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Macrophage elastase (MMP-12) in expanding murine adipose tissue

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ABSTRACT

Background: Matrix metalloproteinases (MMPs) are known to play a role in adipose tissue development, but little information is available on the role of individual proteinases. Expansion of adipose tissue is associated with an increased macrophage content. Macrophage elastase (MMP-12) has an important role in macrophage infiltration, which induces pro-inflammatory effects in adipose tissue.
Methods: The role of MMP-12 was investigated in adipose tissues of MMP-12 deficient and wild-type control mice kept on normal chow or on high fat diet for 15 weeks.
Results: MMP-12 deficiency had no significant effect on total body weight or on subcutaneous (SC) or gonadal (GON) adipose tissue mass. Adipocyte and blood vessel size and density in SC and GON adipose tissues of obese mice were also comparable in MMP-12 deficient and control mice. Macrophage infiltration in SC and GON adipose tissues was not affected by MMP-12 deficiency, but the amount of crown-like structures (CLS) was significantly lower. MMP-12 deficiency did not affect elastin content in the extracellular matrix of SC or GON adipose tissue.
Conclusions: Adipose tissue mass and composition in mice with nutritionally induced obesity was not markedly affected by MMP-12 deficiency, except for an apparently lower degree of CLS.
General Significance: MMP-12 does not seem to be essential for macrophage infiltration in adipose tissue, but contributes to the formation of CLS surrounding moribund adipocytes.

1. Introduction

Obesity and its associated diseases such a non-insulin dependent diabetes, atherosclerosis and cardiovascular disease are a main cause of mortality and morbidity. Development of adipose tissue is a complex process in which under control of growth factors, cytokines, adipokines, proteinases and hormones extensive modifications occur in adipogenesis, angiogenesis and proteolytic remodeling of extracellular matrix (ECM) [1]. Matrix metalloproteinases (MMPs) are known to play a role in these processes, but little is known on the contribution of individual MMPs [2].

MMPs belong to a family of over 20 neutral endopeptidases that are collectively able to cleave all ECM components as well as many non-ECM proteins [3]. Generally, MMPs are expressed at low levels but are rapidly induced at times of active tissue remodeling. On the basis of domain structure and substrate specificity MMPs are classified as collagenases, gelatinases, stromelysins, matrilysins, membrane-type MMPs and others. Most MMPs as well as their 4 known tissue inhibitors (TIMPs) are expressed in adipose tissue and many are up- or downregulated during development of obesity [4–6]. Thus, macrophage elastase (MMP-12; EC3.4.24.65) expression is upregulated in obese as compared to lean adipose tissue [4,6], and is expressed both in the adipocyte and in the stromal-vascular cell fractions [6]. MMP-12 is strongly associated with inflammatory diseases implicating macrophage infiltration. It contributes to macrophage migration through basement membranes, allowing their recruitment to inflammatory sites. MMP-12 is the most active MMP against elastin, but it also cleaves most other components of the ECM [7–9]. In adipose tissues of obese subjects, increased macrophage accumulation associated with enhanced expression of pro-inflammatory compounds was observed due to an influx of bone marrow derived macrophages [10–12]. Infiltrated macrophages surround moribund adipocytes as crown-like structures (CLS), thereby resorbing the lipid remnants [13–15]. Therefore, we hypothesized that MMP-12 may play a functional role in macrophage infiltration and development of adipose tissue. This was evaluated with the use of MMP-12 deficient mice.

2. Materials and methods

2.1. Diet model

MMP-12 deficient (MMP-12$^{-/-}$) mice were originally obtained as a kind gift from Dr. S. Shapiro (Washington University School of Medicine, St. Louis, MO). MMP-12$^{-/-}$ and littermate wild-type (MMP-12$^{+/+}$) mice (75% Swiss: 25% C57Bl/6J genetic background) were generated from heterozygous breeding couples and genotyped as described [16]. From birth on until 5 weeks of age, male mice were kept on a standard fat diet (SFD) (KM-04-k12, Muracon, Carfil, Oud-Turnhout, Belgium, containing 13% kcal as fat with a caloric value of 10.9 kJ per g). Five weeks old mice were then continued on SFD or switched to a high fat

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diet (HFD) for 15 weeks (TD88137, Harlan Taklad, Zeist, The Netherlands, containing 42% kcal as fat with a caloric value of 20.1 kJ per g).

Mice were kept in microisolation cages on a 12 h day/night cycle, fed ad libitum and weighed at weekly intervals. At the end of the experiment, the mice were killed by i.p. injection of 60 mg/kg sodium pentobarbital (Abbott Laboratories, North Chicago, IL). Blood was collected from the retro-orbital sinus on trisodium citrate (final concentration 0.01 mol/L) and plasma was stored at −20 °C. Epididymal (gonadal, GON) and inguinal (subcutaneous, SC) fat pads and lungs were removed and weighed; 10 μm paraffin sections were prepared for histology [17,18]. The weight of other organs was also recorded.

Food intake was measured at weekly intervals throughout the experimental period, and expressed as g per mouse and per day (averaged per mouse for 15 weeks and then averaged for the number of mice). Body temperature was monitored using a rectal probe (TR-100, Fine Science Tools, Foster City, CA). All animal experiments were approved by the local ethical committee and performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals (1996) and the guiding principles of the International Society of Thrombosis and Haemostasis [19].

2.2. Assays

Adipose tissue sections were stained with haematoxylin/eosin or Bandeiraea Simplicifolia lectin [20] using a standard protocol, followed by signal amplification with the Tyramide Signal Amplification Cyanine System (Perkin Elmer, Boston, MA). The number of adipocytes or blood vessels and their mean size were determined by computer-assisted image analysis, using for each animal 3–5 areas in 3 different sections; the data were first averaged per section and then per animal.

Macrophages were stained with F4/80 and expressed as percentage stained area per section area in adipose tissue, or counted in lung tissue sections. Density of crown-like structures (CLS) in adipose tissue was normalized to the section area. Apoptosis in adipose tissues was monitored by staining sections with rabbit anti-active mouse caspase-3 (ab13847) from Abcam (Cambridge, UK). Elastin was detected by Verhoeff-Van Gieson staining, and quantified by computer-assisted image analysis. Expression in extracts of SC and GON adipose tissues of macrophage markers, including TNF-α (Mm00443258_m1), IL-1 (Mm01336189_m1), Cd68 (Mm03047340_m1), F4/80 (Mm00802529_m1), Csf1r (Mm01266652_m1) and MCP-1 (Mm00441242_m1), and of the matrix metalloproteinases MMP-2 (Mm00439498_m1), MMP-9 (Mm00442991_m1) and MMP-12 (Mm01168718_m1) was monitored by quantitative RT-PCR using gene expression assays from Applied Biosystems (Foster City, USA). Similarly, expression levels of caspase-3 (Mm01195085_m1), GM-CSF (Mm01290062_m1), IFN-γ (Mm01168134_m1) and IL-4 (Mm00445259_m1) were determined. Fold differences in gene expression were calculated with the ΔΔCt method, using β-actin (Mm01205647_g1) as housekeeping gene.

MMP-2 and MMP-9 activity in adipose and lung tissue extracts was monitored by gelatin zymography and expressed as arbitrary units (AU), as described elsewhere [18]. Quantitative analysis of zymographies was performed by NIH ImageJ 1.46 software on scanned gels (Epson Perfection V700 PHOTO scanner, Nagano, Japan).

Blood glucose concentrations were determined using Glucocard strips (Menarini Diagnostics, Florence, Italy); trigyceride, total and HDL cholesterol levels, as well as liver enzymes (alkaline phosphatase, AST, ALT) were evaluated using routine clinical assays. Blood cell analysis was performed on a Cell-Dyn 3500 R (Abbott Diagnostics, Abbott Park, IL).

2.3. Statistical analysis

Data are expressed as means ± SEM. Statistical significance for differences between groups, analyzed by non-parametric Mann–Whitney *U*-testing, was set at $p<0.05$. Analysis was done with Prism 5 (GraphPad Software Inc., San Diego, CA).

Table 1
Effect of MMP-12 deficiency on organ weight and fat mass of mice kept on standard fat diet (SFD) or high fat diet (HFD) for 15 weeks.

	SFD		HFD	
	MMP-12$^{+/+}$	MMP-12$^{-/-}$	MMP-12$^{+/+}$	MMP-12$^{-/-}$
Body weight start (g)	30 ± 1.9	28 ± 1.7	27 ± 0.57	29 ± 0.65
Food intake (g/day)	5.9 ± 0.21	5.8 ± 0.13	5.2 ± 0.26	4.9 ± 0.22
Body weight end[a] (g)	38 ± 2.0	38 ± 1.8	54 ± 0.90	53 ± 1.2
SC fat weight (mg)	351 ± 51	445 ± 82	1405 ± 61	1374 ± 107
GON fat weight (mg)	808 ± 127	1038 ± 161	1304 ± 151	1616 ± 159
Liver (mg)	1453 ± 72	1437 ± 70	3678 ± 344	3382 ± 274
Heart (mg)	235 ± 20	205 ± 7.6	220 ± 3.9	238 ± 10
Spleen (mg)	91 ± 6.2	84 ± 6.2	139 ± 9.2	147 ± 14
Lung (mg)	271 ± 23	272 ± 14	277 ± 22	264 ± 18
Pancreas (mg)	395 ± 24	383 ± 33	508 ± 14	530 ± 25
Kidneys (mg)	731 ± 58	668 ± 15	687 ± 14	733 ± 43
Hypothalamus (mg)	73 ± 4.5	67 ± 5.8	82 ± 4.5	81 ± 4.4

Data are means ± SEM of 8 experiments in each group.
[a] Body weight after overnight fasting.

3. Results

Food intake throughout the 15 weeks diet was comparable for MMP-12$^{-/-}$ and MMP-12$^{+/+}$ mice ($n=8$ each) when kept either on SFD or HFD (Table 1). This resulted in comparable weight gain for both genotypes on SFD or HFD (Fig. 1 and Table 1), compatible with a similar feeding efficiency (weight gain normalized to caloric intake). The weight of other main organs was also comparable for both genotypes (Table 1). Metabolic parameters and liver enzymes after 15 weeks of SFD or HFD were not significantly different between MMP-12$^{+/+}$ and MMP-12$^{-/-}$ mice (Table 2). Analysis of blood cell composition including white blood cells, neutrophils, lymphocytes, monocytes, eosinophils, basophils, red blood cells, platelets, hemoglobin and hematocrit, did not reveal significant differences between both genotypes on HFD (data not shown). Body temperature was comparable for MMP-12$^{-/-}$ and MMP-12$^{+/+}$ mice on SFD (37.8 ± 0.24 °C versus 37.4 ± 0.19 °C) as well as on HFD (38.1 ± 0.16 °C versus 38.4 ± 0.13 °C).

The HFD resulted in significantly enhanced fat mass as compared to SFD for both genotypes, but MMP-12 deficiency did not affect development of SC or GON adipose tissue (Table 1). For obese MMP-12$^{-/-}$ and MMP-12$^{+/+}$ mice (HFD), adipocyte size or density in SC or GON adipose tissues was not significantly different (Fig. 2A and Table 3). Similarly, blood vessel size or density was not affected by MMP-12 deficiency (Table 3). Verhoeff-Van Gieson staining did not reveal significant effects of MMP-12 deficiency on the elastin content in the ECM of SC (5.2 ± 0.32% versus 4.1 ± 0.35% for MMP-12$^{+/+}$,

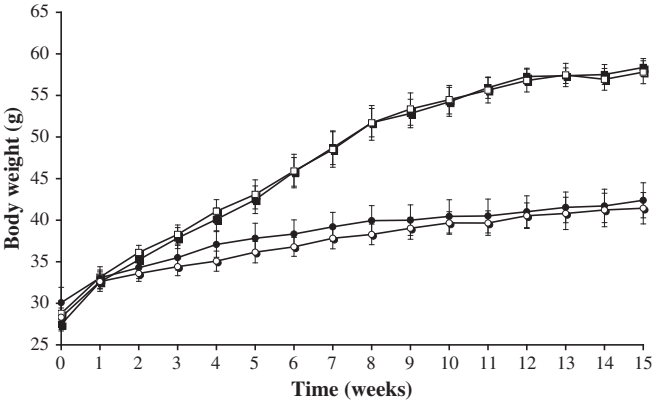

Fig. 1. Evolution of body weight over time of MMP-12$^{-/-}$ (open symbols) and MMP-12$^{+/+}$ (closed symbols) mice kept on standard fat diet (circles) or high fat diet (squares) for 15 weeks. Data are means ± SEM of 8 experiments in each group.

Table 2
Effect of MMP-12 deficiency on metabolic parameters of mice kept on standard fat diet (SFD) or high fat diet (HFD).

	SFD		HFD	
	MMP-12$^{+/+}$	MMP-12$^{-/-}$	MMP-12$^{+/+}$	MMP-12$^{-/-}$
Glucose (mg/dl)	105 ± 5.1	107 ± 8.4	109 ± 12	167 ± 26
Cholesterol (mg/dl)	40 ± 4.8	35 ± 3.0	88 ± 5.5	80 ± 6.3
HDL-cholesterol (mg/dl)	39 ± 4.2	36 ± 2.7	82 ± 4.3	75 ± 4.9
Triglycerides (mg/dl)	24 ± 2.3	23 ± 2.4	23 ± 2.7	30 ± 3.8
Alkaline phosphatases (U/l)	92 ± 7.1	88 ± 7.0	121 ± 18	91 ± 10
AST (U/l)	28 ± 3.3	25 ± 1.1	40 ± 2.9	36 ± 4.2
ALT (U/l)	17 ± 2.1	14 ± 0.7	36 ± 4.0	32 ± 3.9

Data are means ± SEM of 6–8 determinations.

$p = 0.06$) or GON (2.9 ± 0.57% versus 3.8 ± 0.37% for MMP-12$^{+/+}$, $p = 0.1$) adipose tissues of mice on HFD (Fig. 2B).

Diet-induced obesity led to a significantly increased macrophage content in GON adipose tissue of MMP-12$^{+/+}$ (1.40 ± 0.24% versus 0.26 ± 0.07% for SFD, $p<0.001$) as well as MMP-12$^{-/-}$ (0.74 ± 0.15% versus 0.17 ± 0.04% for SFD, $p<0.001$) mice. After HFD feeding, macrophage infiltration in SC adipose tissue was slightly increased in MMP-12$^{+/+}$ (0.45 ± 0.11% versus 0.22 ± 0.09% for SFD, $p = 0.08$) mice, but no difference was observed in MMP-12$^{-/-}$ (0.30 ± 0.06% versus 0.25 ± 0.03% for SFD, $p = 0.6$) mice. Comparison of wild-type and MMP-12$^{-/-}$ mice on HFD showed no difference in F4/80 immunostaining in SC (0.45 ± 0.11% versus 0.30 ± 0.06%, $p = 0.6$) adipose tissue, whereas a moderate decrease was observed in GON (1.40 ± 0.24% versus 0.74 ± 0.15%, $p = 0.055$) adipose tissue. In contrast, analysis of expression levels in MMP-12$^{-/-}$ mice on HFD demonstrated significantly lower expression of F4/80 in both SC and GON adipose tissues and significantly lower expression of Cd68 in SC adipose tissue, as compared to MMP-12$^{+/+}$ mice (Fig. 3). In addition, expression of the macrophage markers TNF-α, IL-1, MCP-1 and Csf1r, as well as GM-CSF and IFN-γ was comparable in SC and GON adipose tissues of both genotypes on HFD (Fig. 3).

MMP-12 mRNA was detected in SC and GON adipose tissues of MMP-12$^{+/+}$ but not of MMP-12$^{-/-}$ mice. Its expression in MMP-12$^{+/+}$ mice on HFD was upregulated 343-fold in SC adipose tissue and 22-fold in GON adipose tissue, as compared to MMP-12$^{+/+}$ mice kept on SFD. Expression of GM-CSF was significantly increased in SC fat of both wild-type (4.7-fold, $p = 0.03$) and MMP-12$^{-/-}$ (5.4-fold, $p = 0.008$) mice kept on HFD, as compared with mice fed SFD. Both MMP-12$^{+/+}$ and MMP-12$^{-/-}$ mice fed HFD showed a 2.4-fold increase in IFN-γ expression in GON fat, as compared to their respective counterparts on SFD. Expression of IL-4 was not detected in MMP-12$^{+/+}$ or MMP-12$^{-/-}$ mice kept on either SFD or HFD.

CLS were not consistently observed in SC or GON adipose tissue of mice kept on SFD. Determination of CLS for MMP-12$^{-/-}$ mice on HFD, however, revealed a significant reduction in SC ($4.3 \pm 0.4 \times 10^{-6}$/μm^2 versus $7.0 \pm 0.9 \times 10^{-6}$/μm^2, $p = 0.03$) and GON ($15.9 \pm 2.3 \times 10^{-6}$/μm^2 versus $26.4 \pm 2.8 \times 10^{-6}$/μm^2, $p = 0.003$) adipose tissue, as compared to MMP-12$^{+/+}$ mice (Fig. 2C). Expression of caspase-3 mRNA in SC and GON adipose tissue of mice kept on HFD was, however, not affected by MMP-12 deficiency (Fig. 3). Furthermore, immunostaining for active caspase-3 did not indicate differences in apoptosis between MMP-12$^{+/+}$ and MMP-12$^{-/-}$ adipose tissues (stained area of 0.4 ± 0.05% versus 0.44 ± 0.07% for SC, and 0.69 ± 0.08% versus 0.71 ± 0.2% for GON).

We also addressed the effect of MMP-12 deficiency on expression and activity of other MMPs. Expression of MMP-2 mRNA was 30% decreased in SC ($p = 0.07$) and GON ($p = 0.09$) adipose tissue of MMP-12$^{-/-}$ versus MMP-12$^{+/+}$ mice kept on HFD. Expression levels of MMP-9 were comparable for both genotypes (Fig. 3). Gelatin zymography with SC and GON adipose tissue extracts (not shown) did not reveal significant differences between MMP-12$^{+/+}$ and MMP-12$^{-/-}$ mice kept on HFD for 92 kDa proMMP-9, 72 kDa and

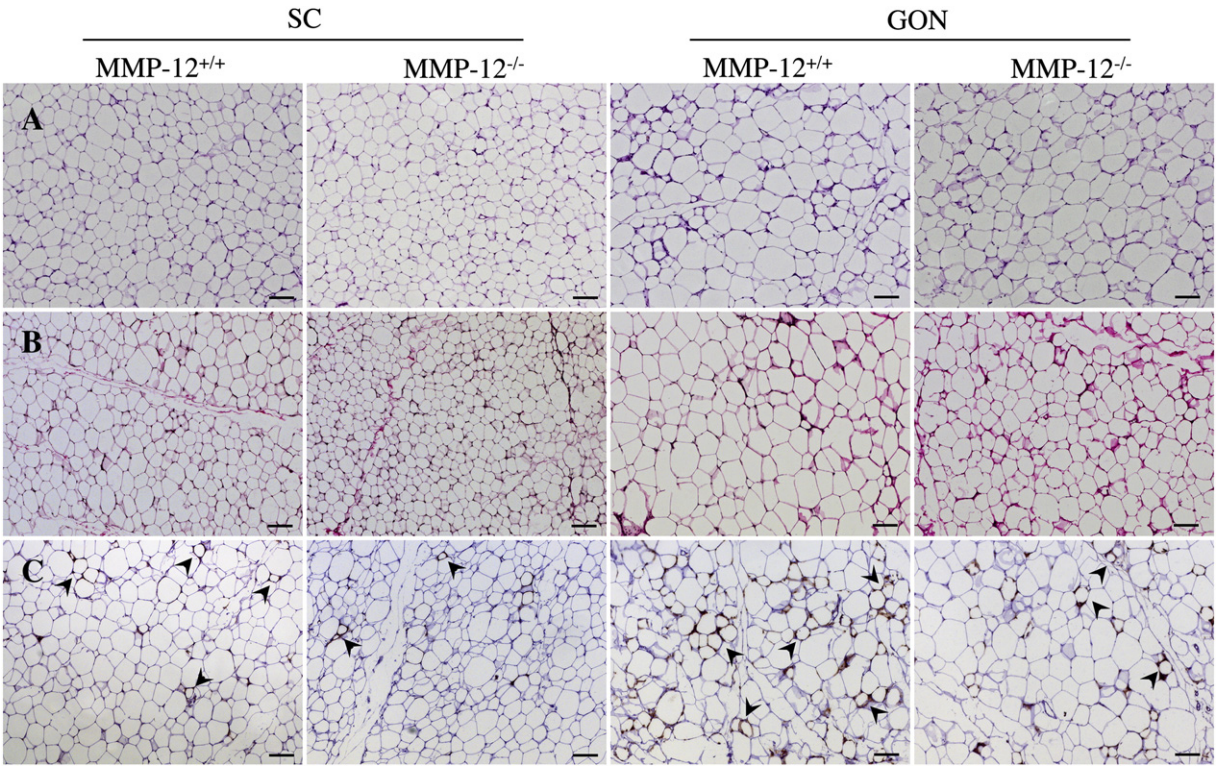

Fig. 2. Histological staining with haematoxylin/eosin (A), Verhoeff-Van Gieson (B) and F4/80 (C) of subcutaneous (SC) and gonadal (GON) adipose tissue sections from MMP-12$^{+/+}$ or MMP-12$^{-/-}$ mice kept on high fat diet. Arrowheads in panels C indicate crown-like structures. Scale bars correspond to 100 μm.

Table 3
Effect of MMP-12 deficiency on adipocyte and blood vessel size and density of mice kept on high fat diet for 15 weeks.

	MMP-12$^{+/+}$	MMP-12$^{-/-}$
Adipocyte size (μm^2)		
SC fat	4368 ± 241	3700 ± 395
GON fat	6290 ± 270	6928 ± 234
Adipocyte density ($\times 10^{-6}/\mu m^2$)		
SC fat	247 ± 14	295 ± 28
GON fat	163 ± 8.0	147 ± 5.0
Blood vessel size (μm^2)		
SC fat	59 ± 2.2	58 ± 1.4
GON fat	55 ± 2.1	60 ± 1.5
Blood vessel density ($\times 10^{-6}/\mu m^2$)		
SC fat	378 ± 35	362 ± 14
GON fat	259 ± 16	222 ± 12

Data are means ± SEM of 8 experiments.

68 kDa proMMP-2, or 62 kDa and 58 kDa active MMP-2 (Table 4). Active MMP-2 in the total measured gelatinase moieties corresponds to 26 ± 3.7% and 31 ± 3.6% for SC fat of MMP-12$^{+/+}$ and MMP-12$^{-/-}$ mice respectively, with corresponding values of 30 ± 5.0% and 29 ± 5.3% for GON fat.

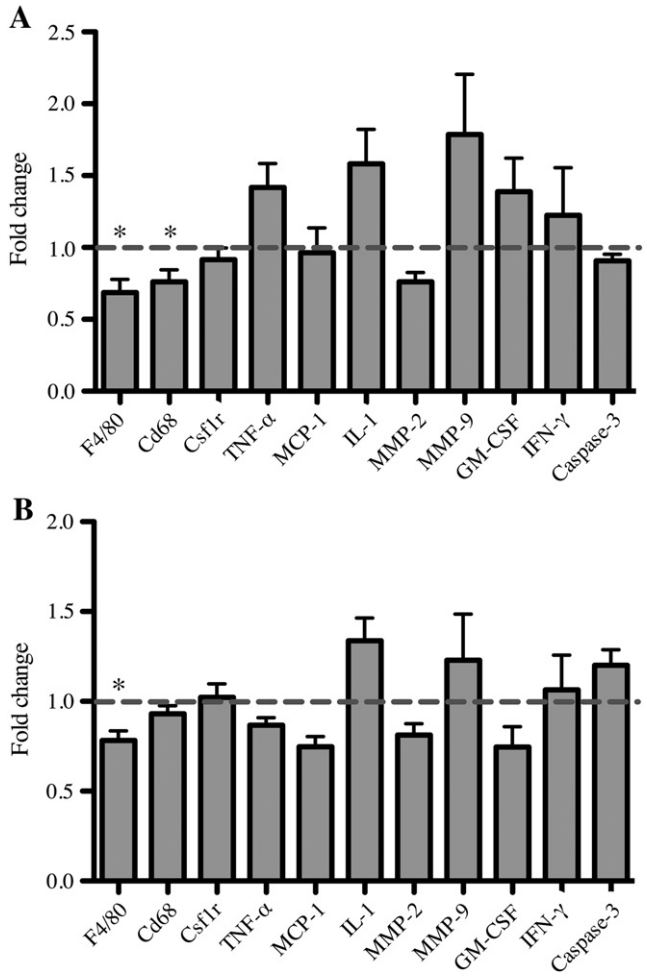

Fig. 3. mRNA expression levels in subcutaneous (A) or gonadal (B) adipose tissues from MMP-12$^{-/-}$ mice kept on high fat diet are shown as fold-change compared to wild-type MMP-12$^{+/+}$ controls. Data are means ± SEM; *p<0.05.

For comparison, lung tissue of MMP-12$^{+/+}$ and MMP-12$^{-/-}$ mice kept on HFD was analyzed. Gelatin zymography of lung tissue extracts (not shown) did not reveal significant differences between both genotypes for 92 kDa proMMP-9, proMMP-2 (only 68 kDa form detected) or active MMP-2 (only 58 kDa form detected) (Table 4). Active MMP-2 in the total measured gelatinase moieties in the lung thus corresponds to 29 ± 3.4% for MMP-12$^{+/+}$ and to 34 ± 1.6% for MMP-12$^{-/-}$ mice (p = 0.20).

Verhoeff-Van Gieson staining did not reveal differences in pulmonary total ECM elastin content between MMP-12$^{+/+}$ and MMP-12$^{-/-}$ mice kept on SFD (8.1 ± 2.2% versus 6.8 ± 0.5%; n = 5 each). Macrophage content in the lungs was comparable for MMP-12$^{+/+}$ and MMP-12$^{-/-}$ mice (217 ± 43 × 10^{-6}/μm^2 versus 190 ± 71 × 10^{-6}/μm^2).

4. Discussion

Development of adipose tissue requires ECM degradation and is believed to be related to macrophage migration, which is largely regulated by MMP-12 expression and activation [16]. A link between MMP-12 and adipose tissue development is further suggested by the finding that treatment of obese diabetic mice with polyunsaturated fatty acids prevented MMP-12 expression in macrophages and adipocytes, associated with impaired matrix remodeling and reduced adipocyte growth [21]. Furthermore, MMP-12 can activate other MMPs in vivo, including proMMP-2 and pro MMP-3 [22]. We have previously shown that MMP-2 deficiency in mice results in reduced adiposity [18], whereas MMP-3 deficiency promotes adipose tissue development [17].

In this nutritionally induced obesity model, absence of MMP-12 had no effect on body weight or on adipose tissue expansion. Weight of other major organs was not different between MMP-12$^{+/+}$ and MMP-12$^{-/-}$ mice, either on SFD or HFD, indicating no significant effect on general development of the mice. MMP-12 deficiency did not affect size or density of adipocytes, suggesting that ECM degradation, allowing hypertrophic growth of adipocytes with high fat feeding, is not markedly different. This is supported by unaltered levels of total elastin in the ECM of adipose tissues for both genotypes. MMP-mediated ECM remodeling, a prerequisite for adipose tissue expansion [1], thus appears preserved in MMP-12 deficient mice. In addition, both MMP-12$^{+/+}$ and MMP-12$^{-/-}$ mice gained comparably more body and fat mass when kept on HFD as compared to SFD. This is thus independent of the drastic increase in MMP-12 expression seen in MMP-12$^{+/+}$ mice on HFD versus SFD. It was shown previously that mice deficient in MCP-1 (an important macrophage chemoattractant) did not show significant differences in body weight and fat depots compared to wild-type mice, when kept on a HFD for 12 weeks [23]. Furthermore, size and density of blood vessels in SC or GON fat were not different between MMP-12$^{-/-}$ and MMP-12$^{+/+}$ mice, suggesting that MMP-12 is not essential for adipose tissue associated angiogenesis.

Since increased macrophage infiltration and CLS surrounding moribund adipocytes in obese adipose tissue were shown to be associated with the chronic low-grade inflammation that contributes to metabolic disorders [14,15], we have analyzed macrophage content in SC and GON adipose tissue. Adipose tissue of mice kept on normal chow showed little macrophage infiltration and CLS were only scarcely observed. On the other hand, HFD feeding resulted in increased macrophage recruitment, mainly in GON fat tissue. Interestingly, MMP-12 deficiency was associated with a lower amount of CLS in obese SC and GON adipose tissue, with GON fat being richer in CLS than SC fat. This is consistent with the increased risk for metabolic disorders with increased visceral fat depots. Taken together, our data do not appear to support markedly reduced macrophage infiltration in MMP-12$^{-/-}$ adipose tissues. This is suggested by our findings that: 1) total macrophage content evaluated by F4/80 immunostaining was not significantly affected by MMP-12 deficiency (despite a trend towards a decrease); 2) mRNA expression of the macrophage

Table 4
Effect of MMP-12 deficiency on gelatinolytic activity in adipose tissue and lungs of mice kept on high fat diet.

	SC fat		GON fat		Lung	
	MMP-12$^{+/+}$	MMP-12$^{-/-}$	MMP-12$^{+/+}$	MMP-12$^{-/-}$	MMP-12$^{+/+}$	MMP-12$^{-/-}$
92 kDa proMMP-9	259 ± 106	483 ± 210	ND	ND	14 ± 2.3	17 ± 3.1
72 kDa proMMP-2	626 ± 62	588 ± 54	921 ± 166	849 ± 224	ND	ND
68 kDa proMMP-2	9,025 ± 428	8,617 ± 359	13,309 ± 1,114	13,400 ± 1,327	18 ± 3.4	16 ± 1.1
62 kDa MMP-2	1,300 ± 411	1,957 ± 456	2,789 ± 755	3,209 ± 814	ND	ND
58 kDa MMP-2	3,068 ± 459	3,550 ± 411	4,606 ± 631	4,041 ± 608	13 ± 1.5	17 ± 2.7

Data are expressed as arbitrary units and represent means ± SEM of 7 or 8 determinations. ND, not detectable.

makers TNF-a, IL-1, MCP-1 and csf1r was comparable in SC and GON adipose tissues of MMP-12$^{+/+}$ and MMP-12$^{-/-}$ mice. The lower mRNA expression of F4/80 in SC and GON adipose tissues and cd68 in SC adipose tissue of MMP-12$^{-/-}$ mice thus is not reflected in the actual macrophage content in the tissues. It should be noted that activated macrophages in CLS often fuse to form multinucleated giant cells [24]. Our data thus indicate that, in contrast to lung injury, MMP-12 does not seem to be required for adipose tissue macrophage infiltration. A similar finding was recently reported for macrophage infiltration in the unilateral ureteric obstruction model in mice [25]. Whereas the lower prevalence of CLS in MMP-12$^{-/-}$ adipose tissues of mice kept on HFD would suggest reduced adipocyte apoptosis, determination of casapse-3 expression and activity in SC and GON adipose tissues did not substantiate this finding. Indeed, adipocyte apoptosis is an early event that contributes to macrophage infiltration into obese adipose tissue [26]. Moreover, it was shown that HFD-induced adipocyte cell death occurs through a cyclophilin D intrinsic signaling pathway, and is independent of adipose tissue inflammation [27]. Additionally, a recent study in FAT ATTAC mice ("Fat Apoptosis Through Targeted Activation of Caspase-8") showed that adipocyte death precedes the CLS formation and that the latter is a direct consequence of adipocyte apoptosis [28]. Upon induction of adipocyte apoptosis, infiltration of macrophages was observed with CLS formation and subsequent reabsorption of dead adipocytes. It is thus conceivable that the lower amount of CLS in MMP-12$^{-/-}$ adipose tissue reflects a delayed formation of CLS in response to adipocyte death. The link between MMP-12 deficiency and adipocyte apoptosis, however, remains enigmatic.

Wild-type mice fed a HFD showed a massive increase in MMP-12 expression in SC and GON adipose tissues, as compared to mice kept on SFD. Interestingly, the increase was much greater in SC fat (343-fold) compared with GON fat (22-fold). Increased expression of GM-CSF, in particular in SC fat, could partially explain this difference. Indeed, it was shown previously that MMP-12 expression (in U937 monocytes) was induced by GM-CSF through the AP-1 binding activity [29]. We observed markedly enhanced expression of GM-CSF in SC adipose tissue of both wild-type and MMP-12 deficient mice kept on HFD as compared to SFD. Thus, it is possible that diet-induced enhanced expression of GM-CSF stimulates MMP-12 expression in the wild-type mice. Alternatively, it was previously reported (in abdominal aortic aneurism) that increased MMP-12 expression was associated with blockade of IFN-γ signaling pathways, and subsequent IL-4 mediated events [30]. We observed significantly enhanced expression of IFN-γ in GON adipose tissue of both MMP-12$^{+/+}$ and MMP-12$^{-/-}$ mice when kept on HFD as compared to SFD, whereas this was not observed for SC adipose tissue of either genotype. IL-4 expression was not detected in these adipose tissues. In adipose tissue the IFN-γ signaling pathways thus do not appear to be specifically involved in enhanced MMP-12 expression on HFD, as it is similarly affected in wild-type and MMP-12 deficient tissues. However, enhanced IFN-γ in obese GON adipose tissue together with abundance of CLS appears in agreement with the reported role of IFN-γ in regulating visceral adipose tissue inflammatory responses [31].

Because of the potential role of MMP-12 in gelatinase activation and matrix degradation, we have also monitored expression and activity of MMP-2 and MMP-9 in adipose tissues. Except for a borderline-significant 30% downregulation of MMP-2 expression in SC and GON fat, we did not observe differences between both genotypes, and the contribution of active MMP-2 to total gelatinase activity in SC or GON fat was not affected by MMP-12 deficiency. Also in lung tissues we could not find an effect of MMP-12 deficiency on gelatinase activity.

Taken together, these data indicate that MMP-12 deficiency does not markedly affect macrophage content or ECM degradation in adipose tissue, and moreover does not affect angiogenesis or adipose tissue expansion. However, the prevalence of CLS was lower in fat of MMP-12$^{-/-}$ mice kept on high fat diet, suggesting delayed clearance of apoptotic adipocytes.

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