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RAPID COMMUNICATION

Effect of weight loss on gelatinase levels in obese mice

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SUMMARY

1. Gelatinases (matrix metalloproteinase (MMP)-2 and MMP-9) might play a role in the development and structural organization of adipose tissue. Obese mice were subjected to caloric restriction (from 82 to 27 kJ/day) for 6 weeks, in order to investigate the effect of drastic weight loss on gelatinase mRNA, protein and activity levels.

2. Caloric restriction resulted in significantly lower bodyweight, as well as subcutaneous (SC) and gonadal (GON) fat mass (all $P < 0.001$).

3. The expression of MMP-2 mRNA was significantly upregulated in both SC (3.3-fold) and GON (2.2-fold) adipose tissues; however, without significant effect on total MMP-2 protein levels in fat tissues or in plasma. In contrast, MMP-9 mRNA expression in SC or GON fat was not affected by caloric restriction, whereas protein levels were reduced in SC and GON fat, but not in plasma. Zymography showed significantly elevated levels of active MMP-2 in both SC and GON adipose tissues, whereas active MMP-9 levels were reduced in GON fat.

4. These findings imply that the evaluation of the role of gelatinases in obesity and metabolic disorders requires the determination of both antigen and activity levels in plasma and fat tissues.

Key words: adipose tissue, gelatinases, matrix metalloproteinase, obesity.

INTRODUCTION

Gelatinases (matrix metalloproteinase (MMP)-2 and MMP-9) are expressed in rodent and human adipocytes.[1,2] MMP-2 (gelatinase A) activity plays a role in multicellular organization of adipocytes,[3] whereas a role for MMP-9 (gelatinase B) was reported in human adipocyte differentiation.[4] In a murine model of nutritionally-induced obesity, deficiency of MMP-2 impaired adipose tissue development,[5] whereas MMP-9 deficiency had no significant effect.[6] In obese patients, plasma levels of MMP-2 and MMP-9 antigen are significantly higher than in lean controls.[7] Pronounced weight loss in morbidly obese subjects after gastric banding[8] or a low energy diet[9] was associated with markedly reduced plasma or serum levels of MMP-9 antigen; in contrast, serum MMP-2 levels were not affected by bariatric surgery.[10] At present, no data are, however, available on the effect of weight loss on MMP-2 and MMP-9 antigen and activity levels in adipose tissues. Therefore, we have evaluated the effect of drastic weight reduction after caloric restriction on adipose tissue expression and activity of gelatinases, using a murine obesity model. The aim of the present study was to investigate the relationship between plasma antigen levels of MMP-2 and MMP-9 and adipose tissue expression and activity levels.

MATERIALS AND METHODS

Male C57Bl/6 wild-type mice were kept on a high fat diet (HFD; Harlan Teklad TD 88 137, Zeist, the Netherlands; 42% kcal as fat, caloric value 20.1 kJ/g) for 50 weeks in order to achieve morbid obesity and to allow marked weight reduction. Mice (bodyweight 59 ± 1.9 g) were then continued on a HFD *ad libitum* ($n = 7$), or were switched to a standard fat diet (SFD; KM-04-k12; Muracon, Carfil, Oud-Turnhout, Belgium; 13% kcal as fat, caloric value 10.9 kJ/g; $n = 8$) restricted to 2.5 g/day for 6 weeks. This corresponds to a reduction in energy intake from 82 to 27 kJ/day. At the end of the experiments, after 18 h fasting, mice were killed by intraperitoneal injection of 60 mg/kg Nembutal (Abbott Laboratories, North Chicago, IL, USA). Blood was collected through the retro-orbital sinus on trisodium citrate (final concentration 0.01 mol/L) and plasma was stored at −80°C. Subcutaneous (SC) and gonadal (GON) fat pads were removed and weighed; portions were snap frozen in liquid nitrogen for protein or RNA extraction. The experiments were approved by the local ethical committee (KULeuven P07071) and carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals (1996).

Matrix metalloproteinase (MMP)-2 and MMP-9 mRNA levels in SC and GON fat pads were determined by quantitative real time polymerase chain reaction as previously described[5,6] using Taqman gene expression assays (Applied Biosystems, Lennik, Belgium) for MMP-2 (Mm00439498_m1) or MMP-9 (Mm00442991_m1), with β-actin (Mm01205647_g1) as the housekeeping gene. MMP-2 and MMP-9 antigen levels were measured in plasma or in adipose tissue extracts using commercially available ELISA (R&D Systems Europe, Lille, France). The ELISA for mouse MMP-2 is based on antibodies against human MMP-2 and is calibrated with human pro

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MMP-2, whereas that for mouse MMP-9 is based on antibodies against mouse MMP-9 and calibrated with mouse MMP-9. Gelatinase activity in adipose tissue extracts was measured by gelatin zymography, as previously described.[5,6]

Data are reported as means ± SEM, and statistical significance between groups was evaluated by non-parametric Mann–Whitney *U*-test. Correlations were examined by the non-parametric Spearman's rank correlation coefficient. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Bodyweight of mice subjected to caloric restriction (SFD) for 6 weeks was significantly lower as compared with control mice that continued on the HFD (30 ± 1.7 vs 50 ± 2.5 g; $P < 0.001$). Both SC (700 ± 110 vs 2290 ± 180 mg) and GON (865 ± 150 vs 3140 ± 310 mg) fat mass were significantly reduced ($P < 0.001$). Plasma MMP-2 antigen levels were 71 ± 1.2 ng/mL at the start, as compared with 75 ± 2.7 ng/mL after caloric restriction and 64 ± 4.8 ng/mL for mice that continued on the HFD ($P = 0.07$); corresponding values for MMP-9 antigen were 59 ± 9.1 ng/mL at the start versus 87 ± 14 ng/mL after caloric restriction and 77 ± 15 ng/mL when continued on the HFD ($P = 0.61$).

Caloric restriction resulted in significant upregulation of the expression of MMP-2 mRNA in both SC (3.3-fold) and GON (2.2-fold) adipose tissue (Table 1). MMP-2 mRNA levels correlated negatively with SC ($r = -0.81$, $P = 0.0002$) and GON ($r = -0.85$, $P < 0.0001$) fat mass, whereas MMP-9 m-RNA levels also correlated negatively with GON ($r = -0.59$, $P = 0.02$), but not with SC ($r = -0.48$, $P = 0.09$) fat mass. In contrast, total MMP-2 antigen levels in SC or GON adipose tissues were not affected by caloric restriction, whereas total MMP-9 antigen levels were significantly reduced (Table 1). However, MMP-2 or MMP-9 antigen levels did not correlate with either SC or GON fat mass. Zymography with adipose tissue extracts (not shown) showed consistent detection of active MMP-9 and pro MMP-9, as well as active MMP-2, 72 kDa pro MMP-2 and 68 kDa pro MMP-2 (differently glycosylated forms). Both in SC and GON adipose tissues, active MMP-2 levels were significantly elevated after caloric restriction (Table 1). In SC fat, active MMP-2 after caloric restriction represented 38 ± 3.8% of total MMP-2 species as compared with 20 ± 8.1% for mice that continued on the HFD; corresponding values for GON fat were 28 ± 3.8% as compared with 13 ± 2.9%. Active MMP-2 levels correlated negatively with fat mass for both SC ($r = -0.70$, $P = 0.04$) and GON ($r = -0.65$, $P = 0.03$) adipose tissues.

In contrast, active MMP-9 levels were lower after caloric restriction in SC ($P = 0.43$) and GON ($P = 0.04$) fat. The contribution of active MMP-9 to total MMP-9 levels was not different between the SFD and HFD groups (43 ± 2.7% and 41 ± 5.7% for SC, 38 ± 1.9% and 42 ± 2.6% for GON). Active MMP-9 levels did not correlate with SC or GON fat mass.

DISCUSSION

Several lines of evidence suggest a functional role for the matrix metalloproteinase system in adipogenesis and adipose tissue development.[11] Furthermore, several components of the MMP system are affected by weight loss.[8–10] Thus, in plasma or serum of morbidly obese patients, drastic weight loss was associated with reduced antigen levels of MMP-9[8,9] and increased levels of MMP-7,[10] whereas MMP-2, MMP-3, TIMP-1, TIMP-2 and TIMP-4 were not affected.[10] However, no data are available on gelatinase levels in the fat tissues. In the present study, we therefore focused on the effect of drastic weight loss on MMP-2 and MMP-9 levels in adipose tissues of severely obese mice, a model that is commonly used in metabolic studies.[12] We also investigated the relationship between plasma antigen levels of gelatinases and adipose tissue levels of mRNA, antigen and activity. Both experimental groups are age-matched, precluding a confounding effect of ageing.

Several observations with respect to the effect of weight loss on gelatinases can be derived from our data: (i) Changes in mRNA and

Table 1 Effect of weight loss on gelatinase levels in murine adipose tissues

	SC		GON	
	HFD	SFD	HFD	SFD
mRNA (copy number)†				
MMP-2	187 ± 13	568 ± 68***	179 ± 9	393 ± 35**
MMP-9	39 ± 4	64 ± 8	15 ± 3	23 ± 2
Antigen (ng/mg)‡				
MMP-2	2.1 ± 0.18	2.4 ± 0.12	1.4 ± 0.05	1.8 ± 0.14
MMP-9	1.1 ± 0.15	0.73 ± 0.08*	0.94 ± 0.13	0.56 ± 0.07*
Active enzyme (AU)§				
MMP-2	4140 ± 1460	10 470 ± 1660*	2600 ± 550	8900 ± 1670*
MMP-9	8010 ± 2610	5500 ± 1660	10 180 ± 1270	6730 ± 700*
Pro-enzyme (AU)§				
72 kDa pro MMP-2	5970 ± 1880	4320 ± 840	8430 ± 1610	8890 ± 970
68 kDa pro MMP-2	11 250 ± 1240	12 110 ± 790	8210 ± 770	12 530 ± 910*
92 kDa pro MMP-9	8540 ± 2280	6350 ± 1510	12 850 ± 1080	10 390 ± 530

†mRNA expression levels are normalized to the expression of β-actin and shown as copy number relative to 10^5 copies of β-actin.
‡Total antigen levels expressed as ng/mg protein in adipose tissue extracts.
§Arbitrary units (AU) of gelatinolytic activity. Data represent means ± SEM.
*$P < 0.05$, **$P < 0.005$, ***$P < 0.0005$ versus the high fat diet.

protein levels in adipose tissue do not necessary occur in parallel. Thus, MMP-9 protein levels are reduced, whereas mRNA expression is not affected and MMP-2 protein levels are not affected, but mRNA expression is significantly upregulated. This might not be surprising, as protein translation can occur at different levels for both gelatinases; (ii) Effects on protein levels in fat and in plasma do not necessarily correspond. Thus, MMP-9 is reduced in fat, but not affected in plasma, whereas MMP-2 levels are not affected in fat or plasma. This suggests a different level of secretion of both MMP from fat tissue; (iii) Extrapolation from mice to humans should be carried out only with care. Whereas MMP-2 serum/plasma levels are not affected in either species, weight loss in obese patients is associated with reduced MMP-9 plasma levels, but no significant effect is seen in mice; (iv) The contribution of active MMP-2 and MMP-9 to total gelatinases can be variable. Thus, total MMP-2 antigen levels in SC or GON fat are not affected by caloric restriction, but active MMP-2 levels are significantly elevated; whereas for MMP-9, both antigen and activity levels are reduced by weight loss. It cannot be excluded that the ELISA used in the present study react differently withdifferent molecular forms of MMP-2 and/or MMP-9 (pro, active, complexed with inhibitors), which might account for some differences between antigen levels and activity measured by zymography.

It remains to be shown whether higher MMP-2 and lower MMP-9 activity levels in adipose tissues will affect matrix degradation, as many other proteinases can degrade one or more components of the extracellular matrix. The discrepancies between gelatinase mRNA, protein and activity levels on the one hand, and between plasma/-serum and adipose tissue levels on the other hand show that proper evaluation of gelatinases in models of obesity and metabolic diseases will require the determination of both antigen and activity levels in plasma/serum and in fat tissues.

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