

Enhanced nutritionally induced adipose tissue development in mice with stromelysin-1 gene inactivation

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Summary
To investigate a potential role of stromelysin-1 (MMP-3) in development of adipose tissue, 5 week old male MMP-3 deficient mice (MMP-$3^{-/-}$) and wild-type (MMP-$3^{+/+}$) controls were kept on a high fat diet (HFD) for 15 weeks. MMP-$3^{-/-}$ mice were hyperphagic and gained more weight than the MMP-$3^{+/+}$ mice. At the time of sacrifice, the body weight of the MMP-$3^{-/-}$ mice was significantly higher than that of the MMP-$3^{+/+}$ mice, as was the weight of the isolated subcutaneous (SC) and gonadal (GON) fat deposits. Significant adipocyte hypertrophy was observed in the GON but not in the SC adipose tissue of MMP-$3^{-/-}$ mice. Fasting plasma glucose and cholesterol levels were comparable in both genotypes, whereas triglyceride levels were significantly lower in MMP-$3^{-/-}$ mice. Staining with an endothelial cell specific lectin revealed a significantly higher blood vessel density and larger total stained area in the GON adipose tissues of MMP-$3^{-/-}$ mice. Thus, in a murine model of nutritionally induced obesity, MMP-3 impairs adipose tissue development, possibly by affecting food intake and/or adipose tissue-related angiogenesis.

Keywords
MMP-3, stromelysin-1, obesity, adipose tissue, adipocyte, angiogenesis

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Introduction

Obesity is a significant health problem that is reaching epidemic proportions. Obesity and its related diseases such as non-insulin-dependent diabetes mellitus, cardiovascular disease, atherosclerosis and hypertension are a main cause of death and disability. Development of obesity is associated with extensive modifications in adipose tissue involving adipogenesis, angiogenesis and extracellular matrix (ECM) remodeling (1). Matrix metalloproteinases (MMP) contribute to tissue remodeling by degradation of ECM and basement membrane components or activation of latent growth factors (2-4). Expression of gelatinolytic activity was reported in rat adipocytes (5), and in adipose tissue of obese mice (6) and humans (7).

Like most other MMPs, stromelysin-1 (MMP-3) is secreted as a proenzyme that can be activated via several pathways (8). MMP-3 cleaves several ECM components such as proteoglycans, laminin, fibronectin, the nonhelical domains of collagen types IV and IX, denatured collagens as well as non-ECM components such as plasminogen and insulin-like growth factor binding protein-3. MMP-3 also activates several other proMMP's, initiating a proteolytic cascade (8, 9). Stromelysin-1 is found at sites of active ECM remodeling, such as wound healing, the mammary and uterine involution (10), and organ morphogenesis (11). MMP-3 deficiency was shown to accelerate adipocyte differentiation during involutive mammary gland remodeling (12). Recently, a detailed analysis of the expression of MMP system components in adipose tissue of lean and obese mice revealed significant upregulation with obesity of MMP-3 mRNA (13). In gonadal adipose tissue, MMP-3 is mainly expressed in the stromal-vascular cells and only marginally in adipocytes, whereas in subcutaneous adipose tissue it is ex-

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pressed in both, but at a higher level in the stromal-vascular cell population (13).

In the present study we have investigated a potential role of MMP-3 in development of adipose tissue with the use of a nutritionally induced obesity model in wild-type mice and in mice with *MMP-3* gene inactivation.

Materials and methods

Diet model

MMP-3-deficient (MMP-$3^{-/-}$) and wild-type (MMP-$3^{+/+}$) mice with the genetic background B10.RIII (14) were a kind gift of Dr. J. Mudgett (Merck Research Laboratories, Rahway, NJ); they were rederived by back-crossing with C57/Bl6 mice. Genomic DNA was extracted from tail tips for genotyping of offspring by Southern blotting (not shown).

Five week old male mice (n = 11 for MMP-$3^{+/+}$ and n = 8 for MMP-$3^{-/-}$) were kept in microisolation cages on a 12 h day-night cycle and fed water and a high fat diet (HFD) at libitum. The HFD (TD88137, Harlan Tekland, Madison, WI) contains 21%, w/w, fat from milk and 49%, w/w, carbohydrate from sucrose and corn starch, yielding 42% kcal as fat, and a caloric value of 4.8 kcal/g. Mice were weighed every week during 15 weeks. Food intake was measured for 48 h periods at weekly intervals, and expressed as g per 24 h. Feeding efficiency was calculated as the increase in body weight per kcal intake. To evaluate physical activity, mice (n = 4, for each genotype) were placed in a separate cage equipped with a turning wheel linked to a computer to register full turning cycles; data are represented as number of cycles per 12 h (at night). At the end of the diet period, following overnight fasting the mice were anesthetized by i.p. injection of 60 mg/kg Nembutal (Abbott Laboratories, North Chicago, IL). Blood was collected from the retroorbital sinus with addition of trisodium citrate (final concentration 0.01 mol/L). Intra-abdominal (gonadal, GON) and inguinal subcutaneous (SC) fat pads were removed and the wet weight determined. Other organs, including the lung, kidney, liver, spleen and heart were also removed and weighed.

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 (15).

Haematologic and metabolic parameters

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 (16), 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 total triglyceride and cholesterol levels were evaluated using routine clinical assays.

Western blotting and *in situ* zymographic analysis

Extraction of adipose tissue (about 250 mg/mL) was performed as described previously (6). Extracts of adipose tissue (100 µg total protein) were applied to SDS-PAGE and immunoblotted with a rabbit polyclonal antiserum specific for murine MMP-3 (17). In situ zymography on 10 µm 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 to 48 h in a moist chamber at 37°C, and the lysis area was normalized to the total section area.

Morphometric and immunohistochemical analysis

The mean size of adipocytes was determined by computer-assisted image analysis in 15 µm 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 paraffin sections, and quantified by computer-assisted image analysis. Blood vessels were visualized by staining paraffin sections with the biotinylated Bandeiraea (Griffonia) Simplicifolia BSI lectin (Sigma-Aldrich) (19) followed by an incubation with horseradish peroxydase-conjugated streptavidin. For each animal, at least 12 randomly selected fields in nine to twelve sections were analyzed by computer-assisted image analysis, and data were then averaged. Parameters analyzed included the lectin-positive area (expressed in percent of total section area), the vessel density (expressed in number of vessels per mm^2 of tissue section), and the vessel sectional area (expressed in μm^2).

In order to evaluate the angiogenic potential, microvessel fragments were isolated from gonadal fat pads of MMP-$3^{+/+}$ and MMP-$3^{-/-}$ mice, and cultured for 6 days in 3-dimensional collagen gels, essentially as described (20). Quantitation of endothelial outgrowths by image analysis was performed using fluorescein-labeled Griffonia Simplicifolia I isolectin B4, and expressed as fluorescence intensity per surface area (21).

DNA quantification

Weighed adipose tissues were digested in lysis buffer (100 mM Tris, pH 8.5, 5 mM EDTA, 0.2% SDS, 200 mM NaCl) supplemented with Proteinase K (2 mg/ml) for 8 h at 55°C. DNA concentrations were determined by using the PicoGreen kit (Molecular Probe) according to manufacturer's instructions. DNA contents were expressed in ng DNA/ mg tissue.

	MMP-3$^{+/+}$	MMP-3$^{-/-}$
Body weight at end[a] (g)	41 ± 1.6	51 ± 1.0**
Body weight at sacrifice[b] (g)	37 ± 1.5	47 ± 1.0**
SC fat (g)	1.5 ± 0.24	2.5 ± 0.15**
GON fat (g)	1.4 ± 0.13	2.6 ± 0.16**
Adipocyte area (μm^2)		
SC	3000 ± 340	3800 ± 400
GON	4580 ± 270	6200 ± 360 **
DNA content[c] (ng/mg tissue)		
SC	140 ± 11	160 ± 9
GON	140 ± 11	150 ± 19

Data are mean ± SEM of 11 or 8 experiments for MMP-3+/+ or MMP-3-/- mice.

*, $p < 0.05$ and **, $p < 0.005$ versus MMP-3+/+.

(a) Body weight after 15 weeks of HFD; (b) body weight at sacrifice, after overnight fasting; (c) data are mean ± SEM of 6 experiments for each genotype.

Table 1: Effect of MMP-3 deficiency in mice on adipose tissue weight and cellularity after 15 weeks of HFD.

RNA extraction and reverse transcription-PCR (RT-PCR)

Total DNA-free RNA from GON and SC adipose tissues was prepared using the RNeasy mini kit (Qiagen, Valencia, CA) and RNA concentrations were determined using the RiboGreen RNA quantification kit (Molecular Probes).

Murine leptin mRNA level was quantified by real-time quantitative RT-PCR. Total RNA (200 ng) was reverse transcribed for 1 h at 48°C using the Taqman Reverse Transcription kit and 5 μM random hexamers (Applied Biosystems, Foster City, CA). Quantitative real-time PCR was performed in the ABI-prism 7700 sequence detector, using the Taqman PCR core reagent kit (Applied Biosystems). Leptin-specific forward (5'-CAAAACCCTCATCAAGACCATTG-3') and reverse (5'-AGTCCAAGCCAGTGACCCTCT-3') primers and probe (FAM-5'-TTCACACACGCAGTCGGTATCCGC-3'-TAMRA) were designated using the Primer Express software (Applied Biosystems). Samples were normalized using the housekeeping gene 18S rRNA. Quantitative real-time PCR for 18S rRNA was performed using the Taqman ribosomal RNA control (Applied Biosystems) according to manufacturer's protocol. PCR amplifications were performed in duplicate wells, using the following conditions: 2 min at 50°C and 10 min at 94°C, followed by 40 two-temperature cycles (15 s at 95°C and 1 min at 60°C).

The expression level of murine MMP-3 mRNA was determined by semi-quantitative RT-PCR using the GeneAmp Thermostable RNA PCR kit (Applied Biosystems) and MMP-3-specific forward (5'-CCTATTCCTGGTTGCTGCTCA-3') and reverse (5'-TGGAAACGGGACAAGTCTGTG-3') primers. Results were normalized by 28S rRNA level as described previously (13).

Statistical analysis

Data are expressed as mean ± SEM. Statistical significance between groups is evaluated using non-parametric t-testing. Values of $p < 0.05$ are considered statistically significant.

Results

Body and fat pad mass

At the start of the diet (5 weeks of age) the body weight of the MMP-3$^{-/-}$ mice was higher than that of the MMP-3$^{+/+}$ mice (22 ± 0.71 versus 18 ± 1.2 g, p= 0.026; *n* = 8-11). When fed a HFD, MMP-3$^{-/-}$ mice gained slightly more weight than wild-type mice, resulting in significantly higher body weights at the end of the 15 weeks of diet (Table 1 and Fig. 1A and B). The weight of the isolated SC or GON fat pads was significantly higher in the MMP-3$^{-/-}$ group (Table 1). In contrast the weight of other organs, including lung, kidney, liver, spleen and heart was not significantly different from the MMP-3$^{+/+}$ group (not shown), indicating that increased adipose tissue is not an aspecific result of increased body growth. Based on the assumption that the weight of the GON fat pad is a reliable estimate of body fat in normal and obese mice (22), the percentage of body fat in MMP-3$^{-/-}$ mice was increased by 53% as compared with wild-type mice (5.6 ± 0.31% versus 3.7 ± 0.22%, p = 0.001; *n* = 8-11).

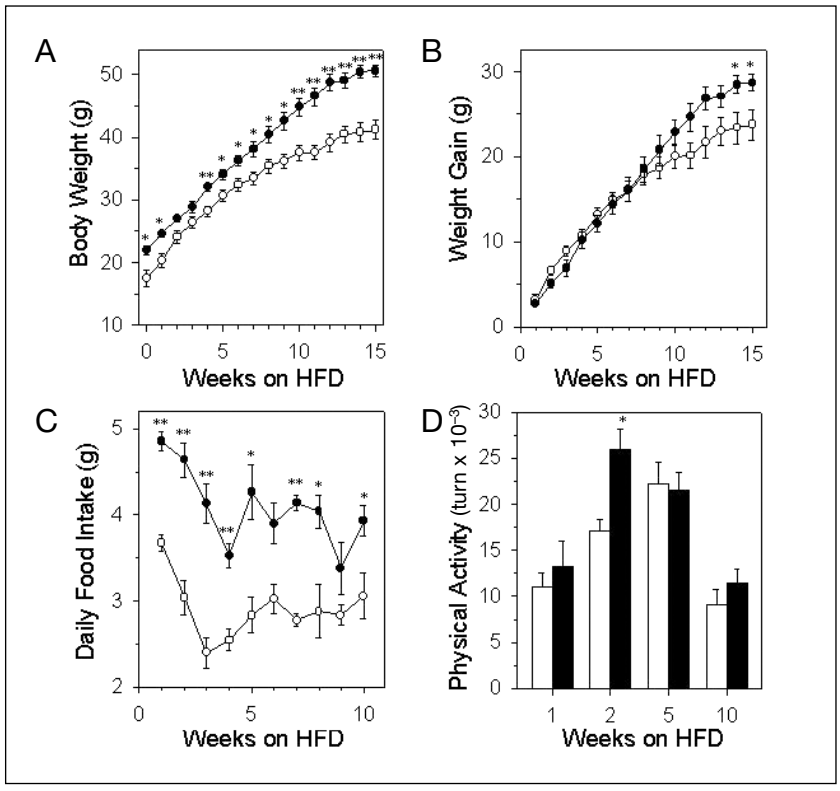

Figure 1: Growth curve, feeding behavior and physical activity of MMP-3$^{+/+}$ (*open circle*) and MMP-3$^{-/-}$ (*filled circle*) mice. (*A*) Body weight of mice kept on HFD during 15 weeks (n = 11 and 8). (*B*) Weight gain relative to day 0 during 15 weeks of HFD (n = 11 and 8). (*C*) Food uptake at different intervals is expressed as g per mouse and per day (n = 4). (*D*) Physical activity of MMP-3$^{+/+}$ (*open bars*) or MMP-3$^{-/-}$ (*filled bars*) mice is expressed as the number of cycles per 12 h (n = 4). *, $p < 0.05$ and ** $p < 0.005$ versus MMP-3$^{+/+}$.

Adipose tissue cellularity

Adipose tissue accretion may be due to an increase in the number of adipocytes, hypertrophy of individual adipocytes, or both. Histological analysis of GON fat pads from animals fed a HFD for 15 weeks suggested that adipocytes were larger in MMP-3$^{-/-}$ than in MMP-3$^{+/+}$ mice (Fig 2A and C). Morphometric analysis demonstrated that the mean adipocyte area of SC and especially of GON fat pads of MMP-3$^{-/-}$ mice, was larger (26% and 35%, respectively) than those of MMP-3$^{+/+}$ mice (Table 1). Thus, the mean volume of adipocytes in both the SC and GON

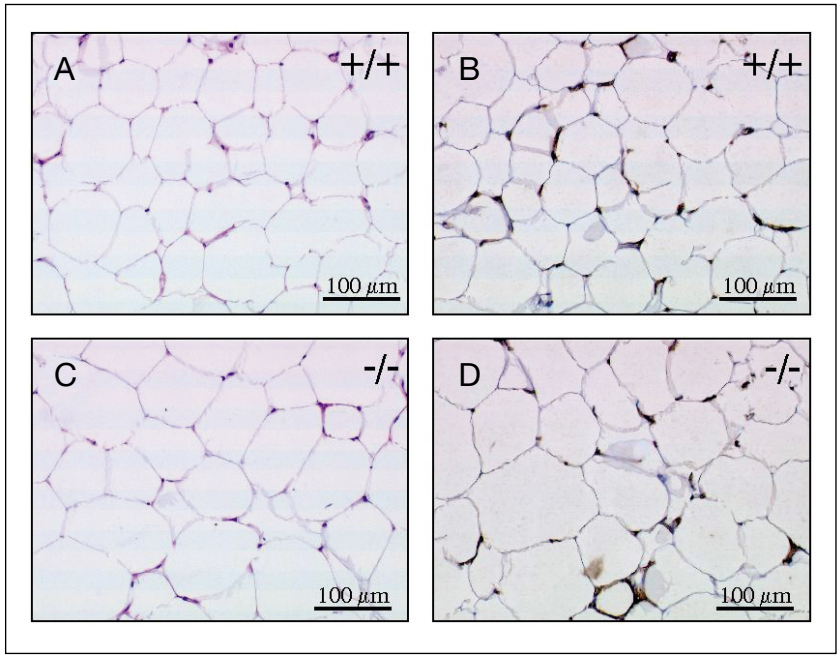

Figure 2: Immunohistochemical analysis of GON adipose tissue from MMP-3$^{+/+}$ (*A*, *B*) or MMP-3$^{-/-}$ (*C*, *D*) mice kept on HFD for 15 weeks. Staining was performed with haematoxylin-eosin (*A* and *C*) or with the Bandeirae Simplicifolia lectin (*B* and *D*).

	MMP-3$^{+/+}$	MMP-3$^{-/-}$
Glucose (mg/dl)	88 ± 9.2	93 ± 5.7
Triglycerides (mg/dl)	122 ± 8.7	75 ± 6.4**
Cholesterol (mg/dl)	94 ± 7.4	107 ± 5.6
PAI-1 (ng/ml)	5.8 ± 1.4	4.7 ± 0.6

Data are mean ± SEM of 11 or 8 experiments for MMP-3$^{+/+}$ or MMP-3$^{-/-}$ mice.
**, $p < 0.005$ versus MMP-3$^{+/+}$

Table 2: Metabolic parameters of MMP-3$^{+/+}$ and MMP-3$^{-/-}$ mice after 15 weeks of HFD.

fat pads of MMP-3$^{-/-}$ mice was about 1.4-fold higher as compared to MMP-3$^{+/+}$ mice. DNA contents were similar in both genotypes, indicating that adipocyte hypertrophy, and not a higher number of adipocytes, was the major cause of the enlarged fat pads (Table 1).

Staining of adipose tissue sections with Sirius red showed the presence of fibrillar collagen throughout the sections (not shown). Quantification of the stained area did not reveal significant differences between MMP-3$^{+/+}$ and MMP-3$^{-/-}$ mice (17 ± 2.0 versus 12 ± 0.8% for SC tissue and 10 ± 0.62 versus 9.9 ± 0.81% for GON tissue).

Food intake and physical activity

In order to monitor food intake and physical activity, 5-week-old mice on HFD were kept in isolated cages. As shown in Fig. 1C, the average food intake was significantly higher for MMP-3$^{-/-}$ as compared to MMP-3$^{+/+}$ mice. However, the feeding efficiency was similar in wild-type and MMP-3$^{-/-}$ mice (0.086 ± 0.028 and 0.079 ± 0.022 g/kcal, respectively). In contrast the average physical activity of MMP-3$^{+/+}$ and MMP-3$^{-/-}$ mice during weeks 1, 5 and 10 of the HFD was not significantly different, whereas only at week 2 MMP-3$^{-/-}$ mice were slightly more active (Fig. 1D).

Haematologic and metabolic parameters

Plasma haematologic parameters including white and red blood cells, haemoglobin and haematocrit levels of MMP-3$^{+/+}$ and MMP-3$^{-/-}$ mice were comparable after 15 weeks of HFD (not shown). Plasma PAI-1 antigen levels were about 2- to 3-fold enhanced (about 1-2 ng/ml in plasma of mice on normal chow), as a result of the HFD, but were comparable in both groups. Glucose and total cholesterol levels were also comparable for both genotypes, whereas triglycerides were significantly reduced in the MMP-3$^{-/-}$ animals (Table 2).

Expression of leptin mRNA was comparable for SC and GON adipose tissue of MMP-3$^{+/+}$ or MMP-3$^{-/-}$ mice (Fig. 3A). Plasma leptin antigen levels were also not significantly different (Fig. 3*B*), and correlated well with the weights of SC and GON fat pads (Fig. 3C) and with total body weight (r^2= 0.75; not shown).

Expression of MMP system components

Semi-quantitative RT-PCR confirmed expression of MMP-3 mRNA in SC and GON adipose tissue of MMP-3$^{+/+}$ mice, whereas it was undetectable in MMP-3$^{-/-}$ tissue (Fig. 4A).

Western blotting of extracts of SC or GON adipose tissue of MMP-3$^{+/+}$ mice with an antiserum against murine MMP-3, revealed two bands that are compatible with the presence of latent and active MMP-3 (Fig. 4B); these bands were not detected in extracts of MMP-3$^{-/-}$ tissue (not shown). The M_r of the natural active MMP-3 species is somewhat higher than that of the purified recombinant molecule used as control.

In situ zymography with gelatin-containing gels on cryosections of SC or GON adipose tissue did not reveal differences in gelatinolytic activity (Table 3). The observed gelatinolytic activity was inhibited by ≥ 80% upon addition of excess MMP inhibitors.

In contrast, in situ zymography with casein-containing gels revealed somewhat lower activity in SC ($p = 0.25$) and significantly ($p= 0.004$) lower activity in GON adipose tissue of MMP-3$^{-/-}$ as compared to MMP-3$^{+/+}$ mice (Table 3). Caseinolytic activity was inhibited by 50 to 60% in the presence of excess MMP inhibitors.

Adipose tissue related angiogenesis

Staining of SC or GON adipose tissue with an endothelial cell specific lectin revealed larger stained areas in the MMP-3$^{-/-}$ mice. This is illustrated in Figures 2B and D for the GON tissue. Quantitative analysis confirmed a significantly higher blood vessel density and larger total stained area in the tissues of MMP-3$^{-/-}$ mice, whereas vessel size was not significantly different (Table 4).

The angiogenic potential of microvessel fragments isolated from adipose tissue of MMP-3$^{+/+}$ or MMP-3$^{-/-}$ mice after 15 weeks of HFD, was not significantly different. Similar results were obtained with microvessel fragments isolated from mice kept on regular chow (not shown).

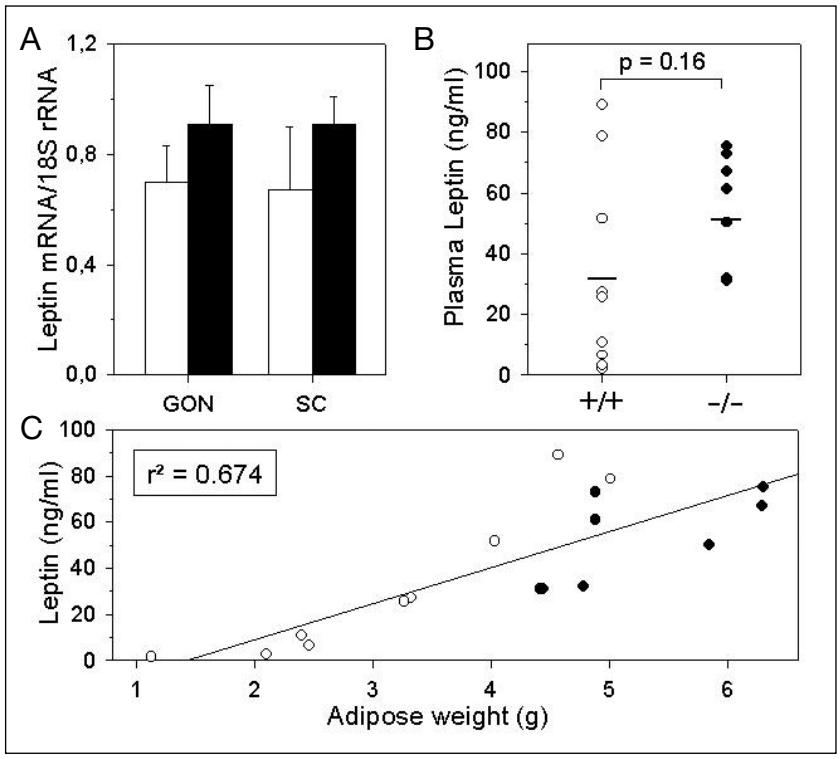

Figure 3: Expression of leptin in mice kept on HFD for 15 weeks. (*A*) Leptin mRNA levels in SC and GON fat pads of MMP-3$^{+/+}$ (*open bars*) and MMP-3$^{-/-}$ (*filled bars*) mice (n = 7) are normalized to the 18S rRNA. (*B*) Plasma leptin levels in MMP-3$^{+/+}$ and MMP-3$^{-/-}$ mice are expressed in ng/ml. (*C*) Correlation of leptin plasma levels with SC and GON adipose tissue weight in MMP-3$^{+/+}$ (*open circle*) and MMP-3$^{-/-}$ (*filled circle*) mice.

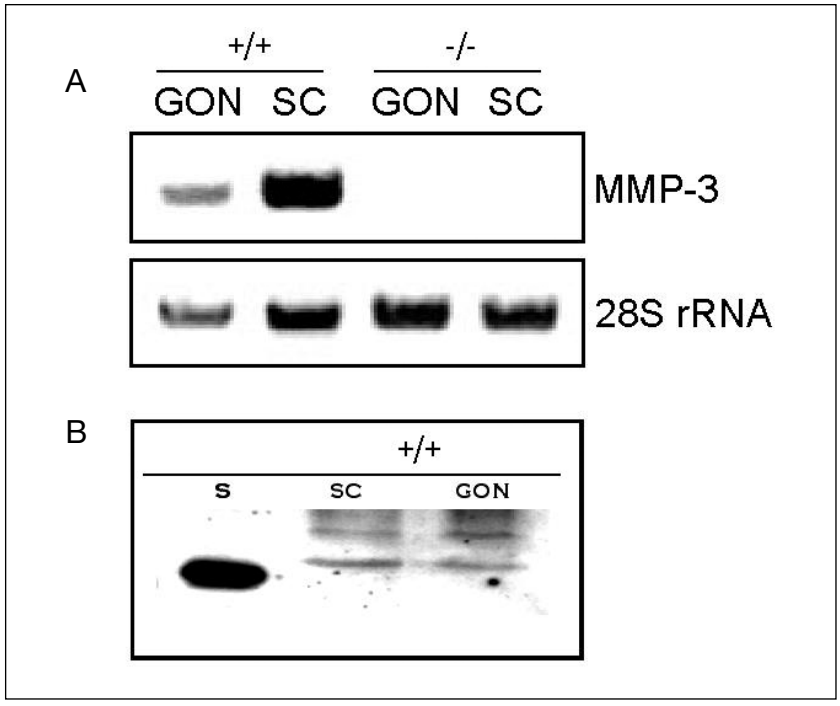

Figure 4: Identification of MMP-3 in SC and GON adipose tissue from MMP-3$^{+/+}$ mice kept on HFD for 15 weeks. (*A*) MMP-3 mRNA and 28S rRNA levels were quantified by RT-PCR. (*B*) Western blot of adipose tissue extracts with antiserum against murine MMP-3. Lane S shows purified recombinant (non-glycosylated) murine MMP-3 (20 ng protein applied).

Discussion

Because it was recently reported that MMP-3 mRNA expression was significantly upregulated in adipose tissue of obese as compared to lean mice (13), we have in the present study investigated a potential role of MMP-3 in development of adipose tissue. Mice with inactivation of the *MMP-3* gene and wild-type controls were kept on a high fat diet for 15 weeks, and total body weight, weight, composition and cellularity of SC and GON adipose tissue, and plasma metabolic parameters were monitored, using a previously described model (23). Because in mice important genetic differences in the metabolic response to

		Lysis/section area			
		Gelatin		Casein	
		-Inh.	+Inh.	-Inh.	+Inh.
SC	MMP-3$^{+/+}$	15 ± 1.9	3.3 ± 0.17	19 ± 3.0	8.6 ± 2.3
	MMP-3$^{-/-}$	22 ± 3.6	3.9 ± 0.98	13 ± 2.4	5.6 ± 1.7
GON	MMP-3$^{+/+}$	15 ± 3.6	2.9 ± 0.35	20 ± 2.6	8.0 ± 1.8
	MMP-3$^{-/-}$	17 ± 3.5	3.1 ± 0.42	9.4 ± 1.3*	5.5 ± 1.5

Data are mean ± SEM of 5 or 6 determinations in the absence (-Inh.) or the presence (+Inh.) of MMP inhibitors (5 mM 1,10 phenanthroline and 25 mM EDTA).

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

	SC		GON	
	MMP-3$^{+/+}$	MMP-3$^{-/-}$	MMP-3$^{+/+}$	MMP-3$^{-/-}$
Stained area[(a)]	2.1 ± 0.16	2.3± 0.29	1.5 ± 0.09	2.3 ± 0.17**
Vessel density[(b)]	310 ± 20	350 ± 40	210 ± 17	280 ± 34*
Vessel size[(c)]	70 ± 3.3	67 ± 3.0	76 ± 3.9	87 ± 3.9

[(a)]Area stained with the Bandeiraea Simplicifolia lectin, in percent of the total section area; [(b)]number of vessels per mm^2 tissue; [(c)]average vessel size in μm^2.

Data are mean ± SEM of determinations with 7 to 11 animals. *, $p < 0.05$ and **, $p < 0.0005$ versus MMP-3$^{+/+}$.

Table 4: Characterization of blood vessels in subcutaneous and gonadal adipose tissue of MMP-3$^{+/+}$ or MMP-3$^{-/-}$ mice kept on HFD for 15 weeks.

fat have been reported (24-26), we have used wild-type and MMP-3 deficient mice with the same genetic background (50% B10.RIII: 50% C57/Bl6). Furthermore, in order to avoid hormonal effects on synthesis or secretion of components that may affect obesity, only male mice were used.

At 5 weeks of age, MMP-3$^{-/-}$ mice had a slightly higher total body weight than their wild-type counterparts. In a previous study (27), a somewhat higher body weight was also observed for 8 to 14 weeks old male MMP-3$^{-/-}$ than for MMP-3$^{+/+}$ mice (31 ± 0.9 versus 27 ± 1.0 g, n= 17). When exposed to a high fat diet, despite their similar feeding efficiency, MMP-3$^{-/-}$ mice gained more weight than their wild-type counterparts, resulting in significantly higher body weights at the time of sacrifice. This may be related to the higher food intake of the MMP-3$^{-/-}$ mice. In rodent models of obesity, such as *ob/ob* mice, accretion of fat is caused by a combination of hyperphagia and reduced energy expenditure. Our MMP-3$^{-/-}$ mice are hyperphagic, and their food intake is about 40% greater than that of the MMP-3$^{+/+}$ mice. In contrast, energy expenditure is not different between the two genotypes, suggesting that enhanced food intake seems to be an important causal element in the development of obesity. Leptin plays a major role in body weight homeostasis by acting as a satiety factor and increasing energy expenditure (28). The secretion of leptin is highly correlated with body fat mass and adipocyte size (28). Our data indicate that the relationship between plasma leptin levels and adiposity is preserved in both MMP-3$^{+/+}$ and MMP-3$^{-/-}$ mice, suggesting that MMP-3 deficiency does not impair leptin secretion. However, the observation that MMP-3$^{-/-}$ mice are hyperphagic despite normal leptin secretion suggests that these mice might present a reduced leptin sensitivity.

The higher body weight of MMP-3$^{-/-}$ mice results essentially from a specific increase of their adiposity. The larger GON and SC fat pads were characterized by the presence of hypertrophic adipocytes, reflecting their increased triglyceride storage. Interestingly, plasma triglyceride levels were decreased by 40% in fasted MMP-3$^{-/-}$ mice when compared with wild-type controls. This observation raises the possibility that MMP-3 deficiency promotes the conversion of circulating plasmatic triglycerides to their storage form as intracellular lipid droplets, possibly as a result from an increased synthesis and/or activity of the lipoprotein lipase.

Fat mass excess requires hemodynamic adaptations to supply an adequate blood flow to the growing tissue. Evaluation of blood vessels revealed no difference in the vessel size between both genotypes, but the vessel density was higher in the MMP-$3^{-/-}$ mice. Enhanced angiogenesis during the development of adipose tissue may thus have contributed to increased tissue mass. However, the *ex vivo* angiogenic potential of microvessels isolated from adipose tissue of MMP-$3^{+/+}$ or MMP-$3^{-/-}$ mice after 15 weeks of HFD was not different anymore. This may indicate a time-dependent effect of enhanced angiogenesis on adipose tissue development or the requirement for specific adipocyte-derived angiogenic factors, which were not present in our *ex vivo* model.

From a mechanistic perspective, the key question is how MMP-3 deficiency contributes to obesity. Several lines of evidence show that ECM proteins play an important role in modulating adipocyte differentiation, by allowing the morphological changes and adipocyte-specific gene expression that accompany differentiation (29). MMP-3 degrades a broad range of ECM substrates, and plays a role in several biological phenomena involving degradation of matrix components (2-4). Recently, the generation of entactin fragments, which inhibit the rate of basement membrane assembly (30), has been shown to be abrogated in MMP-$3^{-/-}$ mice, resulting in an accelerated adipocyte differentiation during mammary gland involution (12). MMP-3 also increases the bioactivity of transforming growth factor-β, a potent inhibitor of adipogenesis (31). Furthermore, cleavage of plasminogen by MMP-3 generates angiostatin, a strong angiogenic inhibitor (32), thus potentially restricting the development of the adipose vasculature. In addition to its potential direct effect on adipogenesis and adipose tissue growth, MMP-3 might also indirectly affect these processes by regulating food intake. Indeed, the hyperphagic behavior observed in the MMP-$3^{-/-}$ mice emphasizes the occurrence of an altered energy homeostasis. Several hypothalamic neurotransmitters are implicated in the regulation of energy homeostasis (33). Therefore, it can be hypothesized that the activity of MMP-3 might also modulate the bioactivity of several potent orexigenic neuropeptides such as the melanin-concentrating hormone, the neuropeptide Y, or the agouti-related peptide, thus affecting food intake.

In conclusion, these results demonstrate that MMP-3 functions as a negative regulator of adipose tissue development, possibly by regulating food intake and/or by affecting adipose tissue-related angiogenesis. However, the precise mechanism of this effect still remains elusive.

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