Role of ADAMTS13 in diet-induced liver steatosis

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Abstract. Previous studies, predominantly based on increased or decreased plasma levels, have reported conflicting data on a potential functional role of ADAMTS13 in the pathogenesis of liver diseases, including non-alcoholic steatohepatitis (NASH). The aim of the current study was to evaluate whether ADAMTS13 deficiency affects development of NASH. Therefore, male wild-type (WT) and *Adamts13* deficient (*Adamts13*$^{-/-}$) mice were kept on a steatosis-inducing diet devoid of methionine and choline (MCD) or a control diet (MCC) for 4 weeks. Induction of NASH did not affect plasma ADAMTS13 antigen levels of WT mice. MCD as compared with MCC feeding resulted in reduced body and liver weight with no differences between the genotypes. Plasma levels of the liver enzymes AST and ALT were significantly higher for MCD vs. MCC fed *Adamts13*$^{-/-}$ and WT mice, however were not different between the genotypes. Liver triglyceride levels were also higher after MCD feeding, but were not different between WT and *Adamts13*$^{-/-}$ mice. *Adamts13*$^{-/-}$ mice on the two diets exhibited higher insulin sensitivity when compared with WT mice. On the MCC diet, the genotype did not show clear histological abnormalities in the liver, whereas severe steatosis and fibrosis were observed on MCD diet, however were comparable for both genotypes. This was supported by comparably enhanced hepatic expression in the two genotypes on MCD diet of the steatosis marker CD36 and of the fibrosis marker tissue inhibitor of metalloproteinase 1. Thus, the results of the current study do not support a functional role of ADAMTS13 in this murine model of NASH.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is characterized by excessive fat accumulation in the liver of patients without a history of alcohol abuse. NAFLD is classified into simple steatosis and non-alcoholic steatohepatitis (NASH). In patients with NASH, in addition to steatosis, additional intralobular inflammation and hepatocellular ballooning are observed, frequently with progressive fibrosis (1). Over time, NASH may progress to liver cirrhosis and hepatocellular carcinoma (2-4). It is recognized that the increased prevalence of obesity and metabolic syndrome is paralleled by an increase in NAFLD (5,6), as up to 80% of obese subjects suffer from NAFLD (7). Worldwide, approximately 20% of all adults have NAFLD and 2-3% suffer from NASH (8).

ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin type-1 motif, member 13) is a proteinase that specifically cleaves multimeric von Willebrand factor (VWF), thereby preventing accumulation of ultralarge VWF multimers and subsequent platelet clumping and formation of microthrombi that disturb the microcirculation and impair oxygen supply to organs (9). ADAMTS13 is predominantly produced by stellate cells in the liver (10), of which proliferation contributes to steatohepatitis and fibrosis. This is compatible with enhanced ADAMTS13 antigen and activity levels observed in rats suffering from diet-induced steatosis and fibrosis (11). Several previous studies have, however, reported conflicting data on a potential functional role of ADAMTS13 in the pathogenesis of liver diseases [reviewed in (12)]. A beneficial effect of ADAMTS13 activity was observed on liver disease severity, and increased ADAMTS13 activity was associated with an improved prognosis of liver cirrhosis (13), alcoholic hepatitis (14) and hepatic veno-occlusive disease (15). In contrast, a detrimental effect of ADAMTS13 was reported on hepatocellular carcinoma risk in patients suffering from chronic liver disease (16). Thus, it remains unclear whether NASH is associated with increased or decreased levels of ADAMTS13, nor whether ADAMTS13 serves a functional role in its development. In order to resolve these apparent contradictions, we have subjected wild-type and *Adamts13* deficient mice to a steatosis-inducing diet.

Materials and methods

Animal model. Male (n=2) and female (n=2) heterozygous *Adamts13*$^{+/-}$ mice were provided by Professor David Ginsburg (Howard Hughes Medical Institute, University of Michigan, Ann Arbor, MI, USA) and were crossed to generate *Adamts13* deficient (*Adamts13*$^{-/-}$) and wild-type (WT) littermates (genetic background, C57Bl6/Jx129X1/SvxCASA/RK). Male *Adamts13*$^{-/-}$ (n=20) and WT (n=10) mice were used and genotyped as previously described (17,18). The mice were kept from the age of 5 weeks in individual micro-isolation cages on a 12 h day/night cycle and fed for 4 weeks with a lipogenic diet devoid of methionine and choline (MCD; #02960034; MP Biomedicals, Illkirch Cedex, France) or the MCD diet supplemented with 3 g/kg DL-methionine and 2 g/kg choline chloride (control diet; MCC; #02960414; MP Biomedicals) (n=10 or 5 for *Adamts13*$^{-/-}$ or WT mice on each diet, respectively). The starting weight of the mice was 26.4±0.8 g or 27.0±1.0 g for WT mice on MCD or MCC diets, respectively, and 26.0±0.5 g or 25.4±0.7 g for *Adamts13*$^{-/-}$ mice on MCD or MCC diets, respectively. Water was available *ad libitum*, room temperature and relative humidity were 23˚C and 26%, respectively. Body weight and food intake were measured at weekly intervals. At the end of the diet, following fasting for 6 h, blood was taken from the tail of unanesthetized mice for determination of blood glucose concentrations using the Accu-chek Performa meter and blood glucose test strips (Roche Diagnostics, Basel, Switzerland). At the end of the experiment, the mice were sedated with 60 mg/kg pentobarbital (Nembutal; Ceva Santé Animale, Libourne, France) and blood was taken from the retro-orbital sinus on trisodium citrate (0.01 M), prior to sacrifice by cervical dislocation. Platelets were counted (Cell Dyn 3200R; Abbott Diagnostics, Illinois, USA). Livers, subcutaneous (SC) and gonadal (GN) adipose tissues were removed and weighed. Portions were used for RNA or protein extraction or were fixed in 4% formaldehyde for histological analysis.

All animal experiments were approved by the University of Leuven Ethical Committee (P158-2011) and performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (19) and the EU Directive 2010/63/EU for animal experiments.

Metabolic and inflammatory parameters. Total, high density lipoprotein (HDL) and low density lipoprotein (LDL) cholesterol, triglycerides, alkaline phosphatases, alanine aminotransferase (ALT) and aspartate aminotransaminase (AST) levels in plasma were evaluated using routine clinical assays. Insulin levels in plasma were determined by ELISA (Ultrasensitive Mouse Insulin ELISA 10-1249-01; Mercodia AB, Uppsala, Sweden). The homeostasis model assessment of insulin resistance (HOMA-IR) was determined using the formula: [Fasting plasma insulin (ng/ml)x fasting blood glucose (mg/dl)]/405. Liver tissue extracts were prepared by adding alcoholic KOH (30%) to a sample of 30 mg. Samples were incubated overnight at 55˚C to digest the tissue, and 1 M $MgCl_2$ was added to the digested sample (1:1 vol/vol), mixed well, incubated on ice for 10 min and centrifuged for 30 min at 13,523 x g and room temperature. The supernatant was analyzed using the Triglycerides FS* kit (DiaSys Diagnostic Systems GmbH, Holzheim, Germany).

Table I. Markers detected by reverse transcription-quantitative polymerase chain reaction using TaqMan gene expression assays.

Gene	Assay
F4/80	Mm00802529_m1
TNF-α	Mm00443258_m1
MCP-1	Mm00441242_m1
IL-6	Mm0446190_m1
Arginase	Mm00475988_m1
Mannose receptor	Mm00485148_m1
FAS	Mm01253300_g1
CD36	Mm00432403_m1
PPAR-α	Mm00440939_m1
TIMP-1	Mm0441818_m1
PAI-1	Mm0435860_m1
α-SMA	Mm00725412_s1
Col1a1	Mm00801666_g1
TGF-β	Mm01178820_m1
β-actin	Mm01205647_g1

All assays were purchased from Life Technologies (Thermo Fisher Scientific, Inc., Waltham, MA, USA). TNF-α, tumor necrosis factor α; MCP-1, monocyte chemoattractant protein 1; IL-6, interleukin 6; FAS, Fas cell surface death receptor; CD36, cluster of differentiation; PPAR-α, peroxisome proliferator-activated receptor α; TIMP-1, tissue activator of metalloproteinase 1; PAI-1, plasminogen activator inhibitor 1; α-SMA, α-smooth muscle actin; Col1a1, collagen type I α1 chain; TGF- β, transforming growth factor β.

Histological and microscopic analysis. Liver samples were fixed in 4% buffered formaldehyde and embedded in paraffin. Sections (4 μm) were stained with hematoxylin-eosin (H&E) or Picrosirius Red to assess steatosis or fibrosis, respectively. All liver biopsies were analyzed by an expert liver pathologist, blinded to the genotype and diet. Steatosis and fibrosis were diagnosed and semiquantitatively scored according to the NASH-Clinical Research Network criteria (20,21). Hepatocyte ballooning was classified as 0 (none), 1 (few) or 2 (numerous cells/prominent ballooning). Foci of lobular inflammation were scored as 0 (no foci), 1 (<2 foci per x200 field), 2 (2-4 foci per x200 field) and 3 (>4 foci per x200 field). Fibrosis was scored as stage F0 (no fibrosis), stage F1a (mild, zone 3, perisinusoidal fibrosis), stage F1b (moderate, zone 3, perisinusoidal fibrosis), stage F1c (portal/periportal fibrosis), stage F2 (perisinusoidal and portal/periportal fibrosis), stage F3 (bridging fibrosis) and stage F4 (cirrhosis). Severity of the disease was assessed using the NAFLD activity score (NAS) as the unweighted sum of scores of steatosis, hepatocyte ballooning and lobular inflammation (22). Percentage of fibrosis was quantitated by morphometry from digitalized Picrosirius Red stained sections (23).

RNA extraction and expression analysis. RNA extraction from livers was performed using the RNeasy Mini kit (Qiagen, Basel, Switzerland) according to the manufacturer's protocol. A total of 10 ng/μl RNA was reverse transcribed into cDNA using the Multiscribe™ Reverse Transcriptase kit (Life Technologies;

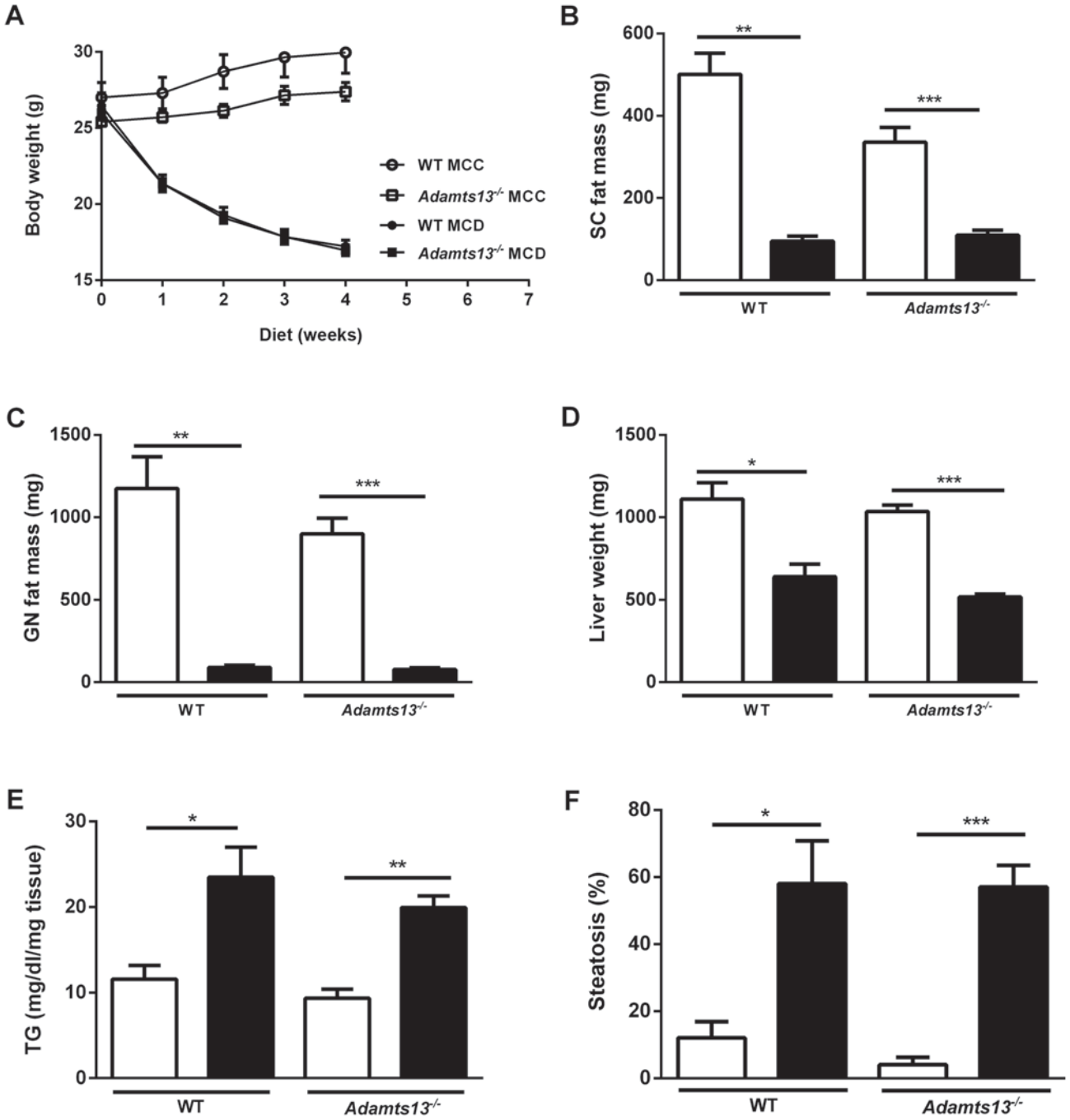

Figure 1. Effect of MCD and MCC diets on body weight and liver of WT and *Adamts13*$^{-/-}$ mice. (A) Body weight evolution and weight of (B) SC fat, (C) GN fat and (D) liver of mice kept on MCD (black symbols and bars) or MCC (white symbols and bars) diets for 4 weeks. (E) Liver TG levels and (F) quantitation of liver steatosis. Data are presented as the mean ± standard error of 10 (*Adamts13*$^{-/-}$) or 5 (WT) experiments for each diet. *P<0.05, **P<0.01 and ***P<0.001 MCD vs. MCC. MCD, methionine and choline deficient diet; MCC, control diet; WT, wild-type; *Adamts13*$^{-/-}$, a disintegrin and metalloproteinase with thrombospondin type-1 motif, member 13 deficient; SC, subcutaneous; GN, gonadal; TG, triglyceride.

Thermo Fisher Scientific, Inc., Waltham, MA, USA), according to the manufacturer's protocol. Reverse transcription-quantitative polymerase chain reaction was completed in the ABI 7500 Fast Sequence detector (Life Technologies; Thermo Fisher Scientific, Inc.) to detect the markers listed in Table I. Thermocycling conditions were as follows: Fast mode run of 40 cycles of 20 sec at 95˚C, 3 sec at 95˚C and 30 sec at 60˚C. The reaction mixture contained 5 μl TaqMan Fast Universal PCR Master Mix (2X; Life Technologies; Thermo Fisher Scientific, Inc.), 0.5 μl TaqMan Gene Expression assay (see Table I; Life Technologies; Thermo Fisher Scientific, Inc.) and 2 μl cDNA diluted with RNase-free water to a total volume of 10 μl. For ADAMTS13 expression, probe (0.2 μl; 1:10 diluted probe; Life Technologies; Thermo Fisher Scientific, Inc.), and forward and reverse primers (0.3 μl; 1:10 diluted primer; Life Technologies; Thermo Fisher Scientific, Inc.) were used instead of TaqMan Gene Expression assays. The sequences of the probe, and forward and reverse primers for ADAMTS13 expression were as follows: Forward primer, GGAGCCCAAGGATGTGTGTCTT; reverse primer, TCTCTGGAGGTGAGAGGGAGGAT; and probe,

6FAM (reporter dye) CTTGGCCACCATGCT MGBNFQ (non-fluorescent quencher with maximum sensitivity). Analyses were performed using the ΔΔCq method (24) using the 7500 System SDS software (Life Technologies; Thermo Fisher Scientific, Inc.). Normalization was conducted to correct for fluctuations caused by sample differences. Fold changes for MCD diet fed mice were calculated as $2^{-\Delta\Delta Cq}$ relative to the MCC diet. β-actin was used as the housekeeping gene.

VWF and ADAMTS13 antigen levels. Murine VWF and ADAMTS13 antigen levels in the plasma were measured using ELISA assays made in the Laboratory for Thrombosis Research (Department of Chemistry, University of Leuven Kulak Campus, Kortrijk, Belgium), as previously described (25,26).

Statistical analysis. Data are presented as the means ± standard error of. Statistical significance between groups was analyzed with the non-parametric Mann-Whitney U test. Analysis of the data was performed using Prism, version 6 (GraphPad Software, Inc., San Diego, CA, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

The effects of the MCD diet were determined on body weight and liver function in WT and *Adamts13*$^{-/-}$ mice (Fig. 1) . Feeding WT and *Adamts13*$^{-/-}$ mice with the MCD diet for 4 weeks, as compared with the control MCC diet, resulted in rapid and progressive weight loss for the two genotypes (Fig. 1A). Food intake was comparable for WT and *Adamts13*$^{-/-}$ mice kept on MCC (3.3 vs. 3.0 g/mouse/day) or on MCD diet (2.4 vs. 2.2 g/mouse/day). At the end of the diets, total body weight was comparable for WT and *Adamts13*$^{-/-}$ mice on the MCC (30.0±1.4 vs. 27.4±0.6 g) and MCD diets (17.2±0.4 vs. 17±0.4 g). As predicted, the SC and GN fat mass levels were significantly reduced for in each genotype comparing the MCD and MCC diets (Fig. 1B and C).

For WT mice, plasma ADAMTS13 antigen levels were not significantly different between the MCC and MCD diets (183±12 vs. 205±17% of pooled plasma), and relative expression of *Adamts13* mRNA in the liver was also comparable on the two diets (0.91±0.07 vs. 1.0±0.05). VWF antigen levels were comparable for the genotypes on either diet (Table II). However, platelet counts were significantly lower for *Adamts13*$^{-/-}$ fed the MCD when compared with MCC, however this was not observed in WT mice (Table II). Analysis of plasma metabolic parameters (Table II) identified for MCD vs. MCC diets for the two genotypes: i) Lower glucose levels; ii) lower total and HDL cholesterol levels; iii) higher LDL cholesterol levels (P<0.05 for *Adamts13*$^{-/-}$ mice only); and iv) comparable triglyceride levels. Insulin levels were markedly lower in MCD vs. MCC diet (significant for the *Adamts13*$^{-/-}$ mice due to small sample sizes for WT mice), and were reduced for *Adamts13*$^{-/-}$ vs. WT mice on MCC. Thus, HOMA-IR identified higher insulin sensitivity for *Adamts13*$^{-/-}$ mice on the MCC diet (Table II).

Total liver weight was reduced upon MCD feeding, however was not affected by the genotype (Fig. 1D). The liver enzymes AST and ALT were markedly higher in MCD fed as compared with MCC fed mice, however were not different between genotypes (Table II). Liver triglyceride levels were additionally enhanced upon MCD feeding, however were not affected by the genotype (Fig. 1E).

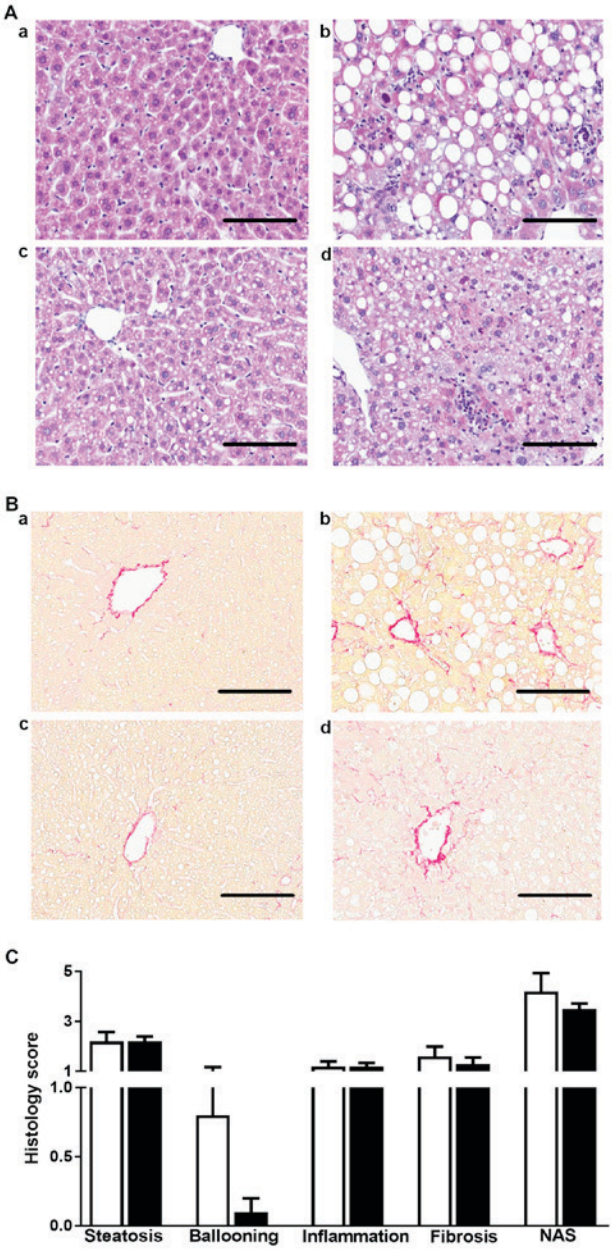

Figure 2. MCD diet-induced steatohepatitis in WT and *Adamts13*$^{-/-}$ mice. (A) Hematoxylin and eosin and (B) Picrosirius Red staining of liver sections of (a and b) WT and (c and d) *Adamts13*$^{-/-}$ mice kept on (a and c) MCC or (b and d) MCD diet for 4 weeks. Scale bars represent 100 μm. (C) Histological scoring of steatosis, hepatocyte ballooning, inflammation, fibrosis and NAS for mice maintained on a MCD diet. Data are presented as the mean ± standard error of 10 (*Adamts13*$^{-/-}$, black bars) or 5 (WT, white bars) determinations. MCD, methionine and choline deficient diet; WT, wild-type; *Adamts13*$^{-/-}$, a disintegrin and metalloproteinase with thrombospondin type-1 motif, member 13 deficient; MCC, control diet; NAS, nonalcoholic fatty liver disease activity score.

The effects of the MCD diet were determined on steatohepatitis in WT and *Adamts13*$^{-/-}$ mice (Fig. 2). H&E staining of liver sections identified more pronounced steatosis in MCD as compared with MCC fed mice of both genotypes (Fig. 2A), as confirmed by quantitative analysis (Fig. 1F). Further histological analysis of liver sections identified that following 4 weeks of MCC diet, WT (3/5 with score 1) and *Adamts13*$^{-/-}$ (3/10 with

Table II. Plasma levels of metabolic parameters, liver enzymes, endogenous VWF and platelet count of WT and *Adamts13*$^{-/-}$ mice kept on MCC or MCD diets for 4 weeks.

	WT		*Adamts13*$^{-/-}$	
Parameter	MCC	MCD	MCC	MCD
Glucose (mg/dl)	194±20.4	94±8.98[a]	177±6.09	93±3.86[b]
Insulin (ng/ml)	0.58±0.12	0.17±0.07	0.26±0.06[c]	0.05±0.01[d]
HOMA-IR	0.30±0.06	0.05±0.02[d]	0.12±0.03[c]	0.01±0.002[a]
Triglycerides (mg/dl)	45±4.6	36±0.95	35±1.7	31±1.0[c]
Cholesterol (mg/dl)	89±14	31±5[d]	84±5	30±1[e]
HDL cholesterol (mg/dl)	82±13	22±2[d]	75±4[c]	17±1[e]
LDL cholesterol (mg/dl)	1.5±1.5	7.5±0.9	3.8±0.9	7.0±0.9[d]
Alkaline phosphatases (U/l)	63±7.6	76±12	56±3.2	77±3.0[d]
AST (U/l)	58±13	185±62.0[d]	78±12	173±14.4[e]
ALT (U/l)	22±2.0	193±53.4[a]	39±9.0	227±24.0[b]
Platelet count (x$10^3/\mu$l)	917±84.3	752±117	829±25	565±65[a]
mVWF (% to NMP)	302±2.53	250±38.9	247±40.3	168±37.5

Data are presented as the means ± standard error of 10 *Adamts13*$^{-/-}$ or 5 WT experiments for each diet. [a]P<0.01 and [b]P<0.0001 MCD vs. MCC fed mice; [c]P<0.05 *Adamts13*$^{-/-}$ vs. WT mice; [d]P<0.05 and [e]P<0.001 MCD vs. MCC fed mice. WT, wild-type; *Adamts13*$^{-/-}$, a disintegrin and metalloproteinase with thrombospondin type-1 motif, member 13 deficient; MCD, methionine and choline deficient diet; MCC, control diet; HOMA-IR, homeostasis model assessment of insulin resistance; HDL, high density lipoprotein; LDL, low density lipoprotein; AST, aspartate aminotransaminase; ALT, alanine aminotransferase; mVWF, murine von Willebrand factor; NMP, normal mouse plasma.

Table III. Histological scoring of liver sections of mice maintained on MCC or MCD diet for 4 weeks.

	MCC		MCD	
Factor	WT	*Adamts13*$^{-/-}$	WT	*Adamts13*$^{-/-}$
Microvesicular steatosis				
Score 0 (<5%)	2/5	7/10	0/5	0/10
Score 1 (5-33%)	3/5	3/10	1/5	1/10
Score 2 (33-66%)	0/5	0/10	2/5	6/10
Score 3 (>66%)	0/5	0/10	2/5	3/10
Hepatocyte ballooning				
Score 0	5/5	10/10	2/5	9/10
Score 1	0/5	0/10	2/5	1/10
Score 2	0/5	0/10	1/5	0/10
Lobular inflammation				
Score 0	5/5	10/10	0/5	0/10
Score 1	0/5	0/10	4/5	8/10
Score 2	0/5	0/10	1/5	2/10
Score 3	0/5	0/10	0/5	0/10
Fibrosis				
F0	5/5	10/10	1/5	1/10
Stage F1a	0/5	0/10	0/5	6/10
Stage F1b	0/5	0/10	4/5	2/10
Stage F1c	0/5	0/10	0/5	0/10
Stage F2	0/5	0/10	0/5	1/10

MCD, methionine and choline deficient diet; MCC, control diet; WT, wild-type; *Adamts13*$^{-/-}$, a disintegrin and metalloproteinase with thrombospondin type-1 motif, member 13 deficient.

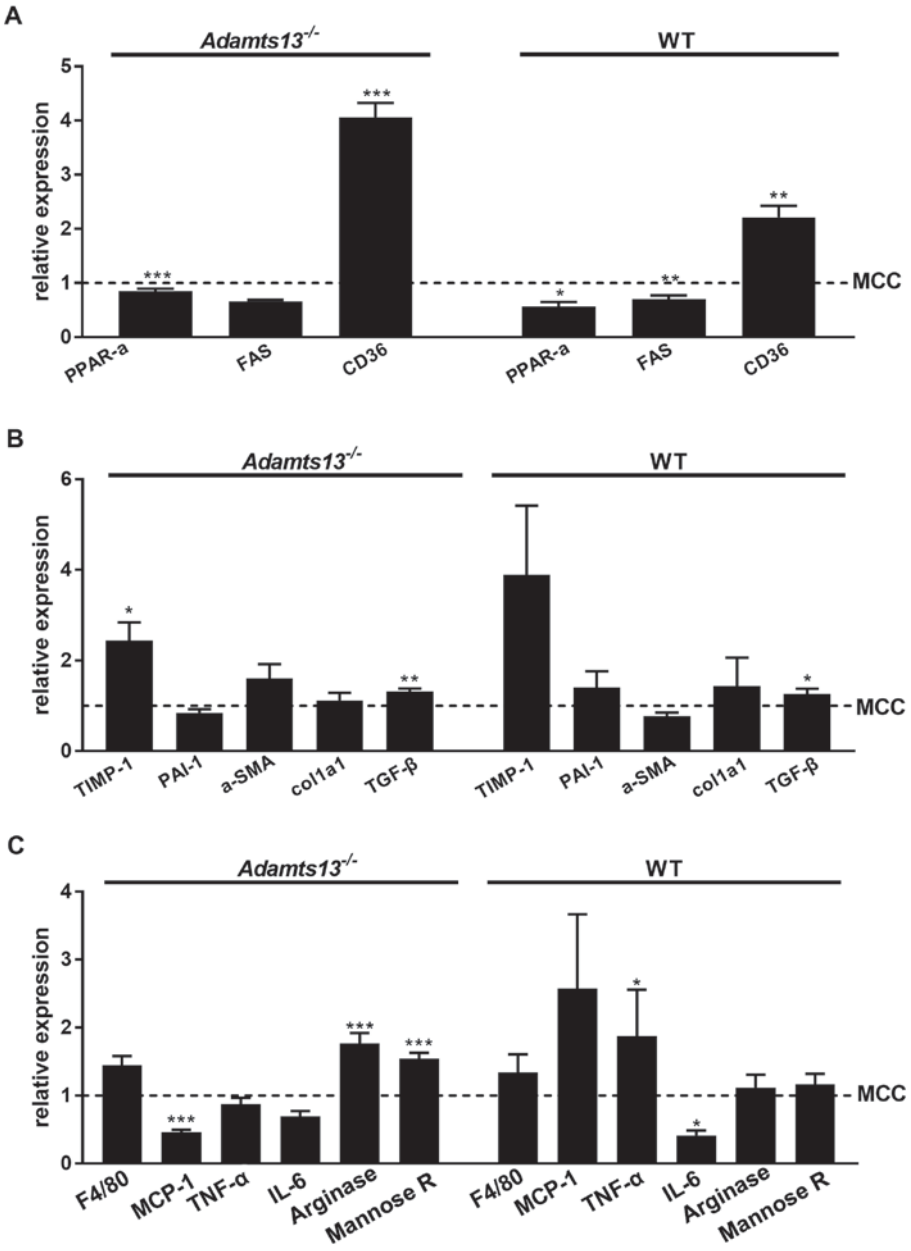

Figure 3. Effect of MCD diet on (A) expression of hepatic markers of steatosis/triglyceride metabolism, (B) fibrosis and (C) inflammation. Gene expression compared with the MCC diet is presented for MCD fed mice. Data are presented as the mean ± standard error of 10 (*Adamts13*$^{-/-}$) or 5 (WT) experiments for each diet. *P<0.05, **P<0.01 and ***P<0.001 MCD vs. MCC. MCD, methionine and choline deficient diet; MCC, control diet; *Adamts13*$^{-/-}$, a disintegrin and metalloproteinase with thrombospondin type-1 motif, member 13 deficient; WT, wild-type; PPAR-α, peroxisome proliferator-activated receptor α; FAS, Fas cell surface death receptor; CD36, cluster of differentiation; TIMP-1, tissue activator of metalloproteinase 1; PAI-1, plasminogen activator inhibitor 1; α-SMA, α-smooth muscle actin; Col1a1, collagen type I α1 chain; TGF-β, transforming growth factor β; MCP-1, monocyte chemoattractant protein 1; TNF-α, tumor necrosis factor α; IL-6, interleukin 6.

score 1) mice exhibited only mild steatosis, whereas they all scored 0 for hepatocyte ballooning, lobular inflammation and fibrosis (Fig. 2A and Table III). However, following MCD feeding, WT and *Adamts13*$^{-/-}$ mice presented with histological abnormalities with markedly enhanced scores for steatosis, ballooning, lobular inflammation and NAS without, however, significant differences between genotypes (Fig. 2C). In addition, fibrosis stage and score were deteriorated in MCD vs. MCC fed mice, however no clear effect of genotype was observed (Fig. 2B and Table III).

Relative expression of markers of triglyceride metabolism, fibrosis and inflammation were not markedly affected by genotype on either diet (data not shown). Histological observations on steatosis and fibrosis were compatible with markedly enhanced gene expression of the steatosis maker CD36 and the fibrosis marker TIMP-1 for MCD vs. MCC fed mice of the two genotypes (Fig. 3A and B). Analysis of hepatic inflammatory markers identified enhanced expression of TNF-α for WT mice on MCD as compared with MCC feeding (Fig. 3C).

Discussion

Deficiency of ADAMTS13, the VWF cleaving proteinase, results in thrombotic thrombocytopenic purpura (TTP), a

rare however severe thrombotic disease (27). A functional role of ADAMTS13 has also been suggested in liver diseases, however the available data remain controversial (12). It has been previously demonstrated that ADAMTS13 antigen and activity levels are enhanced in obese mice, whereas ADAMTS13 deficiency does not affect development of NASH in obese mice (28,29). In the present study, it was observed that a steatosis-inducing diet, independent of obesity, induces NASH to a comparable extent in *Adamts13* deficient and WT mice.

In patients, visceral obesity is frequently associated with NAFLD and NASH. These conditions may be associated with reduced ADAMTS13 activity, as reported in an obese patient with recurrent TTP; defective ADAMTS13 synthesis was suggested as a possible consequence of NASH (30). Furthermore, an apparent paradox with respect to the potential role of ADAMTS13 in development of acute liver failure has been recognized (12). ADAMTS13 activity has been identified to be inversely associated with disease severity and outcome, whereas by contrast, no ultralarge VWF multimers were identified in the systemic circulation, and high molecular weight VWF levels and VWF function were reduced in patients with acute liver failure as compared with healthy controls (31).

Due to the fact that NASH is difficult to study in humans due to slow progression of the disease and ethical considerations, animal models of NASH are crucial to improve the understanding of the pathogenesis of the disease. The model of feeding rodents with an MCD used in the present study is one of the most commonly used in NASH research (32-35). It is characterized by macrovesicular steatosis, hepatocellular death, inflammation, oxidative stress and fibrosis. Due to this diet, the mice lose weight and the metabolic profile is opposite to that of typical human NASH, e.g. the mice do not develop hyperlipidemia or hypertriglyceridemia and do not present with insulin resistance. However, liver injury and steatohepatitis are histologically similar to that of human patients (36-38). The severity of NASH induced in rodents by the MCD diet depends on the administration scheme, however is also affected by species, gender and the animal strain (39,40). Using the administration scheme used in the present study (4 weeks of MCD/MCC), a protective effect of the ADAMTS5 deficiency (C57Bl/6J background) on development of NASH was identified (41). A similar administration scheme was used by Rinella *et al* (35). Although a time course was not performed, different effects of ADAMTS13 deficiency at earlier time points (e.g. two weeks) cannot be excluded.

It was reported that steatosis in mice kept on the MCD diet develops within 2-4 weeks and there is progression to fibrosis by 8-10 weeks (42-44). The mice in the current study developed steatosis on the 4-week diet, which was not restricted to centrolobular or periportal liver portions, however was diffusely spread throughout the liver tissue. More pronounced steatosis and higher liver triglyceride levels were identified upon MCD feeding of the two genotypes, however no differences between the genotypes were observed. No mice developed severe fibrosis, although mRNA expression of the fibrosis markers TIMP-1 and TGF-β in the liver tissue were enhanced following the short-term MCD diet. These results were supported by similar hepatic expression profiles of steatosis and fibrosis markers for the two genotypes. The liver enzymes alkaline phosphatases, AST and ALT were enhanced on the MCD diet, indicating liver damage, however without marked differences between genotypes. Platelet counts appeared lower following MCD diet feeding, which was most pronounced in *Adamts13*$^{-/-}$ mice. To the best of our knowledge, this has not been reported previously and remains to be fully elucidated.

In the present study, it was observed that the WT and the *Adamts13* deficient mice fed with the MCD diet lose weight and present with low fasting blood glucose, however do not develop insulin resistance, which is in agreement with previous studies (32,33,38). Improved insulin sensitivity upon MCD feeding of the two genotypes was observed, whereas HOMA-IR was lower for *Adamts13*$^{-/-}$ vs. WT mice on either diet. By contrast, it has been previously observed that in mice with high fat induced obesity, glucose and insulin sensitivity did not differ between WT and *Adamts13*$^{-/-}$ mice (28).

Taken together, the results of the present study and previous data from the literature (28) do not support a functional role for ADAMTS13 in the development of steatohepatitis in mouse models of diet-induced steatosis. This is in agreement with a recent study in patients with NASH, demonstrating that plasma ADAMTS13 levels were not different from that of the controls (45).

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