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ADAMTS13 deficiency in mice does not affect adipose tissue development

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ABSTRACT

Background: BMI and ADAMTS13 levels are positively correlated in man. Development of obesity is associated with angiogenesis and inflammation, and increased ADAMTS13 synthesis in the liver.
Methods: Male wild-type (WT) and ADAMTS13 deficient (*Adamts13*$^{-/-}$) mice were kept on normal chow (SFD) or high fat diet (HFD) for 15 weeks.
Results: HFD feeding of WT mice resulted in significantly enhanced levels of ADAMTS13 antigen and activity as compared to SFD feeding. ADAMTS13 deficiency had no significant effect on body weight gain, subcutaneous (SC) or gonadal (GN) adipose tissue mass, or on adipocyte size. In GN fat of obese (HFD) *Adamts13*$^{-/-}$ mice, adipocyte density was higher and blood vessel density lower as compared to obese WT mice. No marked effects of genotype were observed on mRNA expression of adipogenic, endothelial, inflammatory or oxidative stress markers in adipose tissue. Analysis of metabolic parameters and of glucose and insulin tolerance did not reveal significant differences between both obese genotypes, except for higher adiponectin and cholesterol levels in obese *Adamts13*$^{-/-}$ as compared to WT mice.
Conclusion: Our data do not support a functional role of ADAMTS13 in adiposity nor in associated angiogenesis or inflammation in mice.
General significance: ADAMTS13 deficiency may cause thrombotic thrombocytopenic purpura (TTP). Obesity, which is associated with enhanced ADAMTS13 levels is nevertheless considered to be an independent risk factor for TTP. To resolve this apparent contradiction, we show that ADAMTS13 does not directly promote development of adipose tissue in a mouse model.

1. Introduction

Metabolic disturbances such as obesity and diabetes and their associated complications are a main cause of mortality and morbidity. The development of adipose tissue is a complex process in which extensive modifications occur in adipogenesis, angiogenesis and proteolytic remodeling of extracellular matrix [1]. Matrix metalloproteinases are known to play a role in these processes, but little is known on a potential contribution of related classes of proteinases [2,3].

ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin type-1 motif, member 13) is the protease that degrades large multimers of von Willebrand factor in plasma, thereby preventing development of thrombotic thrombocytopenic purpura (TTP), a life-threatening multisystem disease characterized by the formation of microthrombi in capillaries and arterioles [4]. It was suggested that obesity may be a risk factor or trigger for acquired TTP [5]; obesity indeed is more frequent in the population of TTP patients than in the general population and represents an independent risk factor for TTP [6]. In addition, it has been suggested that ADAMTS13 itself may play a role in adiposity. Indeed, ADAMTS13 plasma levels correlate positively with body mass index [7], and its synthesis is significantly increased in the liver of obese mice [8], likely due to low-grade chronic inflammation [9]. However, an inhibitory effect of ADAMTS13 on inflammation has been reported in mouse models of atherosclerosis and cerebral ischemia [10,11]. Furthermore, the thrombospondin 1 (TSP1) domain of ADAMTS proteinases, such as ADAMTS5, has anti-angiogenic properties [12]. Indeed, ADAMTS13 inhibits VEGF-induced angiogenesis by binding to VEGF via its TSP1 domain [13]. However, recently Lee *et al.* found ADAMTS13 to have pro-angiogenic features *in vitro* by upregulating VEGF and VEGFR2 [14].

Because of these apparently contradictory data and the known association of angiogenesis and inflammation with obesity, we have investigated a potential role of ADAMTS13 in the development of adipose

Abbreviations: SFD, standard fat diet; HFD, high fat diet; ADAMTS13, a disintegrin and metalloproteinase with thrombospondin motif member 13; SC, subcutaneous; GN, gonadal

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tissue and associated angiogenesis and inflammation, using an established model of diet-induced obesity in wild-type and ADAMTS13 deficient mice.

2. Methods and procedures

2.1. Nutritionally induced obesity model

Male *Adamts13*$^{-/-}$ (n = 20) and wild-type littermates (n = 18) (genetic background, C57Bl6/J × 129X1/Sv × CASA/RK) [15], from the age of 5 weeks on, were kept in individual micro-isolation cages on a 12 h day/night cycle and fed for 15 weeks with a HFD (Harlan Teklad TD88137, Zeist, The Netherlands; 42% kcal as fat, caloric value 20.1 kJ/g) (n = 17) or a standard fat diet (SFD) (KM-04-k12, Muracon, Carfil, Oud-Turnhout, Belgium; 13% kcal as fat, caloric value 10.9 kJ/g) (n = 21). Water was always available *ad libitum*. Body weight and food intake were measured at weekly intervals.

The mice were sedated and blood was taken from the retro-orbital sinus on trisodium citrate (0.01 M), before they were killed by cervical dislocation. Inguinal subcutaneous (SC) and intra-abdominal gonadal (GN) adipose tissues (AT) were removed and weighed. Portions were used for RNA or protein extraction or were fixed in 1% formaldehyde for histological analysis. Other organs were also removed and weighed.

All animal experiments were approved by the KU Leuven ethical committee (P082-2011) and performed in accordance with the NIH Guide for the Care and use of Laboratory Animals (1996) and carried out in accordance with the EU Directive 2010/63/EU for animal experiments.

2.2. ADAMTS13 determinations

Murine ADAMTS13 antigen levels in plasma were measured using a home-made ELISA. A 96-well microtiter plate was coated overnight with the in house developed murine anti-mADAMTS13 monoclonal antibody 20A10 at 5 μg/ml in PBS. After blocking, plasma was diluted in PBS, 0.3% (m/v) skimmed milk and the plate was incubated at 37 °C for 1 h. An in house developed polyclonal rabbit anti-mADAMTS13 (5 μg/ml) was incubated for 1 h. Bound antibody was detected with HRP-labeled goat anti-rabbit antibody (Jackson Immunoresearch Laboratories Inc., West Grove, PA) (1/25,000 in dilution buffer). A pool of normal murine plasma was used as a reference and set as 100% mADAMTS13 antigen.

The activity of murine ADAMTS13 was measured using the FRETS-VWF73 method [16]. Briefly, murine ADAMTS13 in plasma (20 μl) was incubated with 2 μM FRETS-VWF73 in a HEPES buffered saline solution (50 mM HEPES, 5 mM $CaCl_2$, 1 μM $ZnCl_2$, 150 mM NaCl, pH 7.4; HBS), containing 1 mg/ml bovine serum albumin (Sigma, St Louis, MO). Digestion of FRETS-VWF by murine ADAMTS13 generates a fluorescent signal that is measured using the FLUOstar OPTIMA reader (BMG Labtech GmbH, Offenburg, Germany). ADAMTS13 mRNA levels in liver extracts were determined by quantitative real time PCR using the primers and 6-carboxy-fluorescein (FAM) labeled probes, showed in Table 1.

2.3. Metabolic and inflammatory parameters

At the end of the diet, blood was obtained from the tail of unanesthetized mice after fasting for 6 h. Blood glucose concentrations were measured using the Accu-chek performa meter and blood glucose test strips (Roche Diagnostics, Basel, Switserland). Total and HDL cholesterol levels were evaluated using routine clinical assays. Leptin and adiponectin levels in plasma were determined with specific ELISA's (R&D Systems, Minneapolis, USA).

Extracts of SC and GN adipose tissues were prepared as described [17], and the protein concentration was determined using the BCA protein assay. IL-6 and TNF-α levels in plasma or extracts were measured using commercially available ELISA's (ELISA Ready-SET-Go!, Affymetrix eBioscience, San Diego, CA).

Table 1
Markers detected by qPCR using TaqMan gene expression assays (Life Technologies, Carlsbad, CA).

Gene	Assay
GLUT4	Mm00436615_m1
PPAR-γ	Mm01184322_m1
Adiponectin	Mm00456425_m1
CD36	Mm00432403_m1
VEGF-A	Mm00437304_m1
VE-cadherin	Mm00486938_m1
Endoglin	Mm00468256_m1
F4/80	Mm00802529_m1
TNF-α	Mm00443258_m1
IL-6	Mm00446190_m1
MCP-1	Mm00441242_m1
Arginase	Mm00475988_m1
Mannose receptor	Mm00485148_m1
Catalase	Mm00437992_m1
SOD1	Mm01700393_g1
GPX1	Mm00656767_g1
XDH1	Mm00442110_m1
β-Actin	Mm01205647_g1

	FW primer	RV primer	probe
ADAMTS13	GGAGCCCAAGGA TGTGTGTCTT	TCTCTGGAGGTGAG AGGGAGGAT	6FAM CTTGGCCACCAT GCT MGBNFQ

2.4. Gene expression studies

mRNA expression levels in adipose tissue extracts were determined by quantitative real time PCR, as described elsewhere [18]. qPCR was done in the ABI 7500 Fast Sequence detector (Life Technologies) to detect the markers listed in Table 1. Transcript levels were determined in duplicate by qPCR reaction using a gene expression assay with specific primers, probes and the Fast mastermix (Life Technologies, Carlsbad, CA). Analyses were performed with the ΔΔCT method using the 7500 System SDS software (Life Technologies). Normalization was carried out to correct for fluctuations caused by sample differences. Fold changes were calculated as $2^{-\Delta\Delta CT}$ relative to the mice fed a SFD for the obese mice, (effect of diet) and relative to WT mice for the *Adamts13*$^{-/-}$ mice (effect of genotype). β-Actin was used as housekeeping gene.

2.5. Histological analysis

Paraffin sections (8 μm) for histology were prepared from isolated SC and GN fat pads. The size and density of adipocytes in the SCAT and GNAT were determined by staining with hematoxylin/eosin (H&E) under standard conditions. The blood vessels in the adipose tissues were analyzed by staining with *Bandeiraea simplicifolia* lectin [19]. Analyses were performed by using a Zeiss Axioplan 2 microscope with the AxioVision release 4.8 software (Carl Zeiss, Oberkochen, Germany).

2.6. Insulin and glucose tolerance tests

In separate experiments, after 10 weeks of HFD, WT and ADAMTS13 deficient mice (n = 10 each) were fasted for 6 h and a bolus of 0.1 U/kg human insulin (Eli Lilly Benelux S.A., Brussels, Belgium) or 2 mg/g glucose was administered i.p. Blood glucose levels were determined, as described above, before and 30, 60, 90 and 120 min after insulin or glucose injection.

2.7. Statistics

Data are presented as means ± standard error of the mean (SEM). Statistical significance between groups was analyzed with the non-

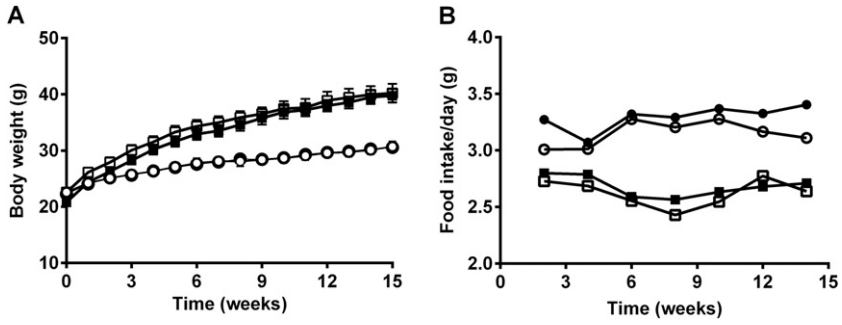

Fig. 1. Body weight and food intake of WT and *Adamts13*$^{-/-}$ mice. Evolution of body weight (A) or food intake (B) of WT (closed symbols) and *Adamts13*$^{-/-}$ (open symbols) mice kept on SFD (circles) or HFD (squares) for 15 weeks. Data are means ± SEM of 8 to 11 experiments in each group. No significant differences were observed between both genotypes (One-way ANOVA).

parametric Mann–Whitney *U* test, one-way or two-way ANOVA. Correlation analysis was performed using the Spearman rank test. Analysis of the data was performed using Prism 6 (GraphPad Software Inc., San Diego, CA). Values of $p < 0.05$ are considered statistically significant.

3. Results

3.1. Effect of ADAMTS13 deficiency on adiposity

Five week old *Adamts13*$^{-/-}$ and WT mice had comparable body weight and showed a very similar weight gain when kept on either SFD (lean) or HFD (obese) for 15 weeks (Fig. 1A) (Table 2). Food intake over this period was also comparable for both genotypes (Fig. 1B). In plasma of WT mice, significantly elevated levels of both ADAMTS13 antigen and activity were observed after 15 weeks of HFD feeding (Fig. 2). ADAMTS13 mRNA expression in the liver was not different between lean or obese WT mice ($p = 0.52$, data not shown). Isolated SC or GN fat mass were not significantly different for WT or *Adamts13*$^{-/-}$ mice on either diet. Adipocytes were markedly enlarged on HFD as compared to SFD, but adipocyte size was similar for both genotypes (Table 2). Adipocyte density in SC adipose tissue was similar for WT and *Adamts13*$^{-/-}$ mice on either SFD or HFD, whereas it was higher in GN fat of obese *Adamts13*$^{-/-}$ versus obese WT mice (Table 2). The weight of other organs, including the liver, heart, spleen, lungs, pancreas, kidneys and hypothalamus was also not different between both genotypes either on SFD or HFD (data not shown).

Gene expression analysis of the adipogenic markers GLUT4, PPAR-γ, adiponectin and CD36 in adipose tissues did not reveal effects of ADAMTS13 deficiency in lean or obese mice, with the exception of lower GLUT4 expression in SC fat of lean *Adamts13*$^{-/-}$ mice (0.80 ± 0.05 versus 1.03 ± 0.09 for lean WT mice, $p < 0.05$ by Mann–Whitney *U* test) (data not shown). Determination of plasma adiponectin levels by ELISA (Table 3) revealed lower levels in obese versus lean WT mice, but not in obese versus lean *Adamts13*$^{-/-}$ mice. Higher levels were observed in obese *Adamts13*$^{-/-}$ versus obese WT mice.

3.2. Effect of ADAMTS13 deficiency on adipose tissue related angiogenesis

Quantitative analysis of blood vessel size and density in SC and GN adipose tissues of WT and *Adamts13*$^{-/-}$ mice on HFD revealed a few significant differences for obese *Adamts13*$^{-/-}$ as compared to obese WT mice: 1) blood vessels in SC adipose tissue were smaller; 2) blood vessel density in GN adipose tissue was lower; and 3) blood vessel density normalized to adipocyte density was lower in GN fat (Table 2). On SFD, no differences were observed between WT and *Adamts13*$^{-/-}$ adipose

Table 2
Body weight, adipose tissue mass, adipocyte size and density, blood vessel size and density in GN and SC adipose tissue of WT and *Adamts13*$^{-/-}$ mice fed a SFD or HFD for 15 weeks.

	SFD		HFD	
	WT	ADAMTS13$^{-/-}$	WT	ADAMTS13$^{-/-}$
n	10	11	8	9
Body weight start (g)	22.3 ± 0.49	22.6 ± 0.65	20.8 ± 0.45	22.4 ± 0.65†
Body weight end [(a)] (g)	31.3 ± 0.48	31.3 ± 0.86	40.9 ± 1.2	40.3 ± 1.7
Body weight gain (g)	8.3 ± 0.28	8.8 ± 0.44	19.8 ± 0.90	17.9 ± 1.3
SC				
Weight (mg)	338 ± 15	384 ± 45	1279 ± 80	1096 ± 92
Adipocyte size (μm^2)	1055 ± 100	1279 ± 156	3092 ± 312	2591 ± 261
Adipocyte density (×10^{-6}/μm^2)	1042 ± 70	900 ± 86	362 ± 42	446 ± 53
Vessel size (μm^2)	24.6 ± 1.9	24.5 ± 1.1	29.5 ± 1.9	24.2 ± 1.2†
Vessel density (×10^{-6}/μm^2)	657 ± 58	510 ± 62	386 ± 43	417 ± 27
Vessel n°/adipocyte n°	0.62 ± 0.04	0.60 ± 0.06	1.2 ± 0.17	1.0 ± 0.09
GN				
Weight (mg)	791 ± 43	855 ± 87	2672 ± 147	2454 ± 241
Adipocyte size (μm^2)	2229 ± 63	2391 ± 155	5626 ± 266	4519 ± 335
Adipocyte density (×10^{-6}/μm^2)	456 ± 13	441 ± 30	182 ± 9.6	234 ± 19†
Vessel size (μm^2)	29.5 ± 1.8	27.6 ± 1.5	34.7 ± 1.3	30.9 ± 3.1
Vessel density (×10^{-6}/μm^2)	337 ± 13	301 ± 20	266 ± 12	203 ± 15††
Vessel n°/adipocyte n°	0.78 ± 0.04	0.70 ± 0.04	1.4 ± 0.14	0.95 ± 0.12†

Data are means ± SEM of n experiments in each group. $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$ as compared to WT mice on HFD (Mann–Whitney-*U* test). [(a)] Body weight after 6 h of fasting.
Abbreviations: SFD, standard fat diet; HFD, high fat diet; WT, wild-type; *Adamts13*$^{-/-}$, ADAMTS13 deficient; SC, subcutaneous adipose tissue; GN, gonadal adipose tissue.

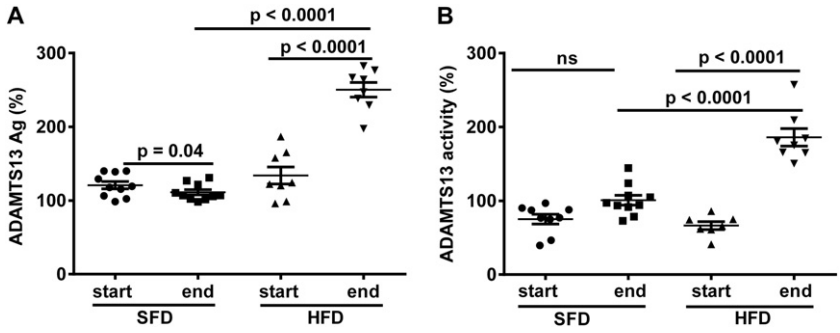

Fig. 2. ADAMTS13 antigen and activity levels in plasma of WT mice. ADAMTS13 antigen (A) and activity (B) levels in plasma of WT mice at the start and the end of SFD or HFD feeding for 15 weeks. *, **** $p < 0.05$, $p < 0.0001$.

tissues. For WT mice, no correlations were observed between plasma ADAMTS13 antigen or activity levels and blood vessel size or density in SC or GN adipose tissues.

We also monitored expression of some endothelial markers by qPCR. This revealed no effects of either diet or genotype on relative expression in GN adipose tissue of endoglin (1.01 ± 0.09 for lean *Adamts13*$^{-/-}$ versus 1.02 ± 0.04 for lean WT mice, with corresponding values of 1.0 ± 0.06 versus 0.89 ± 0.08 for obese *Adamts13*$^{-/-}$ versus obese WT mice). Expression of VEGF-A was lower for obese as compared to lean GN fat for both WT (0.60 ± 0.07 versus 1.05 ± 0.11; $p < 0.01$) and *Adamts13*$^{-/-}$ (0.58 ± 0.06 versus 0.99 ± 0.10; $p < 0.01$ by Mann–Whitney *U* test) mice, but was not different between genotypes. Expression of VE cadherin was higher for obese as compared to lean GN fat for both WT (1.60 ± 0.17 versus 1.02 ± 0.05; $p < 0.01$) and *Adamts*$^{-/-}$ (1.64 ± 0.17 versus 1.13 ± 0.06; $p < 0.01$ by Mann–Whitney U) mice, but was also not different between genotypes.

3.3. Effect of ADAMTS13 deficiency on adipose tissue related inflammation or oxidative stress

Analysis of the effect of diet on expression of inflammatory gene markers in SC adipose tissues of WT mice revealed significantly reduced expression of IL-6 on HFD as compared to SFD, whereas expression of F4/80, TNF-α, MCP-1, arginase or mannose R was not different (Fig. 3A (I)). In GN adipose tissue of WT mice, expression of IL-6 was also significantly reduced on HFD as compared to SFD, whereas F4/80, TNF-α and mannose R expression was enhanced (Fig. 3A (II)). In SC adipose tissue of *Adamts13*$^{-/-}$ mice on HFD versus SFD, enhanced expression of F4/80, arginase and mannose R, and reduced expression of IL-6 was observed (Fig. 3A (I)). Similarly, in GN adipose tissue of obese versus lean *Adamts13*$^{-/-}$ mice, expression of IL-6 was significantly reduced and F4/80 and arginase enhanced (Fig. 3A (II)).

Analysis of the effect of genotype on expression of inflammatory gene markers revealed significantly lower expression of F4/80, TNF-α, IL-6 and mannose R in SC fat, and of F4/80 in GN fat of lean *Adamts13*$^{-/-}$ versus WT mice. For obese *Adamts13*$^{-/-}$ mice as compared to obese WT mice, only higher expression of arginase in SC fat was observed (albeit not statistically different) (Fig. 3B (I)). Expression of MCP-1 in SC or GN adipose tissues was not affected by diet or genotype.

In protein extracts of SC and GN adipose tissues as well as plasma of lean and obese WT and *Adamts13*$^{-/-}$ mice TNF-α and IL-6 could not be detected (detection limit of 78 pg/ml and 145 pg/ml, respectively) by ELISA.

Analysis of the effect of diet on expression of oxidative stress markers in SC adipose tissues revealed enhanced expression of NOX4 for obese *Adamts13*$^{-/-}$ and reduced expression of XDH1 for obese WT mice (Fig. 4A (I)). In GN adipose tissues, NOX4 expression was enhanced for obese WT as well as *Adamts13*$^{-/-}$ mice, whereas SOD1 and GPX1 expression were reduced in obese as compared to lean WT mice (Fig. 4A (II)).

Analysis of the effect of genotype on expression of oxidative stress markers in SC adipose tissues revealed reduced expression of NOX4 and SOD1 for lean *Adamts13*$^{-/-}$ versus lean WT mice, and enhanced expression of GPX1 for obese *Adamts13*$^{-/-}$ versus obese WT mice (Fig. 4B (I)). In GN adipose tissues, only reduced expression of NOX4 for obese *Adamts13*$^{-/-}$ versus obese WT mice was observed (Fig. 4B (II)). Expression of catalase in SC or GN adipose tissues was not affected by diet or genotype.

3.4. Effect of ADAMTS13 deficiency on metabolic parameters

Analysis of plasma metabolic parameters evidenced the effects of HFD versus SFD, as shown by enhanced leptin, total cholesterol and HDL cholesterol levels in WT as well as *Adamts13*$^{-/-}$ mice (Table 3).

No significant effects of genotype were observed on these metabolic parameters, except for higher total cholesterol levels for obese *Adamts13*$^{-/-}$ versus WT mice. Total plasma cholesterol levels for WT mice were strongly positively correlated with both ADAMTS13 antigen ($r = 0.81$, $p < 0.0001$) and activity ($r = 0.67$, $p = 0.002$). Glucose levels for obese *Adamts13*$^{-/-}$ mice were not significantly different as compared to obese WT mice. In separate experiments glucose tolerance and insulin sensitivity tests were performed with obese WT and *Adamts13*$^{-/-}$ mice. No significant differences were observed between WT ($n = 10$) and *Adamts13*$^{-/-}$ ($n = 10$) mice (Fig. 5).

Table 3
Adiponectin, leptin, cholesterol and HDL cholesterol levels in plasma and glucose levels of WT and *Adamts13*$^{-/-}$ mice fed a SFD or HFD for 15 weeks.

	SFD		HFD	
	WT	*Adamts13*$^{-/-}$	WT	*Adamts13*$^{-/-}$
n	10	11	8	9
Adiponectin (μg/ml)	4.1 ± 0.15	4.3 ± 0.33	3.5 ± 0.13**	4.6 ± 0.35††
Leptin (ng/ml)	1.8 ± 0.40	3.4 ± 0.7	18.7 ± 1.2***	16.3 ± 3.7$^{\#}$
Total cholesterol (mg/dl)	56.3 ± 1.1	59.7 ± 3.5	100 ± 7.5***	126 ± 7.9$^{\#\#\#,\dagger}$
HDL cholesterol (mg/dl)	45.6 ± 2.4	47.2 ± 2.8	93.4 ± 6.4***	108 ± 7.3$^{\#\#\#}$
Glucose (mg/dl)	124 ± 7.8	129 ± 7.0	152 ± 8.8	169 ± 4.1

Data are means ± SEM of n experiments in each group. $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$ as compared to WT mice on HFD. $^{**}p < 0.01$, $^{***}p < 0.001$ as compared to WT mice fed a SFD. $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ as compared to *Adamts13*$^{-/-}$ mice fed a SFD (Mann–Whitney-*U* test). Abbreviations: SFD, standard fat diet; HFD, high fat diet; WT, wild-type; *Adamts13*$^{-/-}$, ADAMTS13 deficient.

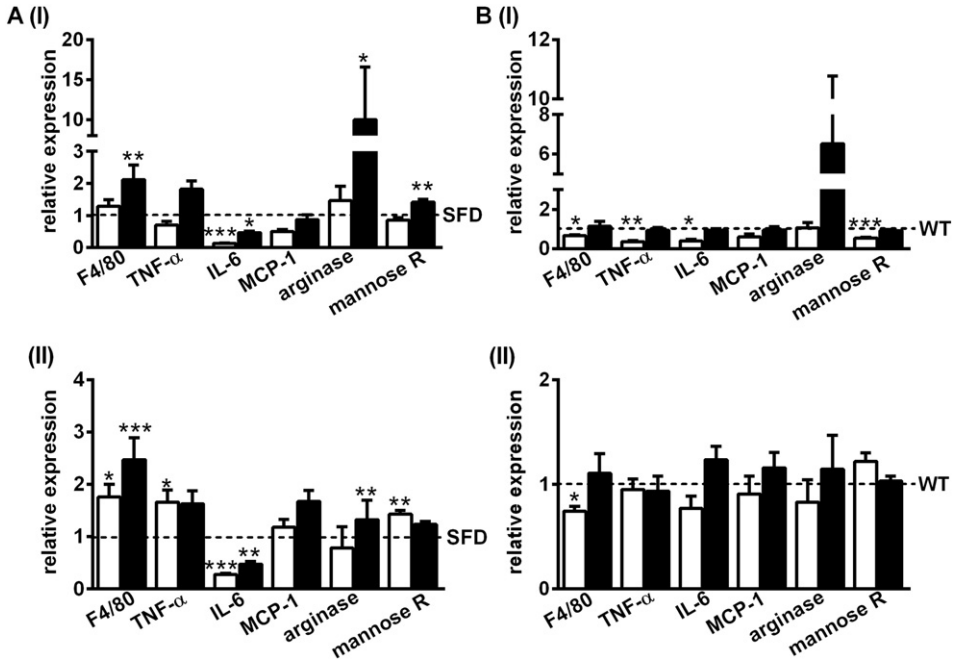

Fig. 3. Gene expression of inflammatory markers in SC and GN adipose tissue. (A) Effect of diet on expression of inflammatory markers in SC (I) or GN (II) adipose tissues. Gene expression is shown for obese relative to lean WT (white bars) or obese relative to lean *Adamts13*$^{-/-}$ (black bars) mice. *, **, *** $p < 0.05$, $p < 0.01$ and $p < 0.001$ for obese versus lean mice. (B) Effect of genotype on expression of inflammatory markers in SC (I) or GN (II) adipose tissues. Gene expression is shown for lean (white bars) or obese (black bars) *Adamts13*$^{-/-}$ relative to WT mice. *, **, *** $p < 0.05$, $p < 0.01$ and $p < 0.001$ for *Adamts13*$^{-/-}$ versus WT mice (Mann–Whitney-*U* test).

4. Discussion

The rationale for this study was based on several published observations relating to a potential functional role of ADAMTS13 in adiposity [7], angiogenesis [13], and oxidative stress/inflammation [20]. Therefore, we have investigated a potential role of ADAMTS13 in adipose tissue development and associated angiogenesis and inflammation using an established mouse model of nutritionally induced obesity [21]. The difference in body weight, weight of SC and GN fat depots, adipocyte size and density between mice fed a SFD or HFD and the enhanced levels of leptin and cholesterol, confirm that the HFD was effective in inducing obesity in both genotypes. However, no differences were found in body weight, food intake, adipocyte size or expression of adipogenic markers between the two genotypes. These data indicate that there is no relevant functional role of ADAMTS13 in adiposity in mice. The enhanced plasma ADAMTS13 levels in obese as compared to lean WT mice could

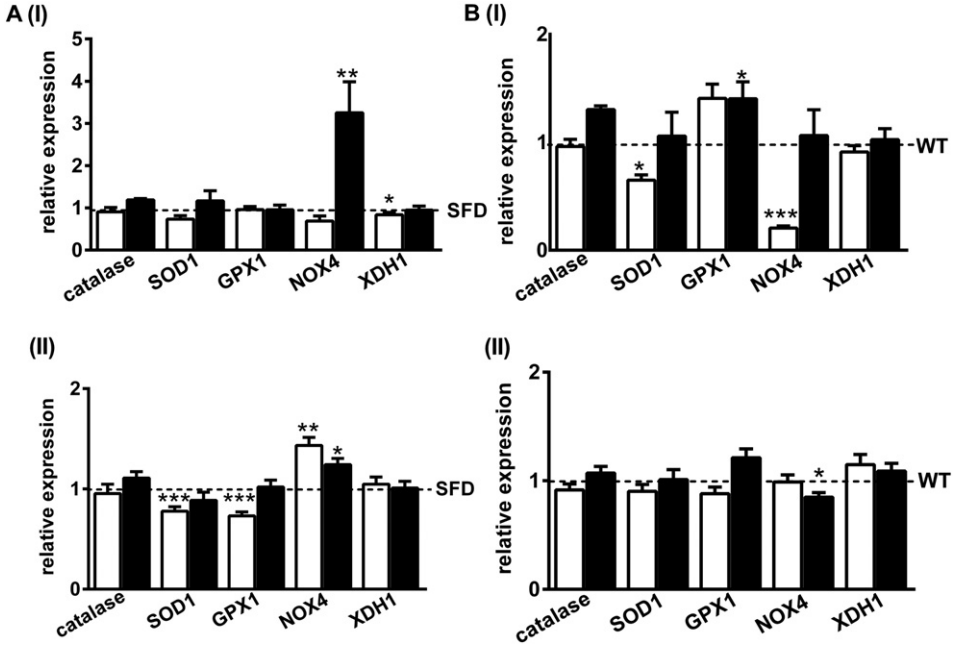

Fig. 4. Gene expression of oxidative stress markers in SC and GN adipose tissue. (A) Effect of diet on expression of oxidative stress markers in SC (I) or GN (II) adipose tissues. Gene expression is shown for obese relative to lean WT (white bars) or obese relative to lean *Adamts13*$^{-/-}$ (black bars) mice. *, **, *** $p < 0.05$, $p < 0.01$ and $p < 0.001$ for obese versus lean mice. (B) Effect of genotype on expression of oxidative stress markers in SC (I) or GN (II) adipose tissues. Gene expression is shown for lean (white bars) or obese (black bars) *Adamts13*$^{-/-}$ relative to WT mice. *, **, *** $p < 0.05$, $p < 0.01$ and $p < 0.001$ for *Adamts13*$^{-/-}$ versus WT mice (Mann–Whitney-*U* test).

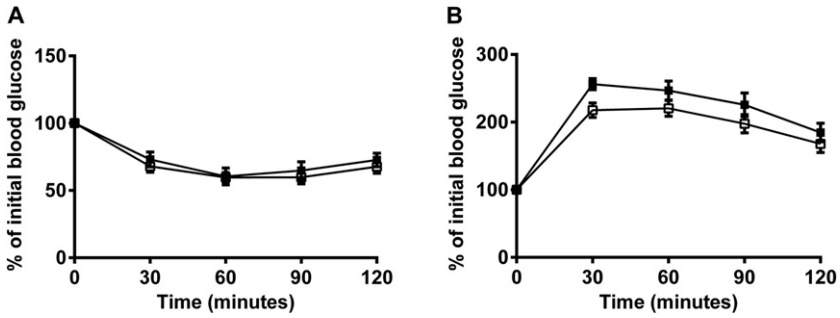

Fig. 5. Insulin and glucose tolerance in WT and *Adamts13*$^{-/-}$ mice after a HFD. Insulin sensitivity (A) and glucose tolerance (B) test of WT (closed symbols) or *Adamts13*$^{-/-}$ (open symbols) mice kept on HFD. Data are means ± SEM of 10 experiments in each group. No significant differences were observed between both genotypes (Two-way ANOVA).

not be explained by increased expression in the liver, the main organ of its synthesis [22], but may be due to enhanced release.

For adipose tissue to expand, each adipocyte needs to be in close proximity to blood vessels for the supply of nutrients and oxygen, for the transport of lipids and signaling molecules and for the elimination of waste products [23]. It was previously established that adipogenesis is related to angiogenesis [24]. Thus, ADAMTS13 inhibits VEGF-induced angiogenesis by binding to VEGF via its TSP1 domain [14]. In our study, obese *Adamts13*$^{-/-}$ mice showed smaller blood vessels in their SC fat depots as compared to WT mice and had fewer blood vessels in their GN adipose tissue. There were, however, no differences in gene expression of endoglin, VEGF-A or VE-cadherin in GN adipose tissues of WT or *Adamts13*$^{-/-}$ mice. It is generally accepted that adipose tissue associated angiogenesis is mainly regulated by VEGF [23]. Thus, the anti-angiogenic effect of ADAMTS13 in adipose tissue related angiogenesis is at best marginal and does not affect *in vivo* adipose tissue development.

Obesity is associated with a state of low-grade chronic inflammation which triggers some related comorbidities. Pro-inflammatory cytokines are secreted and leptin production by dysfunctional adipocytes is increased leading to insulin resistance. Adiponectin is an anti-inflammatory insulin-sensitizing hormone that inhibits several obesity-related processes such as formation of foam cells and production of TNF-α in macrophages [25]. It is known that adiponectin concentrations decrease with increasing body weight in mice as well as in humans [26]. Interestingly, in our study adiponectin levels were comparable in lean and obese *Adamts13*$^{-/-}$ mice, but were higher in obese *Adamts13*$^{-/-}$ mice as compared to their WT counterparts. The fact that adiponectin levels in *Adamts13*$^{-/-}$ mice do not decrease upon HFD feeding may suggest a somewhat improved metabolic profile.

In agreement with a previous study of Crawley *et al.* [7] in man, we observed a strong positive correlation between cholesterol levels and ADAMTS13 levels in WT mice. However, total plasma cholesterol levels were higher in obese *Adamts13*$^{-/-}$ versus obese WT mice. This apparent contradiction questions the functional association between ADAMTS13 and cholesterol levels. Furthermore, insulin and glucose tolerance tests on obese mice did not reveal differences between the two genotypes and blood glucose levels at the start of the tests were comparable for obese WT and *Adamts13*$^{-/-}$ mice. These findings indicate that there is no direct effect of ADAMTS13 on glycemia in nutritionally induced obese mice.

Reactive oxygen species and oxidative stress are involved in adipocyte dysfunction. Stressed adipocytes send out signals that recruit macrophages in white adipose tissue leading to production of pro-inflammatory cytokines resulting in further enhanced stress and adipocyte death [27]. Based on the literature it was anticipated that obesity [28] as well as ADAMTS13 deficiency [20] would be associated with enhanced inflammation. Therefore, we monitored expression of pro- and anti-inflammatory gene markers in SC and GN adipose tissues of lean and obese WT and *Adamts13*$^{-/-}$ mice. More specifically, as general macrophage markers F4/80 and MCP-1 were used. TNF-α and mannose receptor or arginase were used as markers for M1 (classically activated, pro-inflammatory) or M2 (alternatively activated, anti-inflammatory) macrophages, respectively. IL-6 can act as a pro-inflammatory as well as an anti-inflammatory cytokine [29]. It is secreted by adipocytes in obese subjects [30]. The major changes that were observed (≥2-fold difference; $p < 0.05$) for the effect of diet (HFD versus SFD) were: 1) reduced expression of IL-6 in SC and GN fat of both WT and *Adamts13*$^{-/-}$ mice; 2) enhanced expression of the macrophage marker F4/80 in GN fat of *Adamts13*$^{-/-}$ mice (as expected); and 3) enhanced expression of anti-inflammatory arginase and mannose R in SC fat of *Adamts13*$^{-/-}$ mice (reduction was expected). For the effect of genotype (*Adamts13*$^{-/-}$ versus WT), we observed: 1) in SC fat of lean mice reduced expression of TNF-α (increase expected), of IL-6 and of mannose R; 2) enhanced arginase expression in SC fat of obese *Adamts13*$^{-/-}$ mice: and 3) reduced expression of F4/80 in both SC and GN adipose tissue of lean *Adamts13*$^{-/-}$ versus WT mice. In obese adipose tissues no relevant effects of genotype were observed on expression of inflammatory gene markers. In lean SC adipose tissues, however, expression of pro-inflammatory TNF-α and F4/80 and of anti-inflammatory mannose R was significantly reduced in *Adamts13*$^{-/-}$ as compared to WT mice. These differences were not consistently detected in lean GN adipose tissues, except for lower expression of F4/80 in *Adamts13*$^{-/-}$ tissues. Because of the confounding effects of the combination of obesity and ADAMTS13 deficiency, and the differences between SC and GN adipose tissues, these data do not allow to predict whether ADAMTS13 is pro- or anti-inflammatory in adipose tissue. In any case, the observed changes in expression of inflammatory markers are not reflected in differences in adipose tissue development between WT and *Adamts13*$^{-/-}$ mice. Similar analysis for the expression of oxidative stress gene markers revealed only an effect of HFD on enhanced expression of NOX4 in *Adamts13*$^{-/-}$ mice (as expected), and no effects of genotype. As chronic inflammation and enhanced oxidative stress are considered to be consequences of obesity, and ADAMTS13 deficiency did not affect adiposity, significant differences in associated inflammation or oxidative stress between both obese genotypes would not be expected.

Thus, despite several indications from previous studies, our study does not support a functional role of ADAMTS13 in obesity nor in associated angiogenesis or inflammation, at least in mice. However, it cannot be excluded that ADAMTS13 plays a more prominent role in adipose tissue development in humans.

Transparency document

The Transparency document associated with this article can be found, in the online version.

Conflict of interest statement

We have no conflict of interest to declare.

Acknowledgments

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References

[1] D.L. Crandall, G.J. Hausman, J.G. Kral, A review of the microcirculation of adipose tissue: anatomic, metabolic, and angiogenic perspectives, Microcirculation 4 (1997) 211–232.
[2] V. Christiaens, I. Scroyen, H.R. Lijnen, Role of proteolysis in development of murine adipose tissue, Thromb. Haemost. 99 (2008) 290–294.
[3] K. Sun, C.M. Kusminski, P.E. Scherer, Adipose tissue remodeling and obesity, J. Clin. Invest. 121 (2011) 2094–2101.
[4] C. Le Goff, V. Cormier-Daire, The ADAMTS(L) family and human genetic disorders, Hum. Mol. Genet. 20 (2011) R163–R167.
[5] A.M. Lombardi, R. Fabris, A. Scarda, V. Zanato, C. Dal Pra, P. Scarparo, S. Vettore, M. Granzotto, G. Berti De Marinis, M. Foletto, R. Serra, M.T. Sartori, M. Plebani, F. Fabris, R. Vettor, Presence of anti-ADAMTS13 antibodies in obesity, Eur. J. Clin. Invest. 42 (2012) 1197–1204.
[6] K.K. Nicol, B.J. Shelton, M.A. Knovich, J. Owen, Overweight individuals are at increased risk for thrombotic thrombocytopenic purpura, Am. J. Hematol. 74 (2003) 170–174.
[7] J.T. Crawley, D.A. Lane, M. Woodward, A. Rumley, G.D. Lowe, Evidence that high von Willebrand factor and low ADAMTS-13 levels independently increase the risk of a non-fatal heart attack, J. Thromb. Haemost. 6 (2008) 583–588.
[8] M.Y. Liu, Z. Zhou, R. Ma, Z. Tao, H. Choi, A.L. Bergeron, H. Wu, J.F. Dong, Gender-dependent up-regulation of ADAMTS-13 in mice with obesity and hypercholesterolemia, Thromb. Res. 129 (2012) 536–539.
[9] E. Hijona, L. Hijona, J.I. Arenas, L. Bujanda, Inflammatory mediators of hepatic steatosis, Mediators Inflamm. 2010 (2010) 837419.
[10] C. Gandhi, M.M. Khan, S.R. Lentz, A.K. Chauhan, ADAMTS13 reduces vascular inflammation and the development of early atherosclerosis in mice, Blood 119 (2012) 2385–2391.
[11] S.Y. Jin, J. Tohyama, R.C. Bauer, N.N. Cao, D.J. Rader, X.L. Zheng, Genetic ablation of Adamts13 gene dramatically accelerates the formation of early atherosclerosis in a murine model, Arterioscler. Thromb. Vasc. Biol. 32 (2012) 1817–1823.
[12] S. Kumar, S. Sharghi-Namini, N. Rao, R. Ge, ADAMTS5 functions as an anti-angiogenic and anti-tumorigenic protein independent of its proteoglycanase activity, Am. J. Pathol. 181 (2012) 1056–1068.
[13] M. Lee, E.S. Rodansky, J.K. Smith, G.M. Rodgers, ADAMTS13 promotes angiogenesis and modulates VEGF-induced angiogenesis, Microvasc. Res. 84 (2012) 109–115.
[14] M. Lee, J. Keener, J. Xiao, X. Long Zheng, G.M. Rodgers, ADAMTS13 and its variants promote angiogenesis via upregulation of VEGF and VEGFR2, Cell. Mol. Life Sci. 72 (2014) 349–356.
[15] D.G. Motto, A.K. Chauhan, G. Zhu, J. Homeister, C.B. Lamb, K.C. Desch, W. Zhang, H.M. Tsai, D.D. Wagner, D. Ginsburg, Shigatoxin triggers thrombotic thrombocytopenic purpura in genetically susceptible ADAMTS13-deficient mice, J. Clin. Invest. 115 (2005) 2752–2761.
[16] K. Kokame, Y. Nobe, Y. Kokubo, A. Okayama, T. Miyata, FRETS-VWF73, a first fluorogenic substrate for ADAMTS13 assay, Br. J. Haematol. 129 (2005) 93–100.
[17] H.R. Lijnen, E. Maquoi, D. Demeulemeester, B. Van Hoef, D. Collen, Modulation of fibrinolytic and gelatinolytic activity during adipose tissue development in a mouse model of nutritionally induced obesity, Thromb. Haemost. 88 (2002) 345–353.
[18] I. Scroyen, L. Cosemans, H.R. Lijnen, Effect of tissue inhibitor of matrix metalloproteinases-1 on in vitro and in vivo adipocyte differentiation, Thromb. Res. 124 (2009) 578–583.
[19] L. Laitinen, Griffonia simplicifolia lectins bind specifically to endothelial cells and some epithelial cells in mouse tissues, Histochem. J. 19 (1987) 225–234.
[20] A.K. Chauhan, J. Kisucka, A. Brill, M.T. Walsh, F. Scheiflinger, D.D. Wagner, ADAMTS13: a new link between thrombosis and inflammation, J. Exp. Med. 205 (2008) 2065–2074.
[21] V. Christiaens, M. Van Hul, H.R. Lijnen, I. Scroyen, CD36 promotes adipocyte differentiation and adipogenesis, Biochim. Biophys. Acta 1820 (2012) 949–956.
[22] W. Zhou, M. Inada, T.P. Lee, D. Benten, S. Lyubsky, E.E. Bouhassira, S. Gupta, H.M. Tsai, ADAMTS13 is expressed in hepatic stellate cells, Lab. Invest. 85 (2005) 780–788.
[23] G.J. Hausman, R.L. Richardson, Adipose tissue angiogenesis, J. Anim. Sci. 82 (2004) 925–934.
[24] V. Christiaens, H.R. Lijnen, Angiogenesis and development of adipose tissue, Mol. Cell. Endocrinol. 318 (2010) 2–9.
[25] A. Koerner, J. Kratzsch, W. Kiess, Adipocytokines: leptin—the classical, resistin—the controversial, adiponectin—the promising, and more to come, Best Pract. Res. Clin. Endocrinol. Metab. 19 (2005) 525–546.
[26] A. Yadav, M.A. Kataria, V. Saini, Role of leptin and adiponectin in insulin resistance, Clin. Chim. Acta 417 (2013) 80–84.
[27] M.F. Gregor, G.S. Hotamisligil, Thematic review series: adipocyte biology. Adipocyte stress: the endoplasmic reticulum and metabolic disease, J. Lipid Res. 48 (2007) 1905–1914.
[28] S.P. Weisberg, D. McCann, M. Desai, M. Rosenbaum, R.L. Leibel, A.W. Ferrante Jr., Obesity is associated with macrophage accumulation in adipose tissue, J. Clin. Invest. 112 (2003) 1796–1808.
[29] J. Scheller, A. Chalaris, D. Schmidt-Arras, S. Rose-John, The pro- and anti-inflammatory properties of the cytokine interleukin-6, Biochim. Biophys. Acta 1813 (2011) 878–888.
[30] T. Skurk, C. Alberti-Huber, C. Herder, H. Hauner, Relationship between adipocyte size and adipokine expression and secretion, J. Clin. Endocrinol. Metab. 92 (2007) 1023–1033.