Differential expression of plasminogen activator inhibitor-1, tumor necrosis factor-α, TNF-α converting enzyme and ADAMTS family members in murine fat territories

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Abstract

Our objective was to investigate expression of A disintegrin and metalloproteinase (ADAM) and ADAM proteins with a thrombospondin (TS) motif (ADAMTS) family members in adipose tissue of lean and obese mice. Five-week-old male mice were kept on standard chow (SFD) or on high fat diet (HFD) for 15 weeks, and subcutaneous (SC) and gonadal (GON) adipose tissue, as well as mature adipocytes and stromal–vascular (S–V) cells were harvested. mRNA levels of plasminogen activator inhibitor-1 (PAI-1), tumor necrosis factor-α (TNF-α), ADAM-17 (TACE or TNF-α converting enzyme), ADAMTS-1 and ADAMTS-8 were quantified in isolated adipose tissues and cell fractions, and during differentiation of murine preadipocytes. The HFD resulted in a significantly enhanced weight of isolated SC and GON fat pads, and in enhanced blood levels of glucose, cholesterol and PAI-1. ADAM-17, TNF-α, PAI-1, ADAMTS-1 and ADAMTS-8 mRNA were detected in both SC and GON adipose tissue of lean mice (SFD). In SC adipose tissue of obese mice (HFD), the expression of ADAM-17 and PAI-1 was enhanced and that of ADAMTS-1 reduced, whereas in GON adipose tissue expression of TNF-α was enhanced and that of ADAMTS-8 reduced. In lean and obese mice, expression of ADAM-17, ADAMTS-1 and ADAMTS-8 was higher in the S–V cell fraction than in mature adipocytes. During differentiation of murine 3T3-F442A preadipocytes, expression of ADAM-17 and ADAMTS-1 remained virtually unaltered, whereas that of ADAMTS-8 decreased as adipocytes matured. Several ADAM and ADAMTS family members are expressed in adipose tissue and during differentiation of preadipocytes. Modulation of their expression upon development of obesity is adipose tissue-dependent.
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1. Introduction

The A disintegrin and metalloproteinase (ADAM) family comprises proteins containing disintegrin-like and metalloproteinase-like domains [1]. ADAMs are involved in diverse processes such as development, cell–cell interactions and protein ectodomain shedding [2]. The ADAMTS family includes a subset of ADAM proteins that contain a thrombospondin (TS) motif [3]. These proteins or their processed forms most likely function as zinc proteinases, but the natural substrates and inhibitors of many of these enzymes remain to be identified.

Adipose tissue is highly vascularized and adipogenesis is tightly associated with angiogenesis [4]. Adipose tissue and isolated mature adipocytes also produce chemotactic and mitogenic activity toward endothelial cells [5,6]. Proteolytic systems, such as the plasminogen/plasmin (fibrinolytic) and matrix metalloproteinase (MMP) systems appear to play a role in development of adipose tissue [7–10]. Recently, some evidence is emerging that proteins of the ADAM and ADAMTS families may also be implicated.

ADAM-17 (TACE or TNF-α converting enzyme) releases tumor necrosis factor-α (TNF-α) from cells [11]. TNF-α is overexpressed in the adipose tissue of obese animals and humans, and its secretion from explanted adipose tissue is higher in obese as compared to lean samples [12]. Circulating TNF-α levels are significantly elevated in genetically obese db/db mice (lacking the leptin receptor) and in obese human subjects [13]. TNF-α may play an important role in obesity-related insulin resistance [14]. ADAMTS-1 knockout mice are exceptionally lean and

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the volume of epididymal fat is significantly reduced [15]. The human orthologs of ADAMTS-1 and ADAMTS-8 inhibit endothelial cell proliferation and angiogenesis [16].

Plasminogen activator inhibitor-1 (PAI-1), a main inhibitor of the fibrinolytic system, is produced by the adipose tissue and its expression is enhanced by TNF-α. PAI-1-deficient mice kept on a high fat diet (HFD) show enhanced adipose tissue formation [7]. PAI-1 may thus constitute a link between obesity and obesity-related cardiovascular disorders. Murine models of genetically determined or nutritionally induced obesity are frequently used to investigate molecular mechanisms involved in development of adipose tissue [17]. The aims of this study, therefore, were (1) to monitor mRNA levels of ADAM-17, ADAMTS-1, ADAMTS-8, TNF-α and PAI-1 in gonadal and subcutaneous murine adipose tissue; (2) to examine the cellular localization of their expression; (3) to investigate their modulation by diet-induced obesity and; (4) to study their expression pattern during differentiation of murine 3T3-F442A preadipocytes, as in an in vitro model of adipogenesis.

2. Methods

2.1. Obesity model

Five-week-old male wild-type mice of about 20 g (mixed 75% C57/Bl6:25% 129Svj genetic background) were kept on a standard chow (4% (w/w) fat, SFD; $n = 5$) or were given a HFD (containing 21% (w/w) fat from milk fat and 49% (w/w) carbohydrate from sucrose and corn starch, HFD; Harlan TD 88137, Zeist, The Netherlands; $n = 6$) for 15 weeks [7]. The SFD contained 13% kcal as fat and the HFD contained 42% kcal as fat. Following overnight fasting, the mice were weighed and euthanized with an overdose (60 mg/kg i.p.) of Nembutal (Abbott Laboratories, North Chicago, IL). Intra-abdominal (gonadal, GON) and

inguinal subcutaneous (SC) fat pads were removed and the wet weight determined. Blood samples were obtained from the retroorbital sinus and collected on trisodium citrate (final concentration 0.01 M). Glucose concentrations were measured with Glucocard strips (Menarini Diagnostics), PAI-1 levels with a specific ELISA [18] and triglycerides and cholesterol using routine clinical assays. 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 [19].

2.2. Adipose tissue dissociation

Pooled GON or SC fat pads dissected from mice kept on SFD ($n = 5$) or HFD ($n = 4$) were used to separate mature adipocytes from stromal–vascular (S–V) cells by collagenase treatment [20,21]. Briefly, minced fat pads were digested in Krebs–Ringer bicarbonate buffer (KRB buffer, Sigma Chemicals, St. Louis, MO) (pH 7.3) supplemented with 3% (w/v) albumin (KRB-BSA), and 1.5 mg/ml collagenase (Sigma) at 37 °C for 1 h. Undigested tissue fragments were removed by filtration through a nylon screen, and adipocytes were separated by their ability to float upon low-speed centrifugation. The floating fat cells were resuspended in KRB-BSA, washed and centrifuged again. The first and second pellets containing S–V cells were pooled. Red blood cells contaminating the S–V fraction were eliminated using the Red Blood Cell Lysis Buffer (Roche Molecular Biochemicals, Germany) according to the manufacturer's instructions. The two cell populations were washed and resuspended in PBS.

2.3. Culture and differentiation of 3T3-F442A cells

3T3-F442A preadipocytes (a gift from Prof. R. Négrel, University of Nice, France) were cultured in basal medium:

Table 1
Sequence of primers used for the RT-PCR assays

RNA species[a]	Primers (5′–3′)		Product size[b]	Positive control	Cycle profile[c]	Cycle number[d]
	Sense	Antisense				
ADAM-17 (AB021709)	TGCAGGCAAAACAAAGCCA	CCACTCCCACCCCCTCTT	159	kidney	A	26/25/25
TNF-α (NM_013693)	GGGCCACCACGCTCTTC	CCTCCACTTGGTGGTTTGCTAC	180	spleen	A	37/25/–
PAI-1 (M33960)	AGGGCTTCATGCCCCACTTCTTCA	AGTAGAGGGCATTCACCAGCACCA	197	adip	B	25/25/27
ADAMTS-1 (D67076)	ACTCGCGACCCGAGAGC	CCGTGGAAGTCGGCCAT	175	lung	A	34/25/30
ADAMTS-8 (AF175282)	GCTGGCAACGGCGTTTC	ACTGCTCTCCCTTCCCACAGA	102	spleen	A	41/40/48
Pref-1 (L12721)	AACCATGGCAGTGCATCTG	AGCATTCGTACTGGCCTTTC	132	preadip[e]	A	–/–/26
GPDH (J02655)	GGTGGCAGAGGCCTTTG	TGCCCATTTAGCATCTCCTT	69	adip[e]	A	–/–/37
PPAR-γ (NM_011146)	CTGTCGGTTTCAGAAGTGCCT	CCCAAACCTGATGGCATTGTGAGACA	99	adip[e]	A	–/–/26
28S rRNA (X00525)	GTTCACCCACTAATAGGGAACGTGA	GGATTCTGACTTAGAGGCGTTCAGT	212	lung	B	17/17/17

[a] GenBank accession numbers of the targets are indicated in parentheses.
[b] The length of PCR products is expressed in bp.
[c] Cycle profiles A and B are as follows: after 120 s at 95 °C, PCR mixtures were subjected to a cycle profile including denaturation for 15 s at 94 °C, annealing for 20 s at 60 and 68 °C, extension for 15 and 10 s (cycle profile A and B, respectively) at 72 °C and 2 min extension at 72 °C for the last cycle.
[d] Number of cycles used to amplify the target sequences in: adipose tissue/isolated cell fractions/3T3-F442A cells.
[e] Preadip, preadipocytes; Adip, adipocytes.

Dulbecco's MEM/nutrient mix F12 (1:1; Life Technologies, Merelbeke, Belgium) containing 100 mM pantothenate, 1 mM biotin, 2.5 mM glutamine, 15 mM Hepes, and supplemented with 10% (v/v) FBS (Life Technologies). To induce differentiation, cells were seeded at 3.6×10^4 cells/cm^2 and grown to confluency in basal medium with 10% FBS. Confluent cultures (day 0) were washed in serum-free basal medium and treated for 5 days with an induction medium: basal medium supplemented with BSA (100 mg/l), ITS (10 mg/l insulin, 5.5 mg/l transferin, 5 mg/l selenium; Sigma), 10 nM dexamethasone, 250 mM methylisobutylxanthine and 1 nM triiodothyronine (T$_3$). Cultures were then switched to a differentiation medium (basal medium supplemented with ITS and T$_3$) for 2 weeks (induction and differentiation media were renewed every 2–3 days). Cellular viability was assessed by cell counting, Trypan Blue dye exclusion assay and WST-1 (Roche Molecular Biochemicals) assay as described previously [22]. To assess the extent of preadipocyte differentiation, cytosolic triglyceride content was quantified by determining Nile Red uptake, as monitored by flow cytometric analysis (FACSCalibur, Becton Dickinson [21,23].

2.4. RNA isolation

DNA-free total RNAs were extracted from frozen adipose tissue (GON and SC) by the RNA Easy Quiagen Kit, and from isolated adipocytes, S−V cells and 3T3-F442A cells by the HighPure RNA tissue and HighPure RNA isolation kits (Roche Molecular Biochemicals), respectively, according to the manufacturer's instructions. RNA concentrations were measured using the RiboGreen RNA quantification kit (Molecular Probes). RNA samples were diluted in water and stored at −80 °C.

2.5. Oligonucleotide primers

The design of oligonucleotide primers specific for the different targets was based on sequences available in the GenBank (Table 1). When the genomic sequence of the

Fig. 1. mRNA determination in SC and GON adipose tissue derived from mice kept on SFD or HFD for 15 weeks. Lane (−) indicates a blank control without sample, and (+) indicates a positive control as indicated in Table 1.

targets was available, primers (Eurogentec, Seraing, Belgium) annealing to distinct exons were selected in order to discriminate between reverse transcription-polymerase chain reaction (RT-PCR) products resulting from the amplification of the target mRNA or from contaminating genomic DNA. The specificity of the different primer pairs was tested by subjecting RNA isolated from known positive control tissue to RT-PCR (Table 1). RT-PCR products were cloned into a pCRII-TOPO vector (Invitrogen, Groningen, The Netherlands) according to the manufacturer's instructions and cycle sequenced with M13R primer (Invitrogen) on a GeneAmp 9700 thermal cycler using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Nieuwerkerk a/d Ijssel, The Netherlands). The PCR products were separated and analyzed on an ABI Prism 310 Genetic Analyzer (Applied Biosystems). The identity of the resulting sequences was verified with the NCBI BLASTN program.

Table 2
Adipose tissue weight and metabolic parameters of mice after 15 weeks of SFD or HFD

	SFD	HFD
Body weight (g)	29 ± 1.2	44 ± 1.7**
Adipose tissue weight (g)		
SC	0.34 ± 0.08	1.9 ± 0.18**
GON	0.46 ± 0.09	1.5 ± 0.16**
Glucose (mg/dl)	66 ± 6.4	135 ± 16*
Triglycerides (mg/dl)	70 ± 13	95 ± 7.1
Cholesterol (mg/dl)	70 ± 2.4	130 ± 15**
PAI-1 (ng/ml)	1.9 ± 0.14	10 ± 1.8**

Data are mean ± S.E. of five or six experiments for mice on SFD or HFD, respectively. SC and GON, subcutaneous and gonadal adipose tissue.
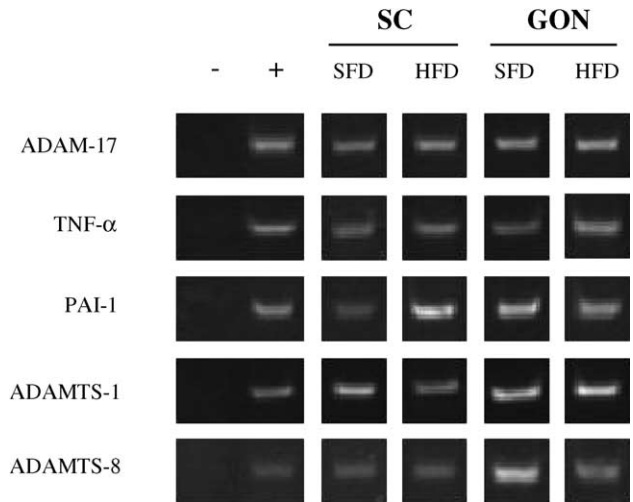* $P < 0.01$ versus SFD.
** $P < 0.005$ versus SFD.

Table 3
mRNA levels in SC and GON adipose tissue from mice kept on SFD or HFD for 15 weeks

	SC		GON	
	SFD	HFD	SFD	HFD
ADAM-17	0.70 ± 0.05	0.94 ± 0.12*	$0.98 \pm 0.05^{++}$	1.0 ± 0.09
TNF-α	0.52 ± 0.20	0.79 ± 0.11	0.52 ± 0.08	0.82 ± 0.10*
PAI-1	0.42 ± 0.12	1.6 ± 0.50**	$1.1 \pm 0.19^+$	0.80 ± 0.14
ADAMTS-1	1.5 ± 0.32	0.72 ± 0.09**	1.3 ± 0.08	$1.1 \pm 0.12^+$
ADAMTS-8	0.60 ± 0.12	0.80 ± 0.12	$2.1 \pm 0.15^{++}$	0.89 ± 0.14**

mRNA levels were analysed by semi-quantitative RT-PCR and normalized to 28S rRNA levels. Data are mean ± S.E. of five or six experiments for mice on SFD or HFD, respectively. SC and GON, subcutaneous and gonadal adipose tissue.
* $P = 0.05$ versus SFD.
** $P < 0.01$ versus SFD.
+ $P < 0.05$ versus SC tissue of the same diet.
++ $P < 0.01$ versus SC tissue of the same diet.

Table 4
Correlations between mRNA levels and the degree of obesity

	GON fat tissue		SC fat tissue	
	ρ	P	ρ	P
ADAM-17	0.077	0.821	0.564	0.071
TNF-α	0.727	0.011	0.4	0.223
PAI-1	− 0.291	0.385	0.864	0.0006
ADAMTS-1	− 0.191	0.573	− 0.601	0.05
ADAMTS-8	− 0.882	0.0003	0.543	0.084

Spearman correlation coefficients for the relationships between mRNA levels and the weight of the fat depot in GON ($n = 11$) and SC ($n = 11$) adipose tissue. Statistically significant differences ($P < 0.05$) are underlined.

2.6. Semi-quantitative RT-PCR

The expression level of the different mRNAs was determined by semi-quantitative RT-PCR. Reverse transcription reactions were performed from 10 ng of total RNA with thermostable reverse transcriptase (r*Tth*) at 70 °C during 15 min using the GeneAmp Thermostable RNA PCR Kit (Applied Biosystems) and target-specific antisense primers. PCR amplifications were performed with target-specific sense primers. The reactions were run for the corresponding number of cycles in a GeneAmp9700 thermal cycler (Table 1). The number of cycles was optimized for each target so that the PCR products did not reach "plateau" levels. RT-

PCR reactions without sample were used as negative controls. RT-PCR products were separated on 10% acrylamide gels and stained with SYBR Green (Molecular Probes). The intensities of the bands were quantified with the Gel Doc 2000 System using the Quantity One software (Bio-Rad, Eke, Belgium). In order to normalize the mRNA levels in the different samples, the intensity of the band corresponding to each mRNA was divided by the intensity of the band corresponding to the 28S rRNA used as an internal standard.

2.7. Statistical analysis

Data are reported as mean ± S.E., and statistical analysis is performed by non-parametric Student's *t*-test. The correlations between mRNA levels and adipose tissue weights were examined using the non-parametric Spearman's rank correlation coefficient. The threshold for significance was set at $P < 0.05$.

3. Results

3.1. Adipose tissue expression

Mice kept on HFD for 15 weeks had a significantly higher total body weight than age-matched mice on SFD, and the weight of their GON and SC fat pad was signifi-
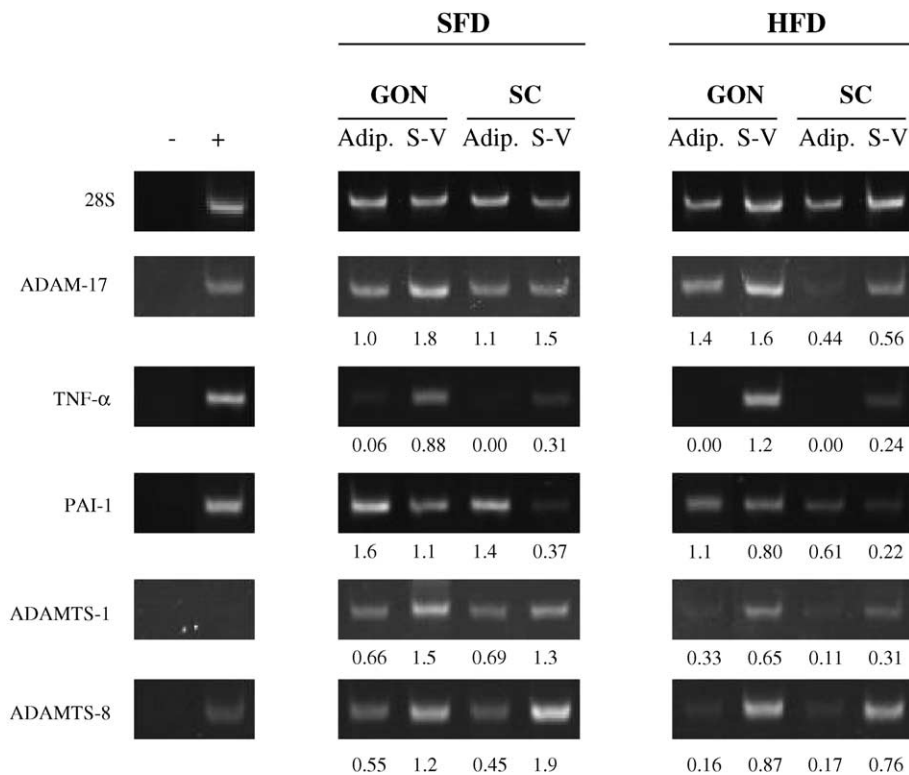

Fig. 2. Cellular localization of mRNA in isolated adipocytes (Adip) or S−V cells derived from SC and GON adipose tissue from mice kept on SFD or HFD for 15 weeks. The ratio between the different mRNA species and 28S rRNA is indicated below the panels. Lane (−) indicates a blank control without sample, and (+) indicates a positive control as indicated in Table 1.

cantly higher (Table 2). A strong positive correlation was observed between the total body weight and the weight of either GON ($\rho = 0.98$, $P = 0.0000001$) or SC ($\rho = 0.97$, $P = 0.000001$) fat, demonstrating that the weight of both depots reflects the extent of obesity. The HFD also resulted in significantly enhanced blood glucose, cholesterol and PAI-1 levels. ADAM-17, TNF-α, PAI-1, ADAMTS-1 and ADAMTS-8 mRNA were detected in both SC and GON adipose tissue from lean mice kept on SFD (Fig. 1). Quantitative analysis (Table 3) indicated that the expression of TNF-α and of ADAMTS-1 was comparable in SC and GON adipose tissue from mice kept on SFD, whereas that of ADAM-17, PAI-1 and ADAMTS-8 was significantly higher in the GON tissue. All five investigated mRNA species were also present in SC and GON adipose tissue from mice kept on HFD (obese); the expression levels were comparable in both fat territories with the exception of a higher expression of ADAMTS-1 in GON tissue (Fig. 1 and Table 3).

Furthermore, the expression of ADAM-17 and PAI-1 was significantly higher, and that of ADAMTS-1 significantly lower in SC, but not in GON, adipose tissue of obese mice as compared to lean mice. In contrast, expression of TNF-α was significantly higher and that of ADAMTS-8 lower in GON, but not in SC, adipose tissue of obese as compared to lean mice. Non-parametric Spearman's rank correlation analysis performed for each mRNA species revealed that the levels of TNF-α were positively and those of ADAMTS-8 negatively correlated with the weight of the GON fat pads. A positive correlation was observed between PAI-1 mRNA levels and a negative correlation between ADAMTS-1 mRNA levels and the weight of the SC fat pads (Table 4). In both SC and GON adipose tissue a significant positive correlation was observed between ADAMTS-8 mRNA levels and the total body weight ($\rho = 0.63$ with $P = 0.038$ for SC and $\rho = -0.84$ with $P = 0.001$ for GON tissue). Furthermore, correlations with total body weight were
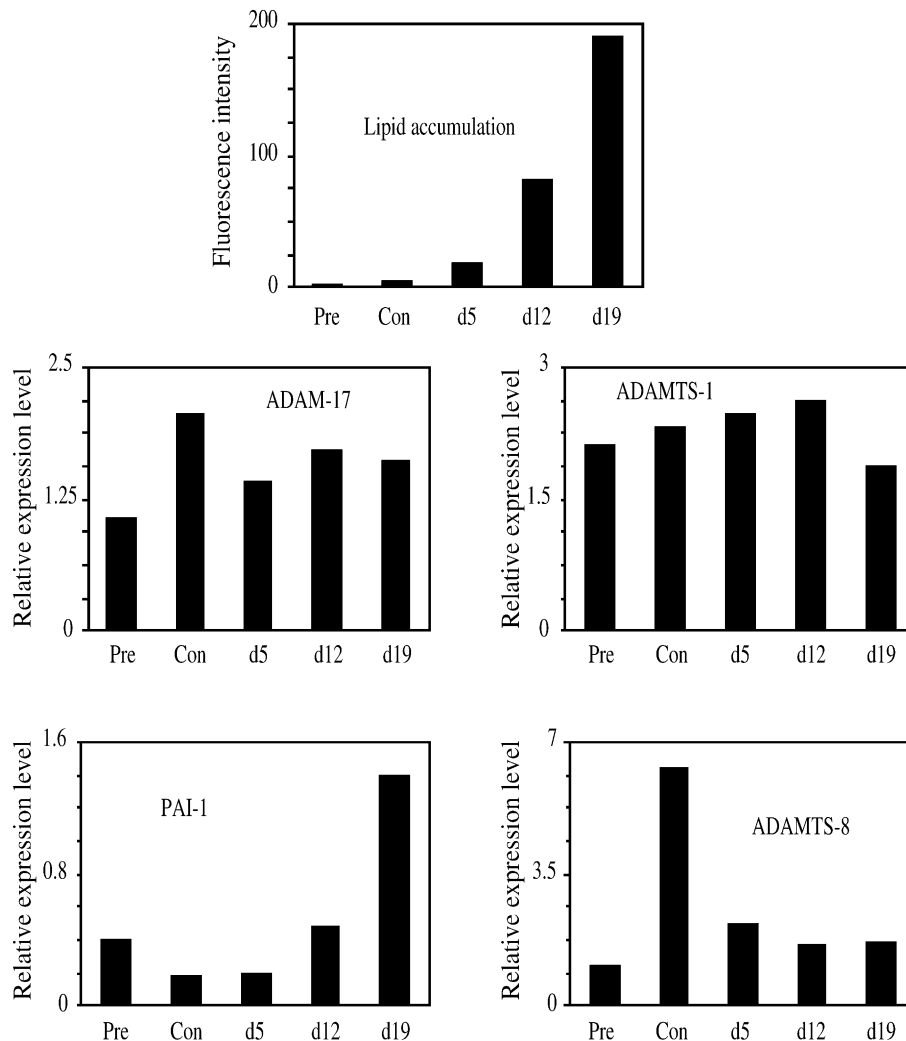

Fig. 3. Expression of ADAM-17, ADAMTS-1, ADAMTS-8 and PAI-1 during in vitro adipogenesis. Total RNA was extracted from 3T3-F442A cells at different stages of differentiation: Pre, preconfluent; Con, confluent (day 0); d5, d12, and d19, postconfluent cultures after 5, 12 and 19 days, respectively. Data, obtained by semi-quantitative RT-PCR, are shown as relative expression levels normalized to the 28S rRNA and are representative of two independent experiments. Intracellular lipid accumulation during preadipocyte differentiation, as monitored by Nile Red fluorescence, is also shown.

observed for PAI-1 ($\rho = 0.8$ with $P = 0.003$) and ADAM-17 ($\rho = 0.69$ with $P = 0.019$) mRNA levels in SC adipose tissue, and for TNF-α ($\rho = 0.72$ with $P = 0.013$) mRNA levels in GON adipose tissue.

3.2. Cellular localization

In mice kept on SFD, the expression of ADAM-17, ADAMTS-1 and ADAMTS-8 was higher in the S–V cell fraction than in mature adipocytes of both SC and GON adipose tissue. TNF-α was expressed nearly exclusively by the S–V cells, whereas PAI-1 expression was higher in mature adipocytes. In mice on HFD, the expression profiles of these mRNA species in isolated adipocytes and S–V cells were similar to those observed in mice on SFD (Fig. 2).

3.3. Expression during in vitro adipogenesis

Differentiation of 3T3-F442A preadipocytes resulted in the appearance of cells characterized by higher intensities of Nile Red fluorescence (a fluorescent staining showing intracellular lipid accumulation) detected at 530 nm; mean values increased from 1 in preconfluent and 5 in confluent cultures to 19, 82 and 190 on days 5, 12 and 19, respectively (Fig. 3). Early signs of differentiation were detected in untreated confluent cultures (day 0) as judged from the presence of small amounts of intracellular lipids and by increased levels of peroxysome proliferator-activated receptor-γ (PPAR-γ; a marker of early differentiation) and glycerophosphate dehydrogenase (GPDH, a marker of the terminal phase of differentiation) mRNAs (not shown). Treatment of confluent preadipocytes with the induction medium resulted in a 5-fold decrease of preadipocyte factor-1 (pref-1, a marker of preadipocytes) mRNA level, concomitant with a strong induction of PPAR-γ expression. As differentiation proceeded, GPDH expression increased simultaneously with the accumulation of intracellular lipids. After 19 days, numerous lipid-filled adipocytes characterized by lower PPAR-γ and GPDH mRNA levels were observed (data not shown).

Quantification of ADAM-17, ADAMTS-1, ADAMTS-8 and PAI-1 mRNA levels revealed different expression profiles (Fig. 3). mRNAs for ADAM-17 and ADAMTS-1 virtually did not change during differentiation. The expression of ADAMTS-8 was high in confluent cultures, but decreased as adipocytes matured. PAI-1 expression increased throughout the differentiation process and was maximal in mature adipocytes (between days 12 and 19). TNF-α was not expressed by 3T3-F442A cells.

4. Discussion

Development of obesity is associated with extensive modifications in adipose tissue, involving adipogenesis, angiogenesis and extracellular matrix remodeling [24]. Pro-

teolytic systems, e.g. the MMP and the plasminogen/plasmin (fibrinolytic) system, contribute to tissue remodeling by degradation of matrix and basement membrane components or activation of latent growth factors [25–27]. Several studies have suggested a potential role of both proteolytic systems in the development of adipose tissue [7–10,21], and recent evidence also suggests involvement of the ADAM and ADAMTS protein families [11–16].

In this study, the expression pattern of ADAM-17, ADAMTS-1, ADAMTS-8, TNF-α and PAI-1 was investigated in adipose tissue of lean and obese mice, and during in vitro differentiation of 3T3-F442A preadipocytes. The development of obesity induced by the HFD modestly increased the expression of ADAM-17 in the SC fat pad and that of TNF-α in the GON fat pad, which might imply an increased TNF-α production and processing in obese tissues. It was previously reported that the exogenous administration of TNF-α to mice increased PAI-1 expression [17]. However, we did not observe a correlation between the expression of either TNF-α and PAI-1 or between TNF-α and ADAM-17, which does not support the hypothesis of an ADAM-17–soluble TNF-α–PAI-1 axis in adipose tissue. In a recent paper by Xu et al. [28], stable expression of ADAM-17, but decreased TNF-α processing by obese murine and human adipose tissue and by mature 3T3F442A adipocytes was reported. The expression of both ADAMTS-1 and ADAMTS-8 was down-regulated in the adipose tissue from mice kept on HFD. The decreased mRNA levels of these angiostatic factors may contribute to the enhanced angiogenesis that accompanies development of obesity. Analysis of the cellular distribution of expression revealed that TNF-α was not expressed by mature adipocytes which is surprising and opposite to the previous finding by Hotamisligil et al. [12]. The differences between genetically and nutritionally obese mice used in the experiments may explain this difference. Furthermore, we observed that PAI-1 expression was higher in mature adipocytes than in S–V cells. Several groups have previously documented PAI-1 synthesis by murine adipocyte cell lines [17,29–31]. In human visceral fat, however, PAI-1 production is mainly due to stromal cells [32]. We also determined the expression pattern during the differentiation of 3T3-F442A preadipocytes. This is an established in vitro model of preadipocyte differentiation although the gene expression profile differs substantially from the in vivo situation. The mRNA levels of PAI-1 showed a marked elevation when the adipocytes became fully differentiated, as reported previously [29]. TNF-α is not produced by 3T3-F442A preadipocytes, indicating that other mechanisms lead to the enhanced expression of PAI-1 in this in vitro system. Expression of ADAM-17 in this system, in the absence of TNF-α expression, indicates that the enzyme has a function different from cleaving membrane-bound TNF-α, as reported in several other studies [33–37].

In conclusion, in this study we show for the first time that ADAM and ADAMTS family members are expressed in

adipose tissue and in 3T3-F442A preadipocytes, and that their mRNA levels show modulation upon development of obesity and during cell differentiation. However, mRNA levels do not necessarily reflect protein levels; furthermore, the activation mechanism of ADAMs and ADAMTSs is not fully understood. Our data may only contribute to a better understanding of these mechanisms. Elucidation of the functional consequences of the modifications reported in this study is at present hampered by the lack of specific reagents to investigate the role of these proteins in murine models of obesity.

Acknowledgements

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