Original Article

MOLECULAR METABOLISM

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

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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.

Keywords CD36; DPP4; GLP-1; Insulin resistance; NAFLD; PPARγ

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

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Abbreviations: Ad, adenovirus; Akt, Akt serine–threonine protein kinase; ALT, alanine aminotransferase; ApoB, apolipoprotein B; AST, aspartate aminotransferase; BAT, brown adipose tissue; CD36, fatty acid translocase; Cpt1a, carnitine palmitoyltransferase 1a; Dgat2, diacylglycerol O-acyltransferase 2; DPP4, dipeptidyl peptidase 4; *Dpp4*-Liv-Tg, transgenic mice with hepatocyte-specific *Dpp4* overexpression; F4/80, adhesion G protein-coupled receptor E1; *Gfp*, green fluorescent protein; GGT, gamma-glutamyl transpeptidase; GLP-1, glucagon-like peptide 1; gWAT, gonadal white adipose tissue; HFD, high-fat diet; HOMA-IR, homeostatic model assessment for insulin resistance; IL6, interleukin 6; MAPK, mitogen-activated protein kinase; MCP1, chemokine (C-C motif) ligand 2; MOGAT1, monoacylglycerol O-acyltransferase 1; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NFκB, nuclear factor-κB; pAkt, phosphorylated Akt serine–threonine protein kinase; WT, wild-type; PPARγ, peroxisome proliferator activated receptor gamma; rhDPP4, recombinant human dipeptidyl peptidase 4; rmDPP4, recombinant mouse dipeptidyl peptidase 4; SM, skeletal muscle; Srebf1, sterol regulatory element binding transcription factor 1; sWAT, subcutaneous white adipose tissue; TNFα, tumor necrosis factor α

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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 (129P2/Ola), which were injected into C57BL/6J blastocysts. Obtained chimeras 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's 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 Ultrasensitive 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 glycin-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-proline-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-nitroaniline (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%.

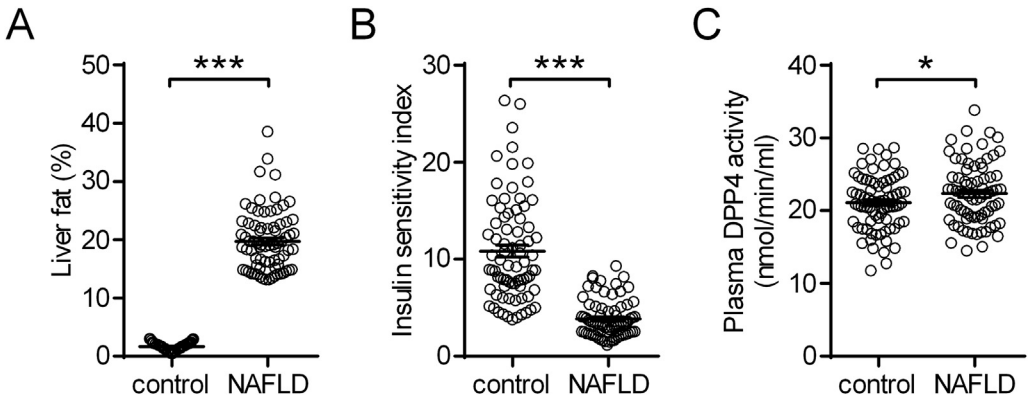

Figure 1: NAFLD and insulin resistant subjects show elevated plasma DPP4 activity. (A–C) Liver fat content (A), insulin sensitivity (B), and plasma DPP4 activity (C) of age- and sex-matched subjects with or without (control) NAFLD. Data are represented as mean ± SEM (n = 75–77). One-tailed *t*-test was performed to test whether liver fat, insulin sensitivity index, and plasma DPP4 activity are increased in NAFLD subjects. *p < 0.05, ***p < 0.001.

2.8. Histology

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% CO_2 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% CO_2. After 4 h, cells were either infected with 3.5×10^6 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 (954-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 two groups, Student's one-tailed (when applicable) or two-tailed unpaired t-test was used. For analysis of fold changes, Wilcoxon signed rank test was performed. 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.lnk software. Significance levels were set for p-values of less than 0.05 (*), 0.01 (**) and 0.001 (***).

3. RESULTS

3.1. Plasma DPP4 activity is increased in patients with NAFLD and insulin resistance

We have recently shown that individuals with elevated levels of hepatic steatosis show an increased expression of *DPP4* in the liver and that the liver substantially contributes to circulating DPP4 levels [23]. Here, we analyzed the plasma DPP4 activity of NAFLD patients with prediabetes in comparison to age- and sex-matched healthy controls. As expected, NAFLD subjects revealed an increased liver fat content (Figure 1A) and reduced insulin sensitivity (Figure 1B). DPP4 activity was augmented in plasma of NAFLD patients (Figure 1C, 52.8 ± 1.2 *vs.* 49.9 ± 1.0, p < 0.05), indicating an increased release of the enzyme into the circulation. However, as BMI was also higher in the NAFLD group (33.9 ± 0.5 *vs.* 26.6 ± 0.6, p < 0.001), increased plasma DPP4 activity could also be originated from elevated adipose tissue mass.

3.2. Liver-specific *Dpp4* overexpression results in elevated plasma DPP4 activity and diminished GLP-1 levels

To investigate whether elevated levels of DPP4 in fatty livers are cause or consequence of the disease, we generated a transgenic mouse model with a hepatocyte-specific *Dpp4* overexpression. Male C57BL/6J wild-type (WT) and *Dpp4*-transgenic (*Dpp4*-Liv-Tg) mice received a HFD (45 kcal% from fat) for 27 weeks and were sacrificed at 30 weeks of age. Both *Dpp4* mRNA and protein contents were significantly increased (2.2-fold and 1.6-fold, respectively) in livers of *Dpp4*-Liv-Tg mice but not altered in adipose depots or skeletal muscle (Figure 2A,B). Primary hepatocytes isolated from *Dpp4*-Liv-Tg mice also showed elevated DPP4 protein content and cellular DPP4 activity than those isolated from WT animals (Figure 2B, Supplementary Figure 1A,B). Moreover, comparing endogenous DPP4 levels in different metabolic tissues of WT mice reveal the highest expression of DPP4 in the liver (Figure 2A,B). Immunohistochemical staining of DPP4 in liver sections suggests increased membrane localization in *Dpp4*-Liv-Tg mice indicated by a co-staining with the plasma membrane marker E-cadherin

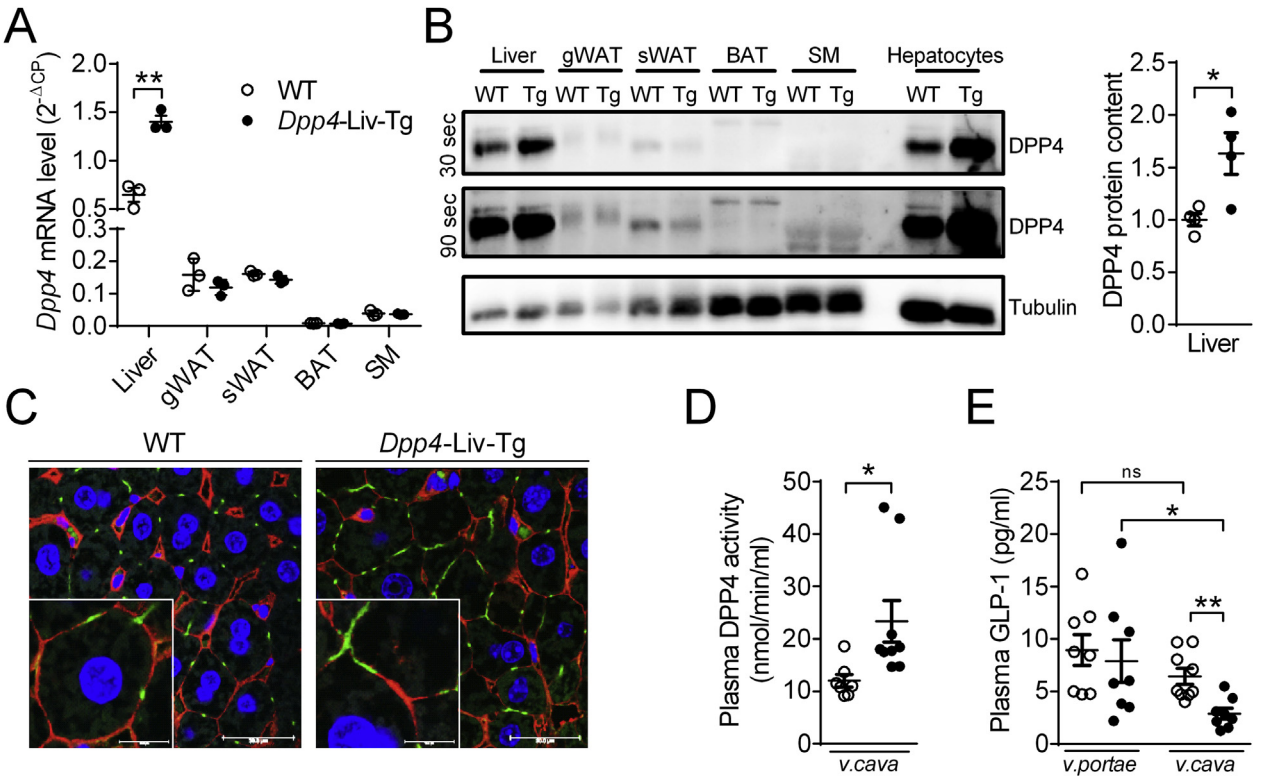

Figure 2: Liver-specific *Dpp4* overexpression causes increased plasma DPP4 activity and reduced glucose-stimulated GLP-1 levels. Male wild-type (WT, open circles) and *Dpp4*-transgenic (*Dpp4*-Liv-Tg, black circles) received a high-fat diet until 30 weeks of age. (A, B) Relative *Dpp4* mRNA expression (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 glucagon-like peptide 1 (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 (*Tnf*) and MCP1 (*Ccl2*)) 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

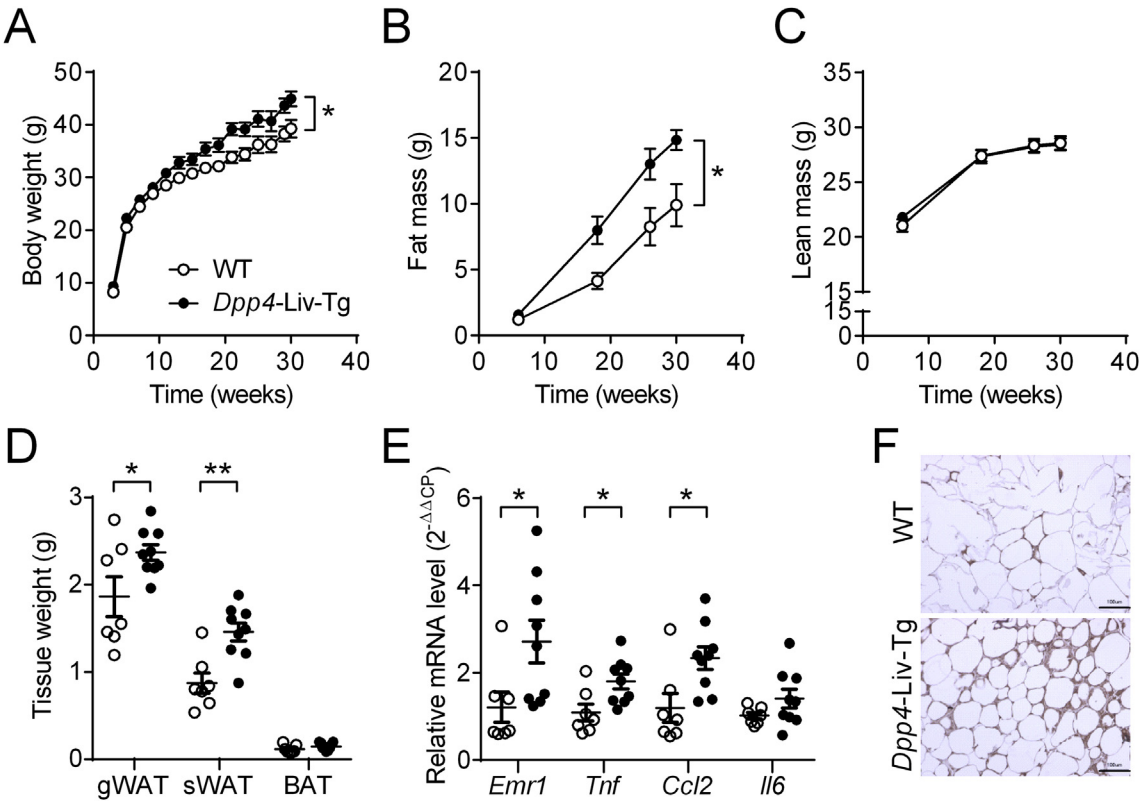

Figure 3: Hepatocyte-specific *Dpp4* overexpression induces adiposity and adipose tissue inflammation. Male wild-type (WT, open circles) and *Dpp4*-transgenic (*Dpp4*-Liv-Tg, black circles) mice were fed a high-fat diet until 30 weeks of age. (A–C) Development of body weight (A), fat mass (B), and lean mass (C). (D) Tissue weight of gonadal (gWAT) and subcutaneous white adipose tissue (sWAT), and brown adipose tissue (BAT). (E) Relative expression of the macrophage marker F4/80 (*Emr1*) and proinflammatory cytokines (TNFα (*Tnf*), MCP1 (*Ccl2*) and IL6 (*Il6*)) 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.

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 of 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 numerical increased alanine aminotransferase (ALT, $p = 0.171$) and aspartate aminotransferase (AST, $p = 0.132$) levels (Table 1). However, markers for inflammation and fibrosis were similar in livers of WT and *Dpp4*-Liv-Tg animals (Supplementary Figure 2). Taken together, elevated levels of DPP4 in the liver cause hepatic steatosis with indications of liver damage but not inflammation and fibrosis in mice on a HFD.

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 3). 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). Monoacylglycerol 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 *Pparγ* 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 upregulated 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 (μg/ml)	366 ± 23	368 ± 28	0.936
Free fatty acids (mmol/l)	0.389 ± 0.038	0.434 ± 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 glucosesss$_{[fed]}$ (mmol/l)	11.3 ± 0.6	11.0 ± 0.6	0.728
Blood glucose$_{[16\ h\ fasted]}$ (mmol/l)	5.3 ± 0.4	5.2 ± 0.1	0.825
Insulin$_{[fed]}$ (μg/l)	1.76 ± 0.75	2.08 ± 0.41	0.698
Insulin$_{[16\ h\ fasted]}$ (μg/l)	0.46 ± 0.09	0.91 ± 0.16	0.121
HOMA-IR	2.64 ± 0.57	5.08 ± 0.93	0.156
Leptin to Adiponectin ratio	3.75 ± 0.86	7.30 ± 1.04	0.024

(Figure 6A). Also, in primary murine hepatocytes obtained from 12-week-old lean WT mice, 500 ng/ml recombinant mouse DPP4 (rmDPP4) reduced the insulin-stimulated Akt-phosphorylation by 15% (Figure 6B). Moreover, adenoviral-mediated overexpression of full-length *Dpp4* (Ad-*Dpp4*) [23] in primary hepatocytes led to a reduction of insulin sensitivity by 30%, when compared to Ad-*Gfp* infected control cells (Figure 6C). Primary hepatocytes obtained from 12-week-old standard diet fed WT and *Dpp4*-Liv-Tg mice did not differ in lipid content (data not shown) but revealed differences in insulin responsiveness, as hepatocytes from *Dpp4*-Liv-Tg mice showed an 18% lower Akt-phosphorylation than those of WT controls (Figure 6D). Taken together, soluble DPP4 reduced the insulin sensitivity of human and mouse liver cells, suggesting a direct role of DPP4 in hepatic insulin signaling.

To test hepatic insulin sensitivity in WT and *Dpp4*-Liv-Tg mice, we injected insulin into 30-week-old animals and sacrificed them 15 min later. Figure 6E shows that insulin-stimulated Akt-phosphorylation was 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 (homeostatic 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*

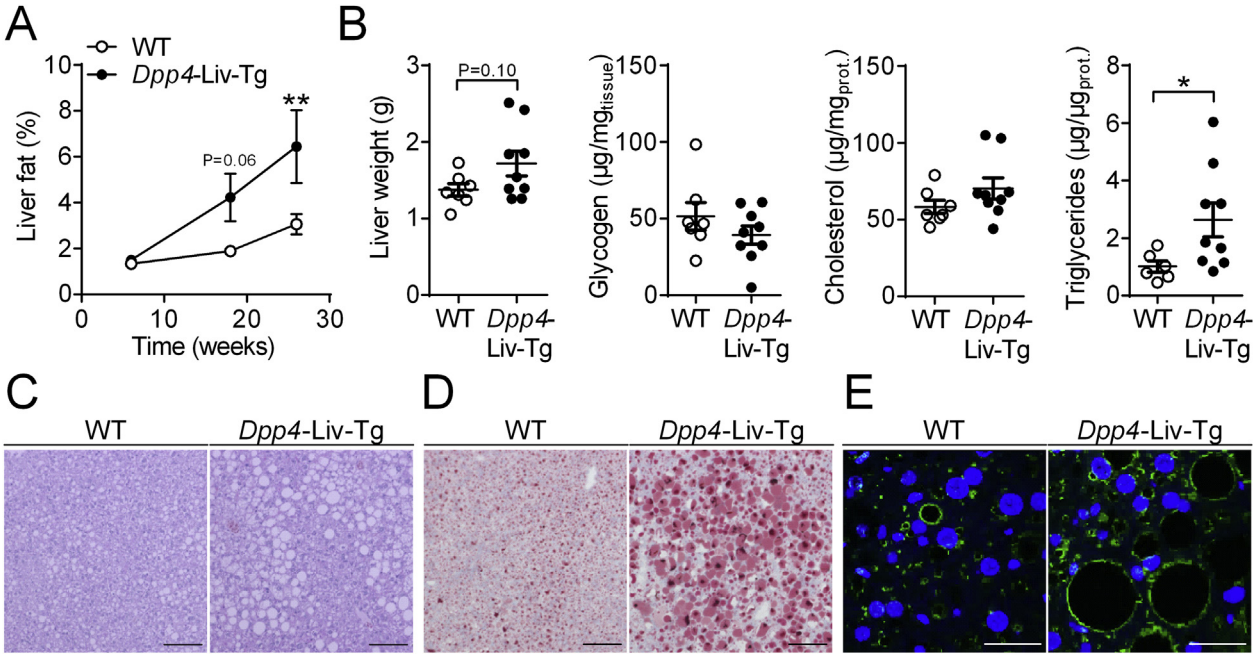

Figure 4: Hepatocyte-specific *Dpp4* overexpression promotes hepatic steatosis. Male wild-type (WT, open circles) and *Dpp4*-transgenic (*Dpp4*-Liv-Tg, black circles) mice were fed a high-fat diet until 30 weeks of age. (A) Development of liver fat content measured by computed tomography. (B) Liver weight, glycogen, cholesterol, and triglyceride content. (C, D) 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.

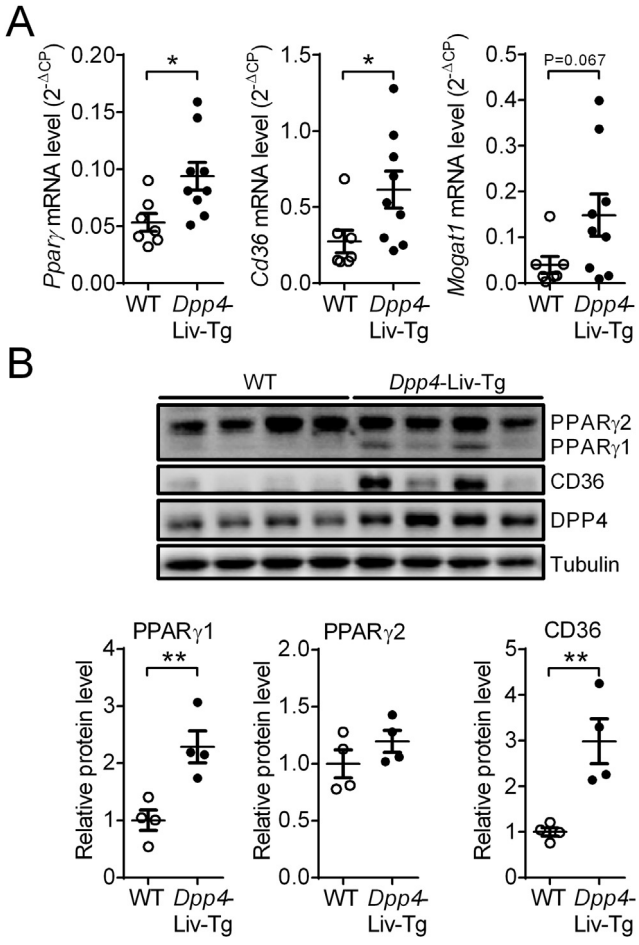

Figure 5: Elevated hepatic DPP4 increases expression of *Pparγ* and its downstream targets *Cd36* and *Mogat1*. (A) Relative mRNA expression of *Pparγ*, *Cd36* and *Mogat1* in livers of 30-week-old wild-type (WT, open circles) and *Dpp4*-transgenic (*Dpp4*-Liv-Tg, black circles) mice on a high-fat diet (n = 7–9). (B) Western blot analysis of CD36, PPARγ and DPP4. Quantification of protein content is depicted relative to tubulin levels (n = 4). The quantification for DPP4 is shown in Figure 2B. All data are represented as mean ± SEM. *p < 0.05, **p < 0.01.

expression by *e.g.* epigenetic mechanisms [23] can lead to perturbations in hepatic metabolism and finally to elevated ectopic lipid accumulation in the long-term. Although we show increased hepatic steatosis as a consequence of *Dpp4* overexpression, our data indicate that the elevated hepatic lipid accumulation in *Dpp4*-Liv-Tg mice is a secondary effect of alterations in (*i*) hepatic insulin sensitivity and (*ii*) energy homeostasis *via* interference in the incretin axis.

Dpp4-Liv-Tg mice display severe hepatic insulin resistance at 30 weeks of age, and insulin tolerance test indicates impaired insulin sensitivity already at a younger age. The fact that human HepG2 cells and murine primary hepatocytes showed impaired insulin sensitivity in response to DPP4 leads to the conclusion that DPP4 has a direct incretin-independent effect on hepatic insulin signaling. Insulin-stimulated Akt-phosphorylation was blunted by the treatment with recombinant DPP4 as well as by adenoviral and transgenic overexpression of *Dpp4*. Importantly, dose of recombinant protein inducing insulin resistance (500 ng/ml) was in a physiological range found in serum of obese and insulin resistant subjects [18,19]. The observed effects on insulin sensitivity were independent of the lipid content, excluding lipotoxicity-induced insulin resistance. This is in line with 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 [18] 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 anorexic 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 canalicular 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 (GGT 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 less liver fibrosis and inflammation in an experimental model of liver injury [36]. Here, we show that hepatocyte-specific *Dpp4* overexpression resulted in significant 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 monoacylglycerol O-acyltransferase 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

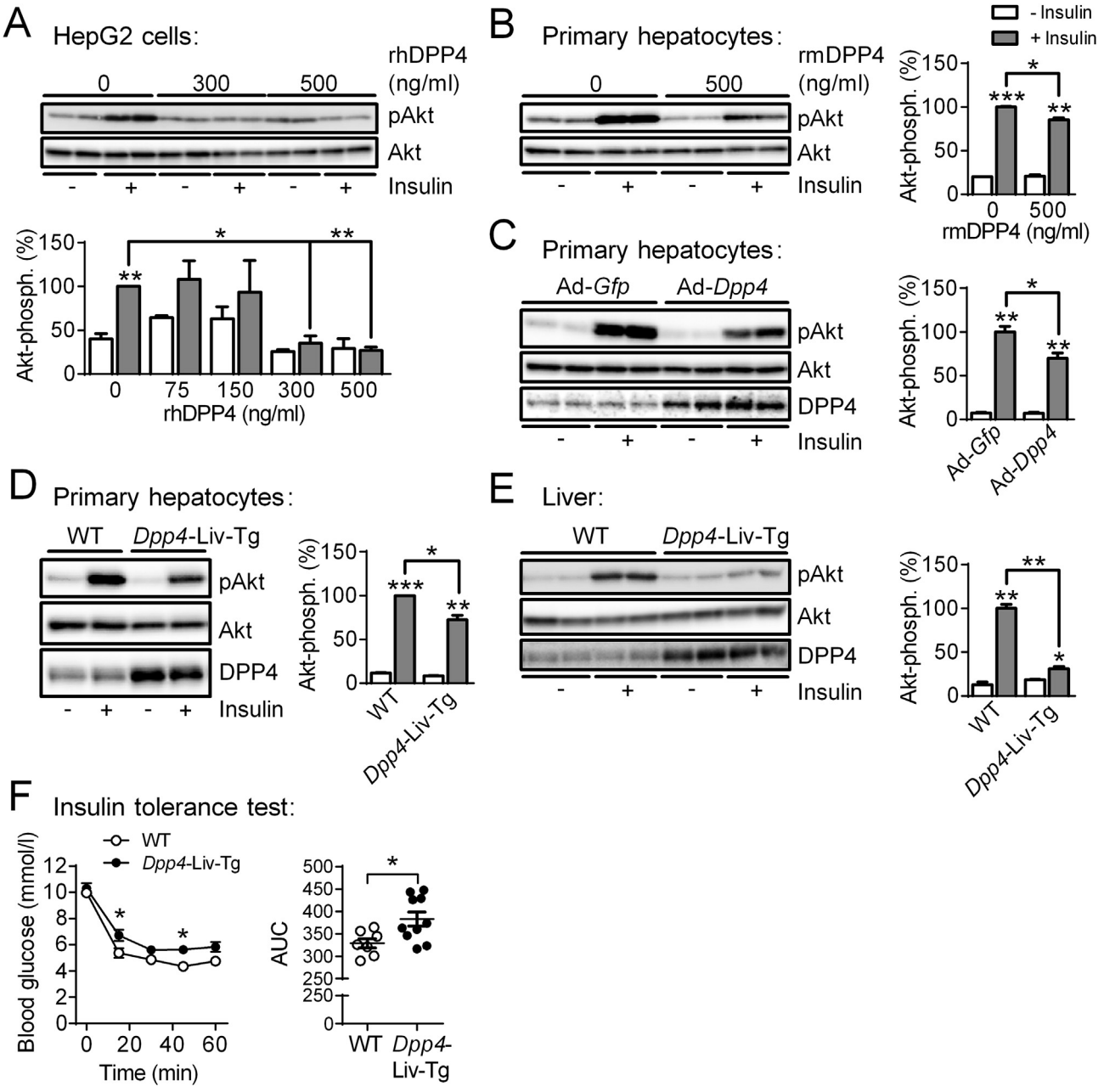

Figure 6: Increased DPP4 levels cause hepatic insulin resistance. (A, B) Basal (white bars) and insulin-stimulated (100 nmol/l, 10 min, gray bars) Akt-phosphorylation after 48 h treatment with various concentrations of recombinant human (rhDPP4) or mouse (rmDPP4) DPP4 in HepG2 cells (A) and murine primary hepatocytes (B) ($n = 3–6$), respectively. (C) Relative insulin-stimulated Akt-phosphorylation in primary mouse hepatocytes, 48 h after infection with adenoviruses coding for DPP4 (Ad-*Dpp4*) or GFP (Ad-*Gfp*) ($n = 4$). (D) Relative insulin-stimulated Akt-phosphorylation in primary hepatocytes isolated from wild-type (WT) and *Dpp4*-transgenic (*Dpp4*-Liv-Tg) mice, 48 h after isolation ($n = 3$). (E) Akt-phosphorylation in livers of 30-week-old WT and *Dpp4*-Liv-Tg mice 15 min after NaCl (−) or insulin injection (+; 1.25 IU/kg body weight) ($n = 3$). (F) Insulin tolerance test (1.25 IU/kg body weight) and area under the curve (AUC) for blood glucose in 22-week-old WT and *Dpp4*-Liv-Tg mice ($n = 7–9$). All data are represented as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

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.

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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.

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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., Brunt, 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] Röhrborn, 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., Seelk, S., Gall, M.G., Chowdhury, S., Poplawski, S.E., et al., 2013. Quantitation of fibroblast activation protein (FAP)-specific protease activity in mouse, baboon and human fluids and organs. FEBS Open Bio 4:43–54.

[8] Itou, M., Kawaguchi, 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., Kohjima, 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 (DDP 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., Zvibel, I., Rabinowich, L., Goldiner, I., Shlomai, 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] Aroor, A.R., Habibi, J., Ford, D.A., Nistala, R., Lastra, G., Manrique, 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., Klöting, 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., Klimontov, V.V., Archipov, 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] Lamers, 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., Klöting, N., Schlich, 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] Röhrborn, 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:T37–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., Saussenthaler, S., Kammel, A., Jähnert, M., Schlüter, L., Hesse, D., et al., 2017. Hepatic DPP4 DNA methylation associates with fatty liver. Diabetes 66:25–35.

[24] Stefan, N., Machicao, F., Staiger, H., Machann, J., Schick, F., Tschritter, O., et al., 2005. Polymorphisms in the gene encoding adiponectin receptor 1 are associated with insulin resistance and high liver fat. Diabetologia 48:2282–2291.

[25] Matsuda, M., DeFronzo, R.A., 1999. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 22:1462–1470.

[26] Lubura, M., Hesse, D., Neumann, N., Scherneck, S., Wiedmer, P., Schürmann, A., 2012. Non-invasive quantification of white and brown adipose tissues and liver fat content by computed tomography in mice. PLoS One 7:e37026.

[27] Schwenk, R.W., Baumeier, C., Finan, B., Kluth, O., Brauer, C., Joost, H.-G., et al., 2015. GLP-1-oestrogen attenuates hyperphagia and protects from beta cell failure in diabetes-prone New Zealand obese (NZO) mice. Diabetologia 58: 604–614.

[28] Schwenk, R.W., Jonas, W., Ernst, S.B., Kammel, A., Jähnert, M., Schürmann, A., 2013. Diet-dependent alterations of hepatic Scd1 expression are accompanied by differences in promoter methylation. Hormone and Metabolic Research 45:786–794.

[29] Baumeier, C., Kaiser, D., Heeren, J., Scheja, L., John, C., Weise, C., et al., 2015. Caloric restriction and intermittent fasting alter hepatic lipid droplet proteome and diacylglycerol species and prevent diabetes in NZO mice. Biochimica Et Biophysica Acta 1851:566–576.

[30] Winkler, R., Benz, V., Clemenz, M., Bloch, M., Foryst-Ludwig, A., Wardat, S., et al., 2012. Histone deacetylase 6 (HDAC6) is an essential modifier of glucocorticoid-induced hepatic gluconeogenesis. Diabetes 61:513–523.

[31] Finucane, F.M., Luan, J., Wareham, N.J., Sharp, S.J., O'Rahilly, S., Balkau, B., et al., 2009. Correlation of the leptin:adiponectin ratio with measures of insulin resistance in non-diabetic individuals. Diabetologia 52:2345–2349.

[32] Rufinatscha, K., Radlinger, B., Dobner, J., Folie, S., Bon, C., Profanter, E., et al., 2017. Dipeptidyl peptidase-4 impairs insulin signaling and promotes lipid accumulation in hepatocytes. Biochemical and Biophysical Research Communications 485:366–371.

[33] Kim, M.-K., Chae, Y.N., Ahn, G.-J., Shin, C.Y., Choi, S.-H., Yang, E.K., et al., 2017. Prevention and treatment effect of evogliptin on hepatic steatosis in high-fat-fed animal models. Archives Pharmacal Research 40:268–281.

[34] Matsumoto, Y., Bishop, G.A., McCaughan, G.W., 1992. Altered zonal expression of the CD26 antigen (dipeptidyl peptidase IV) in human cirrhotic liver. Hepatology 15:1048–1053.

[35] Firneisz, G., Varga, T., Lengyel, G., Fehér, J., Ghyczy, D., Wichmann, B., et al., 2010. Serum dipeptidyl peptidase-4 activity in insulin resistant patients with non-alcoholic fatty liver disease: a novel liver disease biomarker. PLoS One 5: e12226.

[36] Wang, X.M., Holz, L.E., Chowdhury, S., Cordoba, S.P., Evans, K.A., Gall, M.G., et al., 2016. The pro-fibrotic role of dipeptidyl peptidase 4 in carbon tetrachloride-induced experimental liver injury. Immunology and Cell Biology.

[37] Rosmaninho-Salgado, J., Marques, A.P., Estrada, M., Santana, M., Cortez, V., Grouzmann, E., et al., 2012. Dipeptidyl-peptidase-IV by cleaving neuropeptide Y induces lipid accumulation and PPAR-γ expression. Peptides 37:49–54.

[38] Al-Qahtani, A.A., Lyroni, K., Aznaourova, M., Tseliou, M., Al-Anazi, M.R., Al-Ahdal, M.N., et al., 2017. Middle east respiratory syndrome corona virus spike glycoprotein suppresses macrophage responses via DPP4-mediated induction of IRAK-M and PPARγ. Oncotarget 8:9053–9066.

[39] Segond, N., Degrelle, S.A., Berndt, S., Clouqueur, E., Rouault, C., Saubamea, B., et al., 2013. Transcriptome analysis of PPARγ target genes reveals the involvement of lysyl oxidase in human placental cytotrophoblast invasion. PLoS One 8:e79413.

[40] Pettinelli, P., Videla, L.A., 2011. Up-regulation of PPAR-gamma mRNA expression in the liver of obese patients: an additional reinforcing lipogenic mechanism to SREBP-1c induction. The Journal of Clinical Endocrinology and Metabolism 96:1424–1430.

[41] Gavrilova, O., Haluzik, M., Matsusue, K., Cutson, J.J., Johnson, L., Dietz, K.R., et al., 2003. Liver peroxisome proliferator-activated receptor gamma contributes to hepatic steatosis, triglyceride clearance, and regulation of body fat mass. The Journal of Biological Chemistry 278:34268–34276.

[42] Inoue, M., Ohtake, T., Motomura, W., Takahashi, N., Hosoki, Y., Miyoshi, S., et al., 2005. Increased expression of PPARgamma in high fat diet-induced liver steatosis in mice. Biochemical and Biophysical Research Communications 336:215–222.

[43] Yu, S., Matsusue, K., Kashireddy, P., Cao, W.-Q., Yeldandi, V., Yeldandi, A.V., et al., 2003. Adipocyte-specific gene expression and adipogenic steatosis in the mouse liver due to peroxisome proliferator-activated receptor gamma1 (PPARgamma1) overexpression. The Journal of Biological Chemistry 278: 498–505.

[44] Morán-Salvador, E., López-Parra, M., García-Alonso, V., Titos, E., Martínez-Clemente, M., González-Périz, A., et al., 2011. Role for PPARγ in obesity-induced hepatic steatosis as determined by hepatocyte- and macrophage-specific conditional knockouts. FASEB Journal 25:2538–2550.

[45] Wolf Greenstein, A., Majumdar, N., Yang, P., Subbaiah, P.V., Kineman, R.D., Cordoba-Chacon, J., 2017. Hepatocyte-specific, PPARγ-regulated mechanisms to promote steatosis in adult mice. The Journal of Endocrinology 232: 107–121.

[46] Lim, J., Iyer, A., Liu, L., Suen, J.Y., Lohman, R.-J., Seow, V., et al., 2013. Diet-induced obesity, adipose inflammation, and metabolic dysfunction correlating with PAR2 expression are attenuated by PAR2 antagonism. FASEB Journal 27: 4757–4767.

[47] Badeanlou, L., Furlan-Freguia, C., Yang, G., Ruf, W., Samad, F., 2011. Tissue factor-protease-activated receptor 2 signaling promotes diet-induced obesity and adipose inflammation. Nature Medicine 17:1490–1497.

[48] Lee, D.-S., Lee, E.-S., Alam, M.M., Jang, J.-H., Lee, H.-S., Oh, H., et al., 2016. Soluble DPP-4 up-regulates toll-like receptors and augments inflammatory reactions, which are ameliorated by vildagliptin or mannose-6-phosphate. Metabolism 65:89–101.

