presence of fibrillar collagen throughout the sections (not shown). Quantification of the stained area did not reveal significant differences between TIMP-$1^{+/+}$ and TIMP-$1^{-/-}$ mice, either on HFD (15 ± 4.8 versus 15 ± 4.7% for SC tissue and 11 ± 3.9 versus 12 ± 4.0% for GON tissue) or on SFD (22 ± 3.1 versus 25 ± 1.3% for SC tissue and 6.4 ± 0.75 versus 5.7 ± 0.55% for GON tissue).

Food intake and physical activity

The food intake of TIMP-$1^{+/+}$ and TIMP-$1^{-/-}$ mice was comparable, both on the SFD (4.9 ± 0.03 versus 4.6 ± 0.03 g/24 h) and the HFD (4.0 ± 0.18 versus 3.7 ± 0.09 g/24 h). The feeding efficiency (weight gain for food consumed) of TIMP-$1^{-/-}$ mice on the HFD thus appears to be lower than that of the TIMP-$1^{+/+}$ mice. The average physical activity at night was comparable for TIMP-$1^{+/+}$ and TIMP-$1^{-/-}$ mice on the SFD (8,600 ± 760 versus 10,700 ± 1,400 cycles/12 h) as well as on the HFD (11,600 ± 2,000 versus 13,600 ± 1,400 cycles/12 h).

Expression of gelatinase activity

Zymography on gelatin-containing gels of extracts of adipose tissue of mice on HFD revealed the presence of 70 kDa and 65 kDa proMMP-2 and of 58 kDa active MMP-2 species, whereas MMP-9 levels were not detectable (not shown). The total MMP-2 levels (all 3 forms combined) were significantly different for TIMP-$1^{+/+}$ or TIMP-$1^{-/-}$ mice in the SC tissue (140 ± 16 versus 75 ± 14 AU/mg, p = 0.009) but not in the GON tissue (160 ± 21 versus 120 ± 27 AU/mg). The ratio of active 58 kDa versus latent (70 kDa plus 65 kDa) MMP-2 was comparable in TIMP-$1^{+/+}$ and TIMP-$1^{-/-}$ SC tissue (36 ± 3.3 versus 44 ± 3.8%) or GON tissue (16 ± 2.0 versus 12 ± 2.6%).

In stitu zymography with gelatin-containing or casein-containing gels on cryosections of SC or GON adipose tissue did not reveal significant differences in gelatinolytic or caseinolytic activity of both genotypes kept on HFD (Table 3).

Adipose tissue related angiogenesis

Staining of SC or GON adipose tissue with an endothelial cell specific lectin revealed larger stained areas, vessel size and vessel density in TIMP-$1^{-/-}$ mice on HFD as compared to TIMP-$1^{+/+}$ mice (Table 4). These differences were not observed in TIMP-$1^{+/+}$ or TIMP-$1^{-/-}$ mice on SFD (not shown).

Discussion

To establish a potential role of TIMP-1 in development of adipose tissue, we have investigated the effect of *TIMP-1* gene inactivation in a murine model of nutritionally induced obesity. It was recently reported that TIMP-1 mRNA is expressed in adipose tissue of lean mice and that it is significantly upregulated by obesity (10). Furthermore, out of 16 MMPs and 4 TIMPs that were evaluated, only MMP-8 mRNA was not expressed in gonadal and subcutaneous adipose tissue. Striking modulation of the MMP/TIMP expression pattern in this model of nutritionally induced obesity suggested a functional role in the (patho)physiology of the adipose tissue (10). In addition, administration of the broad spectrum MMP inhibitor galardin to mice on high fat diet resulted in impaired adipose tissue development (21). Recently, it was reported that genetically obese *ob/ob* mice treated with the MMP inhibitor Bay12-9566 gained somewhat less weight than the controls (22). Since TIMP-1 inhibits a large array of MMPs, its inactivation may give relevant information on the role of the MMP/TIMP balance in obesity.

On normal chow (SFD), the body weight gain and development of adipose tissue was not strikingly different for TIMP-$1^{-/-}$ or TIMP-$1^{+/+}$ mice. However, when exposed to a high fat diet, TIMP-$1^{-/-}$ mice gained less weight than their wild-type counter-

Table 4: Characterization of blood vessels in subcutaneous (SC) and gonadal (GON) adipose tissue of TIMP-$1^{+/+}$ or TIMP-$1^{-/-}$ mice kept on HFD for 15 weeks.

	SC		GON	
	TIMP-$1^{+/+}$	TIMP-$1^{-/-}$	TIMP-$1^{+/+}$	TIMP-$1^{-/-}$
Stained area[a]	0.87 ± 0.08	2.3 ± 0.37*	0.58 ± 0.04	1.5 ± 0.07***
Vessel density[b]	200 ± 19	360 ± 59	120 ± 6.2	230 ± 22***
Vessel size[c]	44 ± 1.5	63 ± 2.6**	47 ± 2.6	67 ± 3.5**

(a) Lectin stained area in percent of the total area; (b) number of vessels per mm^2 tissue; (c) vessel size in μm^2.

*, ** and ***, $p < 0.05$, $p < 0.005$ and $p < 0.0005$ versus TIMP-$1^{+/+}$.

parts, and developed less adipose tissue. During the diet period, food intake and physical activity were comparable in the experimental groups. We also evaluated circulating leptin levels, since leptin acts as a satiety factor and increases energy expenditure, while its secretion is strongly correlated with body fat mass and adipocyte size (23). Plasma leptin levels were significantly elevated in TIMP-$1^{+/+}$ as compared to TIMP-$1^{-/-}$ mice kept on HFD; this may suggest an effect of TIMP-1 deficiency on leptin secretion, or may reflect merely the lower body fat mass in the TIMP-$1^{-/-}$ mice. Lower circulating leptin levels in the TIMP-$1^{-/-}$ mice did, however, not affect the food intake. For both genotypes a significant correlation between leptin levels and adipose tissue mass was maintained. Lower levels of glucose and cholesterol in the TIMP-$1^{-/-}$mice may also be related with lower fat mass. The lower body weight of the TIMP-$1^{-/-}$ mice may at least in part be explained by the reduced fat mass; it has indeed to be taken into account that not all the fat depots were removed from the body. On the basis of the 35% reduction in total body fat observed in the TIMP-$1^{-/-}$ mice, a significant contribution to the average 30% overall reduction in body weight can be anticipated.

Because gelatinase-like activity was consistently detected in adipose tissue (5-7) and may play a role in adipocyte differentiation (6), we have evaluated a potential effect of TIMP-1 deficiency on gelatinase activity. Overall, we did not observe striking differences in the adipose tissue of both genotypes, indicating that reduced gelatinolytic activity does not play a major role in the impaired adipose tissue development in TIMP-$1^{-/-}$ mice. There were also no differences in fibrillar collagen content in the adipose tissue, indicating that collagenolytic activity was not drastically higher in the TIMP-$1^{-/-}$ mice.

Development of adipose tissue is believed to be associated with angiogenesis (1), and it was recently shown in different obesity models that administration of angiogenesis inhibitors reduces adipose tissue mass, indicating that this can be regulated through the vasculature (22). In a companion study, we have shown that stromelysin-1 (MMP-3) in our murine model of nutritionally induced obesity impairs adipose tissue development, possibly by affecting adipose tissue related angiogenesis (24). The blood vessel size was comparable for wild-type and MMP-3 deficient mice, but the vessel density was significantly higher in the deficient mice. In the present study, we have observed a significantly higher blood vessel density and size in SC and GON adipose tissue of TIMP-$1^{-/-}$ as compared to control mice. Evidence has accumulated that, at least in tumors, microvessel density does not truely reflect angiogenic activity. It was suggested that the number of tumor cells that can be supported (in terms of nutrients and oxygen) by a blood vessel varies, influencing in turn the vascular density (25). Similarly, one could assume that the number and/or size of adipocytes in adipose tissue affects blood vessel density. This is supported by a study on capillary fenestrations in adipose tissue, showing that microvessel density is lower in obese ob/ob mice than in wild-type mice, possibly as a result of increased size of adipocytes in *ob/ob* mice (26). If we normalize the higher vessel density in SC or GON adipose tissue of TIMP-$1^{-/-}$ mice to the adipocyte number, the differences with the TIMP-$1^{+/+}$ mice are no longer observed. In contrast, in the study with MMP-$3^{-/-}$ mice, normalization of the vessel density to the adipocyte number still shows a higher density in the MMP-$3^{-/-}$ tissues. This suggests a real enhancement of angiogenesis in MMP-$3^{-/-}$ but not in TIMP-$1^{-/-}$ mice. The stimulating effect of TIMP-1 on adipose tissue development observed in this study may be due to an effect on other MMPs or on other (proteolytic) systems that regulate obesity. Interestingly, TIMP-1 can act a a growth factor for a wide range of cells (27), and it has been detected in the nucleus of, for example. fibroblasts during S-phase (28). TIMP-1 may thus affect cell proliferation. The mechanisms of such interactions remain however to be further studied.

Acknowledgements

Skillful technical assistance by F. De Cock, A. De Wolf and L. Frederix is gratefully acknowledged.

Part of this work was presented at the 16th Congress of the International Society on Fibrinolysis and Proteolysis, Munich, Germany, September 2002.

Financial support: This study was supported by a grant from the Inter-university Attraction Poles (IUAP, P5/02), and from the Flemish Fund for Scientific Research (FWO-Vlaanderen, G.0112.02).

References

1. Crandall DL, Hausman GJ, Kral JG. A review of the microcirculation of adipose tissue: anatomic, metabolic, and angiogenic perspectives. Microcirculation 1997; 4: 211-32.
2. Dollery CM, McEwan JR, Henney AM. Matrix metalloproteinases and cardiovascular disease. Circ Res 1995; 77: 863-8.
3. Carmeliet P, Collen D. Development and disease in proteinase-deficient mice: role of the plasminogen, matrix metalloproteinase and coagulation system. Thromb Res 1998; 91: 255-85.
4. Lijnen HR. Molecular interactions between the plasminogen/plasmin and matrix metalloproteinase systems. Fibrinolysis & Proteolysis 2000; 14: 175-81.
5. Brown LM, Fox HL, Hazen SA, LaNoue KF, Rannels SR, Lynch CJ. Role of the matrixin MMP-2 in multicellular organization of adipocytes cultured in basement membrane components. Am J Physiol 1997; 272: C937-49.
6. Lijnen HR, Maquoi E, Holvoet P, Mertens A, Lupu F, Morange P, Alessi MC, Juhan-Vague I. Adipose tissue expression of gelatinases in mouse models of obesity. Thromb Haemost 2001; 85: 1111-6.
7. Boulomié A, Sengenès C, Portolan G, Galitzky J, Lafontan M. Adipocyte produces matrix metalloproteinase 2 and 9. Involvement in adipocyte differentiation. Diabetes 2001; 50: 2080-6.
8. Willenbrock F, Murphy G. Structure-function relationships in the tissue inhibitors of metalloproteinases. Am J Respir Crit Care Med 1994; 150: S165-70.

9. Greene J, Wang MS, Liu YLE, Raymond LA, Rosen C, Shi YNE. Molecular cloning and characterization of human tissue inhibitor of metalloproteinase 4. J Biol Chem 1996; 271: 30375-80.
10. Maquoi E, Munaut C, Colige A, Collen D, Lijnen HR. Modulation of the expression of murine matrix metalloproteinases and their tissue inhibitors with obesity. Diabetes 2002; 51: 1093-101.
11. Lijnen HR, Maquoi E, Demeulemeester D, Van Hoef B, Collen D. Modulation of fibrinolytic and gelatinolytic activity during adipose tissue development in a mouse model of nutritionally induced obesity. Thromb Haemost 2002; 88: 345-53.
12. Soloway PD, Alexander CM, Werb Z, Jaenisch R. Targeted mutagenesis of TIMP-1 reveals that lung tumor invasion is influenced by TIMP-1 genotype of the tumor but not by that of the host. Oncogene 1996; 13: 2307-14.
13. Nothnick WB, Soloway P, Curry TE. Assessment of the role of tissue inhibitor of metalloproteinase-1 (TIMP-1) during the periovulatory period in female mice lacking a functional TIMP-1 gene. Biol Reprod 1997; 56: 1181-8.
14. Giles AR. Guidelines for the use of animals in biomedical research. Thromb Haemost 1987; 58: 1078-84.
15. Laitinen L. Griffonia simplicifolia lectins bind specifically to endothelial cells and some epithelial cells in mouse tissues. Histochem J 1987; 19: 225-34.
16. Lijnen HR, Van Hoef B, Lupu F, Moons L, Carmeliet P, Collen D. Function of plasminogen/plasmin and matrix metalloproteinase systems after vascular injury in mice with targeted inactivation of fibrinolytic system genes. Arterioscler Thromb Vasc Biol 1998; 18: 1035-45.
17. Kleiner DE, Stetler Stevenson WG. Quantitative zymography: detection of picogram quantities of gelatinases. Anal Biochem 1994; 218: 325-29.
18. Gallis ZS, Sukhova GK, Libby P. Microscopic localization of active proteases by in situ zymography: detection of matrix metalloproteinase activity in vascular tissue. FASEB J 1995; 9: 974-80.
19. Declerck PJ, Verstreken M, Collen D. Immunoassay of murine t-PA, u-PA and PAI-1 using monoclonal antibodies raised in gene-inactivated mice. Thromb Haemost 1995; 74: 1305-9.
20. Rogers P, Webb GP. Estimation of body fat in normal and obese mice. Br J Nutr 1980; 43: 83-6.
21. Lijnen HR, Maquoi E, Hansen LB, Van Hoef B, Frederix L, Collen D. Matrix metalloproteinase inhibition impairs adipose tissue development in mice. Arterioscler Thromb Vasc Biol 2002; 22: 374-9.
22. Rupnick MA, Panigrahy D, Zhang C-Y, Dallabrida SM, Lowell BB, Langer R, Folkman MJ. Adipose tissue mass can be regulated through the vasculature. Proc Natl Acad Sci USA 2002; 99: 10730-5.
23. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature 1998; 395: 763-70.
24. Maquoi E, Demeulemeester D, Vörös G, Collen D, Lijnen HR. Enhanced nutritionally induced adipose tissue development in mice with *stromelysin-1* gene inactivation. Thromb Haemost 2003; 89 (in press).
25. Hlatky L, Hahnfeldt P, Folkman J. Clinical application of antiangiogenic therapy: microvessel density, what it does and doesn' tell us. J Natl Cancer Inst 2002; 94: 883-93.
26. Cao R, Brakenhielm E, Wahlestedt C, Thyberg J, Cao Y. Leptin induces vascular permeability and synergistically stimulates angiogenesis with FGF-2 and VEGF. Proc Natl Acad Sci USA 2001; 98: 6390-5.
27. Hayakawa T, Yamashita K, Tanzawa K, Uchijima E, Iwata K. Growth-promoting activity of tissue inhibitor of metalloproteinase-1 (TIMP-1) for a wide range of cells. A possible new growth factor in serum. Febs Lett 1992; 298: 29-32.
28. Zhao WO, Li H, Yamashita K, Guo XK, Hoshino T, Yoshida S, Shinya T, Hayakawa T. Cell cycle-associated accumulation of tissue inhibitor of metalloproteinase-1 (TIMP-1) in the nucleiof human gingival fibroblasts. J Cell Sci 1998; 111: 1147-53.