Elevated hepatic DPP4 activity promotes insulin resistance and non-alcoholic fatty liver disease

Christian Baumeier1,4, Luisa Schlüter1, Sophie Saussenthaler1,4, Thomas Laeger1,4, Maria Rödiger1,4, Stella Amelie Alaze1, Louise Fritsche2,3,4, Hans-Ulrich Häring2,3,4, Norbert Stefan2,3,4, Andreas Fritsche2,3,4, Robert Wolfgang Schwenk1,4, Annette Schürmann1,4,*

Abstract

Objective: Increased hepatic expression of dipeptidyl peptidase 4 (DPP4) is associated with non-alcoholic fatty liver disease (NAFLD). Whether this is causative for the development of NAFLD is not yet clarified. Here we investigate the effect of hepatic DPP4 overexpression on the development of liver steatosis in a mouse model of diet-induced obesity.

Methods: Plasma DPP4 activity of subjects with or without NAFLD was analyzed. Wild-type (WT) and liver-specific Dpp4 transgenic mice (Dpp4-Liv-Tg) were fed a high-fat diet and characterized for body weight, body composition, hepatic fat content and insulin sensitivity. In vitro experiments on HepG2 cells and primary mouse hepatocytes were conducted to validate cell autonomous effects of DPP4 on lipid storage and insulin sensitivity.

Results: Subjects suffering from insulin resistance and NAFLD show an increased plasma DPP4 activity when compared to healthy controls. Analysis of Dpp4-Liv-Tg mice revealed elevated systemic DPP4 activity and diminished active GLP-1 levels. They furthermore show increased body weight, fat mass, adipose tissue inflammation, hepatic steatosis, liver damage and hypercholesterolemia. These effects were accompanied by increased expression of PPARγ and CD36 as well as severe insulin resistance in the liver. In agreement, treatment of HepG2 cells and primary hepatocytes with physiological concentrations of DPP4 resulted in impaired insulin sensitivity independent of lipid content.

Conclusions: Our results give evidence that elevated expression of DPP4 in the liver promotes NAFLD and insulin resistance. This is linked to reduced levels of active GLP-1, but also to auto- and paracrine effects of DPP4 on hepatic insulin signaling.

1. INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is characterized by excessive deposition of fat (steatosis) in the liver and can be classified into two major clinical-histological subgroups: (i) non-alcoholic fatty liver (NAFL) and (ii) non-alcoholic steatohepatitis (NASH). The prevalence of NAFLD in the general adult population ranges from 25% to 45% and rises with increasing incidence of obesity and type 2 diabetes [1]. Patients with NASH reveal augmented mortality rate, whereas NAFL has been linked to an increased risk of type 2 diabetes [2].

Current management for NAFLD includes lifestyle modifications, control of metabolic risk factors, and pharmacological therapies. However, since only biopsy-proven NASH patients receive medical treatment [3], there is a need for suitable drugs for the treatment of NAFL.

Dipeptidyl peptidase 4 (DPP4) could serve as target in NAFL therapy. DPP4 is a serine protease that cleaves a variety of substrates including incretin hormones, chemokines, growth factors, and neuropeptides [4]. It is ubiquitously expressed on the apical surface of many cell types and also occurs as a soluble form (sDPP4) in the circulation and other body fluids [5,6]. There is accumulating evidence that sDPP4 is causative for the development of NAFLD is not yet clarified.
evidence that DPP4 is involved in the development of chronic liver disease [5,7,8]. DPP4 is highly expressed in the liver, and its expression as well as circulating levels are increased in NAFL and NASH [8–10]. sDPP4 is suggested as biomarker of NAFLD [11] and was shown to be a valid measure for hepatocyte apoptosis and fibrosis [12]. Genetic ablation of Dpp4 in mice [13] and rats [14] results in improved insulin sensitivity and liver function, and pharmacological inhibition of DPP4 causes reduction of hepatic steatosis and improvement of insulin sensitivity in mouse models of obesity [15,16] and diabetes [17]. Beside its role in the degradation of incretin hormones, DPP4 was shown to exert incretin-independent functions such as the induction of insulin resistance [18–21] and inflammation [22] in different cellular systems. However, whether elevated DPP4 and in particular hepatic DPP4 triggers insulin resistance and NAFLD or simply reflects the state of liver disease is not entirely clarified. We recently demonstrated in diet-induced obesity mice that expression and release of DPP4 is substantially increased in liver when compared to adipose depots [23]. We further showed that elevated expression of Dpp4 in livers of 6-week-old mice associates with early insulin resistance, which, in turn, triggers later liver steatosis [23]. In the current study, we analyzed the DPP4 activity in plasma of healthy and NAFLD subjects and elucidated the effect of hepatocyte-specific Dpp4 overexpression on the development of insulin resistance and liver steatosis in mice under obese conditions.

### 2. MATERIALS AND METHODS

#### 2.1. Human samples

For the quantification of plasma DPP4 activity, 348 subjects with prediabetes from the ongoing Tübingen family (TUEF) study for type 2 diabetes were selected [24]. Each participant underwent a standardized 5 point oral glucose tolerance test with 75 g of glucose after an overnight fasting period. Venous blood samples were obtained at time points 0, 30, 60, 90, and 120 min for the measurement of glucose (ADVIA 1800 Chemistry Analyzer, Siemens Healthcare Diagnostics) and insulin (ADVIA Centaur XP Immunoassay System, Siemens Healthcare Diagnostics). Insulin sensitivity was calculated with the composite whole-body insulin sensitivity index (ISI) [25]. Liver fat was quantified by localized 1H-MR spectroscopy using a 1.5 T MR scanner (Magnetom Sonata, Siemens Healthcare). The cohort was divided into quartiles according to liver fat content. For quantification of plasma DPP4 activity, 158 samples from subjects of the first and fourth quartile, matched for age and sex, were used. DPP4 activity was measured in 25 μl plasma as described before [23]. The work described has been carried out in accordance with the code of ethics of the World Medical Association (Declaration of Helsinki).

#### 2.2. Animals

Hepatocyte-specific Dpp4 transgenic mice (Dpp4-Liv-Tg) were generated by the company genOway (Lyon, France) via Hprt (hypoxanthine phosphoribosyl-transferase) targeted transgenesis. The knock-in targeting vector, containing murine Dpp4 cDNA under the control of an albumin promoter, was transfected into E14 ES cells (12B2/Ola), which were injected into C57BL/6J blastocysts. Obtained chimera were backcrossed seven times with C57BL/6J mice. Three-week-old male Dpp4-Liv-Tg mice (n = 9) and wild-type (WT, n = 7) littermates received a high-fat diet (HFD, 45 kcal% fat, 35 kcal% carbohydrates and 20 kcal% protein, D12451, Research Diets) for 27 weeks. Mice were kept at a temperature of 22 ± 1 °C with a 12:12 h light–dark cycle and had free access to food and water in accordance with the guidelines of the EU Directive 2010/63/EU for animal experiments. All animal experiments were approved by the ethics committee of the State Office of Environment, Health and Consumer Protection (Federal State of Brandenburg, Germany).

#### 2.3. Blood glucose, body weight, body composition, and liver fat content

Body weight was measured every other week. Blood glucose was measured using a CONTOUR® XT glucometer (Bayer). At 6, 18, and 26 weeks of age body composition and liver fat content were analyzed using nuclear magnetic resonance and computed tomography (CT) as described before [26].

#### 2.4. Insulin tolerance test

Non-fasted, 22-week-old mice were intraperitoneally injected with insulin (1.25 IU/kg body weight, Actrapid® Penfill®, Novo Nordisk) and blood glucose levels were measured at indicated time points.

#### 2.5. Plasma analyses

Plasma insulin concentrations were quantified from vena cava blood using a Mouse Ultraseensitive Insulin ELISA (Alpco). Plasma adiponectin and leptin levels were measured by Mouse Adiponectin/Acrp30 (DY1119, R&D Systems) and Mouse/Rat Leptin (MOB00, R&D Systems) ELISA kits. Active GLP-1 levels were detected after oral glucose administration using GLP-1 (Active) ELISA Kit (AKMGP-011, Shibayagi). Mice were fasted for 16 h, orally administered with glucose (2 mg/g body weight), and euthanized after 15 min by isoflurane. Following this, blood was taken with 0.5 M EDTA-coated syringe which was supplemented with 20 μl/ml DPP4 inhibitor (Cat. DPP4, Millipore) from either vena cava or vena portae. Plasma triglyceride (T2449, F6428, G7793, Sigma), free fatty acid (91096, 91898, 91696, Wako), cholesterol (10017, Human), ALT (12212, Human), AST (12211, Human), and GGT (12213, Human) levels were measured according to manufacturer’s protocol. Soluble DPP4 concentration was determined using a Mouse DPP4 ELISA Kit (DY954, R&D Systems). Plasma DPP4 activity was measured by the conversion of glycine-prolin-p-nitroanilide (Sigma) to p-nitroanilide. Twenty μl plasma, 90 μl cell supernatants, and 45 μl cell homogenates were filled to 90 μl with assay buffer (50 mmol/l glycine, 1 mmol/l EDTA, pH 8.7) and supplemented with 10 μl glycine-prolin-p-nitroanilide (5 mmol/l). Production of p-nitroanilide was measured by the absorbance at 405 nm in a kinetic measurement at 37 °C. The DPP4 activity in the samples was calculated using p-nitroanilide (Sigma) standard curve over the concentration range of 20–100 μmol/l. The results are expressed as nmol/min/ml.

#### 2.6. Liver glycogen, cholesterol, and triglyceride content

Liver glycogen content was analyzed using a glucose colorimetric assay (10260, Human) subsequent to an amyloglucosidase (Fluka) digestion of liver homogenates. Hepatic triglyceride content was measured as described before [27] using the TR-210 kit (Randox). Liver cholesterol level was determined using a commercial kit (10017, Human).

#### 2.7. Western blotting

Western blotting was performed as described before [28]. Incubation with primary antibodies (Supplementary Table 1) was performed at 4 °C overnight. Secondary antibodies were peroxidase labeled (Supplementary Table 1). Quantification of blots was performed using the ImageJ 1.50b software. For relative Akt-phosphorylation, untreated insulin-stimulated controls were set to 100%.
Liver and adipose tissue were fixed in 4% formaldehyde and embedded in paraffin. Hematoxylin and eosin (H&E), Sirius Red, and Trichrome staining of liver sections were performed using a standard protocol. Neutral lipids were stained in cryo sections using oil red O. For immunohistochemical staining, paraffin embedded sections were deparaffinized and incubated with appropriate antibodies (Supplementary Table 1) at 4 °C overnight. Secondary antibodies were either Alexa-488, Alexa-546 labeled, or biotinylated (Supplementary Table 1). TO-PRO-3 iodide (Invitrogen) was used for nuclei staining. Microscopy was performed with the confocal Laser Scan microscope Leica-DMi8 (Leica Microsystems) or the Keyence BZ-9000 fluorescent microscope (Keyence International).

2.9. Quantitative real-time PCR and microarray analysis
Total RNA extraction, cDNA synthesis, and TaqMan gene expression assays were performed as described previously [28]. Liver transcriptome analysis was performed by the company Oaklabs (Berlin, Germany) as described [29].

2.10. Cell culture
Human hepatoma cells (HepG2) were cultured at 37 °C and 5% CO2 in Dulbecco’s Modified Eagle Medium (DMEM) containing 5.5 mmol/l glucose, 10% fetal calf serum (FCS) and 1% non-essential amino acids (NEAA). Cells were treated with various concentrations of recombinant human DPP4 (1180-SE-010, R&D Systems) for 48 h before being serum-starved (3 h) and insulin-stimulated (10 min, 100 nmol/l). Primary hepatocytes were isolated from 12-week-old standard diet fed male C57BL/6J WT or Dpp4-Liv-Tg mice by a collagenase perfusion method [30]. Isolated hepatocytes were cultured in 12 well plates in DMEM with 5.5 mmol/l glucose, 10% FCS, 1% NEAA and 1% penicillin/streptomycin at 37 °C and 5% CO2. After 4 h, cells were either infected with 3.5 × 106 PFU/well adenovirus coding for full-length murine Dpp4 (Ad-Dpp4, ADV-257420) or green fluorescent protein (Gfp, Ad-Gfp, 1060) (Vector BioLabs), or treated with 500 ng/ml recombinant mouse DPP4 (554-SE-010, R&D Systems). Cells were cultured for 48 h, serum-starved (3 h) and subsequently stimulated with insulin (10 min, 100 nmol/l).

2.11. Statistical analysis
All data are displayed as mean ± SEM. For comparison of >2 groups, one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test was used. For the analysis of time course two-way ANOVA with Bonferroni’s multiple comparisons test was used. All calculations were performed with GraphPad Prism 6.ink software. Significance levels were set for p-values of less than 0.05 (*), 0.01 (**) and 0.001 (**).
Figure 2: Liver-specific Dpp4 overexpression causes increased plasma DPP4 activity and reduced glucose-stimulated GLP-1 levels. Male wild-type (WT, open circles) and Dpp4-transgenic (Dpp4-Liv-Tg, black circles) received a high-fat diet until 30 weeks of age. (A, B) Relative Dpp4 mRNA expression (A) and protein content (B) in various tissues (n = 3–4). DPP4 western blots are depicted with two different exposure times. Liver DPP4 is quantified with tubulin as loading control (n = 4). (C) Immunohistochemical staining of liver sections for DPP4 (green) and E-cadherin (red). Nuclei were stained with TO-PRO®3 iodide (blue). Scale bar, 10 or 30 μm, respectively. (D) Plasma DPP4 activity in vena cava (v.cava) (n = 7–9). (E) Plasma (active) GLP-1 levels in portal vein (v.portae) and vena cava 15 min after oral glucose bolus (n = 8). Liv, liver; gWAT, gonadal white adipose tissue; sWAT, subcutaneous white adipose tissue; BAT, brown adipose tissue; SM, skeletal muscle (quadriceps). All data are represented as mean ± SEM. *p < 0.05, **p < 0.01. ns, not significant.

(Figure 2C). As transmembrane bound DPP4 can be shed from hepatocytes [23], we studied the capacity of DPP4 release by analyzing plasma DPP4 activity in WT and Dpp4-Liv-Tg mice. Dpp4-Liv-Tg animals exhibited a 2-fold increase in plasma DPP4 activity, demonstrating an elevated release of DPP4 from livers of these mice (Figure 2D). In addition, cell supernatants of primary hepatocytes from Dpp4-Liv-Tg mice showed higher DPP4 concentration and activity than those from WT controls (Supplementary Figure 1C, D).

Since incretin hormones are known substrates of DPP4, we next analyzed the effect of hepatic Dpp4 overexpression on the half-life of GLP-1. Fifteen minutes after oral glucose gavage, portal vein concentration of the active form of GLP-1 was similar in WT and Dpp4-Liv-Tg animals, indicating no differences in GLP-1 secretion from intestinal L-cells (Figure 2E). However, analysis of blood obtained from vena cava (after liver passage) revealed a 2-fold reduction of active GLP-1 in Dpp4-Liv-Tg mice, suggesting an increased cleavage and inactivation of GLP-1 by hepatocyte originated DPP4 (Figure 2E). Together, these data indicate that hepatic overexpression of Dpp4 results in elevated plasma DPP4 activity, which, in turn, leads to reduced post-prandial GLP-1 levels.

3.3. Elevated hepatic DPP4 activity leads to increased fat mass and adipose tissue inflammation

Phenotypic characterization of HFD fed Dpp4-Liv-Tg mice revealed an increased body weight gain, which was due to an elevated fat mass rather than changes in lean mass (Figure 3A–C). At 30 weeks of age, mass of white but not of brown adipose tissue was significantly increased in Dpp4-Liv-Tg mice (Figure 3D). Plasma triglyceride and free fatty acid concentrations did not differ, but cholesterol levels were elevated in mice with liver-specific Dpp4 overexpression (Table 1). Since obesity is associated with adipose tissue inflammation, we next analyzed the expression of inflammatory markers in gonadal white adipose tissue. Indeed, the expression of the macrophage marker F4/80 (Emr1) and of proinflammatory cytokines (TNFα, Tnfα, and MCP1) were increased in white adipose tissue of Dpp4-Liv-Tg mice (Figure 3E). In agreement, immunohistochemical staining of F4/80 showed higher abundance of macrophages in adipose tissue of Dpp4-Liv-Tg animals (Figure 3F).

3.4. Hepatic overexpression of Dpp4 enhances diet-induced fatty liver

To further investigate whether an increased Dpp4 expression in the liver leads to hepatic steatosis or is a consequence of the elevated lipid accumulation, we studied the development of fatty liver in WT and Dpp4-Liv-Tg mice by computed tomography. At a young age (6 weeks), both WT and Dpp4-Liv-Tg mice showed a low liver fat content of about 1.5% (Figure 4A). However, after 15 weeks of HFD feeding hepatic fat content raised to 4.2 ± 1.0% in Dpp4-Liv-Tg mice, whereas liver fat content in WT mice was unchanged (1.8 ± 0.2%) (Figure 4A). After additional 8 weeks on the diet, liver fat content reached 6.4 ± 1.6% in Dpp4-Liv-Tg and 3.1 ± 0.4% in WT mice (p < 0.01) (Figure 4A), indicating a slow but direct effect of hepatic...
DPP4 levels on liver steatosis. At 30 weeks of age, liver glycogen and liver cholesterol were not significantly different, whereas levels of triglycerides were higher in Dpp4-Liv-Tg mice (Figure 4B). As a consequence, liver weight showed a tendency to be higher than WT mice (Figure 4 B). Histological examinations confirmed the increased accumulation of ectopic fat (Figure 4C,D) and larger lipid droplets, indicated by staining of the lipid droplet coating protein perilipin 2 in livers of Dpp4-Liv-Tg mice (Figure 4E). Plasma analysis of markers for liver damage showed significantly elevated levels of gamma-glutamyl transpeptidase (GGT, p < 0.05) and aspartate aminotransferase (AST, p < 0.05) levels in gWAT of 30-week-old mice. (F) Immunohistochemical staining of F4/80 in gWAT. All data are represented as mean ± SEM (n = 7–9). *p < 0.05, **p < 0.01.

3.5. PPARγ and CD36 expression in the liver is induced by elevated hepatic DPP4

Next, we performed global transcriptome profiling with RNA isolated from livers of 30-week-old WT and Dpp4-Liv-Tg mice. Microarray analysis revealed that 105 genes were up- and 60 genes down-regulated in livers of Dpp4-Liv-Tg mice (log2-fold change > 0.50, p < 0.05). Focusing on genes involved in lipid metabolism, we detected no changes in genes of triglyceride hydrolysis, de novo lipogenesis (except Srebf1), and only slightly elevated mRNA levels of Cpt1a, Apob, and Dgat2 in Dpp4-Liv-Tg mice (Supplementary Figure 2). The most striking effect was observed for the transcription factor peroxisome proliferator activated receptor gamma (PPARγ) and its downstream target fatty acid translocase (Cd36), which were both significantly increased in livers of Dpp4-Liv-Tg mice (2.2-fold and 2.3-fold, Supplementary Figure 3). Monacylglycerol O-acyltransferase 1 (Mogat1), another target gene of hepatocyte PPARγ, also tended to be higher in Dpp4-Liv-Tg mice (Supplementary Figure 3). Validation using quantitative real-time PCR confirmed a significantly elevated expression of Ppary and Cd36 and a trend towards higher Mogat1 levels in transgenic animals (Figure 5A). In agreement, western blot analysis showed higher PPARγ and CD36 protein levels in livers of Dpp4-Liv-Tg animals (Figure 5B). Interestingly, only PPARγ isoform 1 was up-regulated in these samples, suggesting an isoform-specific effect of DPP4 on PPARγ expression. In summary, DPP4-induced liver steatosis associates with elevated hepatic PPARγ and CD36 expression.

3.6. DPP4 induces hepatic insulin resistance

We have recently shown that early alterations in hepatic Dpp4 are associated with insulin resistance resulting in later liver steatosis [23]. As studies on primary human adipocytes and skeletal muscle cells discovered direct effects of soluble DPP4 on the insulin sensitivity of these cells [18], we tested whether elevated DPP4 levels also affect hepatic insulin sensitivity. Human HepG2 cells were treated with various concentrations of recombinant human DPP4 (rhDPP4) and analyzed for their insulin responsiveness. Insulin-stimulated Akt-phosphorylation was unaffected with low dose of rhDPP4 (75–150 ng/ml) but completely blunted with a dosage of 300–500 ng/ml.
Table 1 – Biochemical plasma characteristics at 30 weeks of age.

| Parameter          | WT     | Dpp4-Liv-Tg | p-value |
|--------------------|--------|-------------|---------|
| Triglycerides (mg/dl) | 152 ± 0.068 | 366 ± 23 | 0.0001 |
| Free fatty acids (mmol/l) | 0.36 ± 0.038 | 0.43 ± 0.055 | 0.470 |
| Cholesterol (mg/dl) | 135 ± 15 | 181 ± 15 | 0.029 |
| ALT (IU/l)         | 44.3 ± 7.9 | 96.6 ± 30.2 | 0.171 |
| AST (IU/l)         | 88.5 ± 11.8 | 138.7 ± 25.2 | 0.132 |
| GGT (IU/l)         | 0.3 ± 0.1 | 1.1 ± 0.2 | 0.029 |
| Blood glucose (mg/l) | 11.8 ± 1.1 | 13.8 ± 2.5 | 0.029 |
| Leptin (ng/ml)     | 0.46 ± 0.09 | 0.91 ± 0.16 | 0.121 |
| HOMA-IR            | 2.64 ± 0.57 | 5.08 ± 0.93 | 0.156 |

markedly lower in livers of Dpp4-Liv-Tg mice when compared to WT littermates. In agreement, insulin tolerance test at 22 weeks of age confirmed the impaired insulin sensitivity of Dpp4-Liv-Tg mice (Figure 6F), however, at a time-point when fatty liver was already induced (Figure 4A). Fasting insulin levels as well as HOMA-IR (homoeostatic model assessment for insulin resistance) were numerically increased in Dpp4-Liv-Tg mice but did not reach statistical significance (Table 1). Finally, we considered leptin to adiponectin ratio as another measure for systemic insulin resistance [31] and found increased ratio in Dpp4-Liv-Tg mice at 30 weeks of age (Table 1). Thus, overexpression of Dpp4 in livers of diet-induced obesity mice impairs the hepatic insulin sensitivity.

4. DISCUSSION

The present data demonstrate that (i) subjects suffering from NAFLD exhibit elevated plasma DPP4 activity and that (ii) hepatocyte-specific overexpression of Dpp4 contributes to elevated plasma activity of the enzyme, which, in turn, causes diminished glucose-induced active GLP-1 levels. Moreover, overexpression of Dpp4 in the liver (iii) promotes the development of hepatic insulin resistance and NAFLD and (iv) enhances adipose tissue expansion and inflammation under obese conditions.

NAFLD patients are known to have increased DPP4 mRNA levels in the liver [9], and this associates with the degree of hepatic steatosis in obese subjects [23]. In addition, we have recently shown that obesity-prone mice reveal an increased expression of Dpp4 already at the age of 6 weeks, proceeding in hepatic steatosis later in life [23]. However, it was not clear whether elevated DPP4 levels in the liver are causal for the later onset of NAFLD. Here, we show for the first time that the overexpression of Dpp4 in hepatocytes of HFD fed C57BL/6J mice promotes the development of fatty liver. Thus, dysregulation of Dpp4

![Figure 4: Hepatocyte-specific Dpp4 overexpression promotes hepatic steatosis.](image)

Male wild-type (WT, open circles) and Dpp4-transgenic (Dpp4-Liv-Tg, black circles) mice were fed a high-fat diet until 30 weeks of age. (A) Development of liver fat content measured by computed tomography. (B) Liver weight, glycogen, cholesterol, and triglyceride content. (C) Hematoxylin and eosin (H&E) (C) and oil red O (D) staining of liver sections. Scale bar, 100 μm. (E) Immunohistochemical staining of liver for the lipid droplet coating protein perilipin 2 (PLIN2, green). Nuclei were stained with TO-PRO© 3 iodide (blue). Scale bar, 30 μm. All data are represented as mean ± SEM (n = 7–9). *p < 0.05, **p < 0.01.
recently published data showing improved insulin sensitivity and lower fat content in HepG2 cells after siRNA-mediated suppression of DPP4 [32]. Furthermore, studies in primary human adipocytes showed insulin resistance by the administration of recombinant DPP4 [19] and improved insulin sensitivity when DPP4 was downregulated [20]. Moreover, long-term DPP4 inhibition improved insulin sensitivity and reduced liver fat content in animals with diet-induced hepatic steatosis and insulin resistance [16,33]. Thus, DPP4 has an autocrine effect on hepatic insulin signaling which might contribute to later accumulation of ectopic fat in the liver.

GLP-1 is known to be involved in energy homeostasis by its anorexlic action in the brain. Here we show that Dpp4-Liv-Tg mice exhibited lower levels of active GLP-1 in the periphery, whereas portal vein concentrations were not affected. This clearly demonstrates that despite normal secretion from intestinal L-cells, hepatic Dpp4 overexpression leads to a substantial reduction of active GLP-1 after liver passage, suggesting hepatic DPP4 as a major contributor in the degradation of postprandial GLP-1. Since hepatic DPP4 expression is usually confined to the bile canicular domain, it is unlikely that DPP4 reaches the bloodstream to degrade GLP-1. However, under conditions of liver damage polarity of the cells can change leading to the release of DPP4 into interstitial fluids as described before [34]. Thus, increased plasma DPP4 activity in Dpp4-Liv-Tg mice seems not to be solely the result of elevated expression but also of changes in cell polarity as supposed from histological examinations (Figure 2C).

In line with this, human studies have shown associations of serum DPP4 with markers for liver damage (AST and ALT) as well as hepatocyte fibrosis and apoptosis [12,35]. In contrast, Dpp4-knockout rats reveal lower serum AST and ALT after 2 months on a Western diet [14], and Dpp4-deficient mice display lower liver fibrosis and inflammation in an experimental model of liver injury [36]. Here, we show that hepatocyte-specific Dpp4 overexpression resulted in significantly higher levels of GGT and numerical increased AST and ALT concentrations, providing further evidence for a direct connection between hepatic DPP4 and liver damage. The observed hypercholesterolemia of Dpp4-Liv-Tg mice is in line with previous studies, showing increased cholesterol levels in human subjects with elevated hepatic DPP4 [9], and reduced plasma cholesterol levels in Dpp4-deficient rats [14]. The elevated triglyceride content in livers of Dpp4-Liv-Tg mice appears to be the consequence of augmented levels of PPARγ and CD36, both being implicated in liver steatosis. Similarly, exogenous DPP4 increases lipid accumulation and PPARγ expression in pre-adipocytes [37], and activation of DPP4 on the surface of macrophages by middle east respiratory syndrome corona virus (MERS-CoV) induces PPARγ expression [38]. On the other hand, DPP4 was identified as PPARγ target gene in cells derived from human placental tissue [39]. The mechanism of DPP4-mediated PPARγ induction is still unclear, whereas effects of increased hepatic PPARγ are well understood. Hepatocyte-specific expression of PPARγ is associated with fatty liver in human [40] and mice [41,42], and hepatic overexpression of PPARγ induces liver steatosis [43], whereas PPARγ-knockout reduces hepatic fat content in mice on a HFD [44]. The major targets of PPARγ in the liver are fatty acid (Cd36) and monocacylglycerol O-acetyltransferase 1 (Mogat1), both being implicated in fatty liver disease [45], and upregulated in livers of Dpp4-Liv-Tg mice. Thus, high PPARγ and CD36 levels in Dpp4-Liv-Tg livers seem to contribute to elevated levels of hepatic steatosis.

Wronkowitz and colleagues recently identified protease-activated receptor 2 (PAR2) as DPP4 receptor [22], which is implicated in attenuation of obesity, adipose tissue inflammation, macrophage infiltration
and insulin resistance [46,47]. It has been shown that soluble DPP4 induces inflammation in human smooth muscle cells via MAPK and NFκB-mediated pathways [22], and a study in primary human adipocytes revealed suppression of TNFα-induced IL6 secretion after genetic silencing of DPP4 [20]. Moreover, it was suggested that DPP4 enhances inflammatory actions by upregulating toll-like receptors (TLRs) in kidney and adipose tissue, while DPP4 inhibition has anti-inflammatory effects [48]. The present data demonstrate an increased expression of macrophage markers and proinflammatory cytokines in adipose tissue of Dpp4-Liv-Tg mice, despite no effects on adipose Dpp4 expression. Since elevated activity of circulating DPP4 is of hepatic origin, it is likely that hepatic DPP4 contributes to the induction of adipose inflammation via PAR2- and TLR-mediated pathways.

5. CONCLUSIONS

Collectively, the present study shows that hepatic DPP4 is an important contributor to the development of NAFLD under conditions of high-fat
feeding. Overexpressing Dpp4 specifically in hepatocytes of mice resulted in hepatic insulin resistance and pronounced liver steatosis. This finding demonstrates that DPP4 is involved in the regulation of hepatic insulin sensitivity and subsequently lipid storage and not solely a marker of the disease. Based on our findings, we propose the application of DPP4 inhibitors in the therapy of NAFLD patients in order to improve hepatic insulin sensitivity and to prevent further accumulation of ectopic fat in the liver.

FINANCIAL SUPPORT

This work was supported by the German Ministry of Education and Research and the Brandenburg State (DZD grant 82DZD00302).

AUTHOR’S CONTRIBUTIONS

C.B., L.S., S.S., T.L., M.R., S.A.A., N.S. and L.F. performed data acquisition and analysis. C.B. drafted the article. C.B., R.W.S. and A.S. performed study conception and design. A.F. and H.U.H. performed data acquisition and critically reviewed the article. A.S. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All listed authors approved the final version of the manuscript.

ACKNOWLEDGEMENTS

The authors thank Christine Gumz, Andrea Teichmann, Kathrin Warnke, Elisabeth Meyer, and Manuel Ribbeck from the German Institute of Human Nutrition Potsdam-Rehbruecke for their skillful technical assistance.

CONFLICT OF INTEREST

None.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.molmet.2017.07.016.

REFERENCES

[1] Rinella, M.E., 2015. Nonalcoholic fatty liver disease: a systematic review. JAMA 313:2263–2273.
[2] Sanyal, A.J., Friedman, S.L., McCullough, A.J., Dimick-Santos, L., American Association for the Study of Liver Diseases, United States Food and Drug Administration, 2015. Challenges and opportunities in drug and biomarker development for nonalcoholic steatohepatitis: findings and recommendations from an American association for the study of liver Diseases-U.S. Food and drug administration joint workshop. Hepatology 61:1392–1405.
[3] Chalasani, N., Younossi, Z., Lavine, J.E., Diehl, A.M., Brunet, E.M., Cusi, K., et al., 2012. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological association, American association for the study of liver diseases, and American College of Gastroenterology. Gastroenterology 142:1592–1609.
[4] Röhborn, D., Wronkowitz, N., Eckel, J., 2015. DPP4 in diabetes. Frontiers in Immunology 6:1–20.
[5] Gorrell, M.D., 2005. Dipeptidyl peptidase IV and related enzymes in cell biology and liver disorders. Clinical Science (London) 108:277–292.
[6] Lambeir, A.-M., Durinx, C., Scharpé, S., De Meester, I., 2003. Dipeptidyl-peptidase IV from bench to bedside: an update on structural properties, functions, and clinical aspects of the enzyme DPP IV. Critical Reviews in Clinical Laboratory Sciences 40:209–294.
[7] Keane, F.M., Yao, T.-W., Seek, S., Gall, M.G., Chowdhury, S., Popla wski, S.E., et al., 2013. Quantification of fibroblast activation protein (FAP)-specific protease activity in mouse, baboon and human fluids and organs. FEBS Open Bio 4:43–54.
[8] Ito, M., Kagawachi, T., Taniguchi, E., Sata, M., 2013. Dipeptidyl peptidase-4: a key player in chronic liver disease. World Journal of Gastroenterology 19:2298–2306.
[9] Miyazaki, M., Kato, M., Tanaka, K., Tanaka, M., Kohijima, M., Nakamura, K., et al., 2012. Increased hepatic expression of dipeptidyl peptidase-4 in non-alcoholic fatty liver disease and its association with insulin resistance and glucose metabolism. Molecular Medicine Reports 5:729–733.
[10] Balaban, Y.H., Korkusuz, P., Simsek, H., Gokcan, H., Gedikoglu, G., Pinar, A., et al., 2007. Dipeptidyl peptidase IV (DPP IV) in NASH patients. Annals of Hepatology 6:242–250.
[11] Tsai, M.-T., Chen, Y.-J., Chen, C.-Y., Tsai, M.-H., Han, C.-L., Chen, Y.-J., et al., 2017. Identification of potential plasma biomarkers for nonalcoholic fatty liver disease by integrating transcriptomics and proteomics in laying hens. The Journal of Nutrition 147:293–303.
[12] Williams, K.H., Vieira De Ribeiro, A.J., Prakoso, E., Veillard, A.-S., Shackel, N.A., Brooks, B., et al., 2015. Circulating dipeptidyl peptidase-4 activity correlates with measures of hepatocyte apoptosis and fibrosis in non-alcoholic fatty liver disease in type 2 diabetes mellitus and obesity: a dual cohort cross-sectional study. Journal of Diabetes 7:809–819.
[13] Conarello, S.L., Li, Z., Ronan, J., Roy, R.S., Zhu, L., Jiang, G., et al., 2003. Mice lacking dipeptidyl peptidase IV are protected against obesity and insulin resistance. Proceedings of the National Academy of Sciences of the United States America 100:6825–6830.
[14] Ben-Shlomo, S., Zvilbok, I., Rabinowich, L., Goldiner, I., Shlomi, A., 2013. Dipeptidyl peptidase-4-deficient rats have improved bile secretory function in high fat diet-induced steatosis. Digestive Diseases and Sciences 58:172–178.
[15] Anor, A.R., Habibi, J., Ford, D.A., Nistala, R., Lastra, G., Mannrique, C., et al., 2015. Dipeptidyl peptidase-4 inhibition ameliorates Western diet-induced hepatic steatosis and insulin resistance through hepatic lipid remodeling and modulation of hepatic mitochondrial function. Diabetes 64:1988–2001.
[16] Kern, M., Kütting, N., Niessen, H.G., Thomas, L., Stiller, D., Mark, M., et al., 2012. Linagliptin improves insulin sensitivity and hepatic steatosis in diet-induced obesity. PLoS One 7:e38744.
[17] Michurina, S.V., Ishenko, I.J., Klimentov, V.V., Archipova, S.A., Myakina, N.E., Cherepanova, M.A., et al., 2016. Linagliptin alleviates fatty liver disease in diabetic db/db mice. World Journal of Diabetes 7:534–546.
[18] Lammers, D., Famulla, S., Wronkowitz, N., Hartwig, S., Lehr, S., Ouwens, D.M., et al., 2011. Dipeptidyl peptidase 4 is a novel adipokine potentially linking obesity to the metabolic syndrome. Diabetes 60:1917–1925.
[19] Sell, H., Blüher, M., Kütting, N., Schlicht, R., Willems, M., Ruppe, F., et al., 2013. Adipose dipeptidyl peptidase-4 and obesity: correlation with insulin resistance and depot-specific release from adipose tissue in vivo and in vitro. Diabetes Care 36:4083–4090.
[20] Röhborn, D., Brückner, J., Sell, H., Eckel, J., 2016. Reduced DPP4 activity improves insulin signaling in primary human adipocytes. Biochemical and Biophysical Research Communications 471:348–354.
[21] Dunmore, S.J., Brown, J.E.P., 2013. The role of adipokines in β-cell failure of type 2 diabetes. The Journal of Endocrinology 216:T27–T45.
[22] Wronkowitz, N., Görgens, S.W., Romacho, T., Villalobos, L.A., Sánchez-Ferrer, C.F., Peiró, C., et al., 2014. Soluble DPP4 induces inflammation and proliferation of human smooth muscle cells via protease-activated receptor 2. Biochimica Et Biophysica Acta 1842:1613–1621.
[23] Baumeier, C., Sausenenthaler, S., Kammel, A., Jähnert, M., Schütter, L., Hesse, D., et al., 2017. Hepatic DPP4 DNA methylation associates with fatty liver. Diabetes 66:25–35.
