Intervention in hepatic lipid metabolism : implications for atherosclerosis progression and regression

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Chapter 3

The expression level of non-alcoholic fatty liver disease-related gene PNPLA3 in hepatocytes is highly influenced by hepatic lipid status

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ABSTRACT

Background & Aims: Recent studies have suggested that variations in PNPLA3 are associated with non-alcoholic fatty liver disease (NAFLD). To gain insight in the potential function of PNPLA3 in liver, we have determined the effect of metabolic shifts on the hepatic expression profile of PNPLA3 in mice.
Methods and Results: PNPLA3 expression in wild-type C57BL/6 and NAFLD-susceptible LDL receptor knockout (LDLR-/-) mice was determined using microarray and real-time PCR analysis. PNPLA3 expression in livers is 50- to 100-fold lower as compared to (cardiac) muscle and adipose tissue in regular chow diet-fed mice. Feeding a Western-type diet stimulated the hepatic relative PNPLA3 expression level 23-fold ($P<0.001$) both in C57BL/6 mice and LDLR-/- mice, suggesting that PNPLA3 does become an important player in hepatic lipid metabolism under conditions of lipid excess. Subjecting mice to fasting fully reversed the effect of the Western-type diet on hepatic PNPLA3 expression. Under these conditions, also the expression level of PNPLA3 in adipose tissue is 90% decreased ($P<0.001$). Cellular distribution analysis revealed that PNPLA3 is expressed in hepatocytes, but not liver endothelial and Kupffer cells. Microarray-based gene profiling showed that the expression level of PNPLA3 in hepatocytes is correlated to that of genes associated with the lipogenic pathway such as ME1, SPOT14, and SCD1.
Conclusions: It appears that the NAFLD-related gene PNPLA3 is highly responsive to metabolic changes in hepatocytes within the liver and its relative change in expression level suggests an essential function in lipogenesis.
Keywords: Liver, PNPLA3, mice, hepatocytes, gene expression, lipogenesis, NAFLD

INTRODUCTION

Atherosclerosis is the primary cause of cardiovascular diseases such as ischemic (coronary) heart disease, diabetes, and myocardial infarction, which form the major cause of mortality and morbidity in the Western world. Although atherosclerosis is a progressive disease of the arteries, recent studies have indicated that the occurrence of non-alcoholic fatty liver disease (NAFLD) is also a strong risk factor for atherosclerotic lesion development in humans [1,2].

NAFLD is characterized by the accumulation of lipid in liver cells (hepatic steatosis) and is the most common cause of liver disease with a prevalence of 15-25% in the general population [3-5]. The intra-hepatic lipid balance is maintained by different processes including receptor-mediated uptake of lipids from the blood, hepatic degradation of lipids, de novo synthesis of lipids, secretion of lipids into the bile and the blood compartment, and hepatic storage of lipids. Perturbations in the activity of essential mediators functioning in these processes can induce disturbances in the intra-hepatic lipid homeostasis. More specifically, when the input of lipid exceeds the output of lipid from the liver this will induce excessive storage of lipid and thus lead to the development of hepatic steatosis. NAFLD may slowly progress into non-alcoholic steatohepatitis (NASH), which is characterized by excessive liver inflammation. NASH is an established risk factor for the development of end-stage liver disease (i.e. cirrhosis), a condition that can generally only be treated by performing a liver transplantation.

Interestingly, recent genome-wide association studies have suggested that variations in the patatin-like phospholipase domain containing 3 (PNPLA3) gene contribute to differences in hepatic lipid content and the susceptibility to NAFLD [6-8]. PNPLA3, formerly also known as adiponutrin and iPLAε, is predominantly expressed in adipose tissue and is highly responsive to changes in energy balance [9]. It belongs to the patatin-like phospholipase family of proteins that also contains the key protein involved in the hydrolysis of triglycerides to diglycerides in adipocytes, adipose triglyceride lipase (ATGL; PNPLA2). Although PNPLA3 possesses lipase and acylglycerol transacylase activities in adipocytes in vitro [10], the function of PNPLA3 in the liver in vivo remains to be determined. To gain more insight in the potential function of PNPLA3 in liver, in the current study we have determined the effect of metabolic shifts on the hepatic expression profile of PNPLA3 in mice.

MATERIALS AND METHODS

Animals

Wild-type C57BL/6 mice and homozygous LDL receptor knockout (LDLR-/-) mice were obtained from The Jackson Laboratory as mating pairs and bred at the Gorlaeus Laboratories, Leiden, The Netherlands. Mice were maintained on a regular chow diet containing 5.7% (wt/wt) fat and no cholesterol or were fed a semi-synthetic Western-type diet containing 15% (wt/wt) cacao butter and 0.25% (wt/wt) cholesterol (Diet W, Hope Farms, Woerden, The Netherlands). Mice were fed ad libitum or fasted overnight (~16 hours) before whole body perfusion with PBS and subsequent sacrifice. Organs were isolated and immediately frozen in

liquid N_2 and stored at -80°C until RNA extraction. Animal experiments were performed at the Gorlaeus Laboratories of the Leiden/Amsterdam Center for Drug Research in accordance with national laws. All experimental protocols were approved by the Ethics Committee for Animal Experiments of Leiden University.

Hepatic cell separation
Mice were anaesthetized and the vena cava inferior was cannulated. Subsequently, the vena porta was ligated and the liver was perfused for 10 min (14 ml/min) with oxygenated Hanks' buffer pH 7.4, containing HEPES (1.6 g/l). The perfusion was continued for 10 min with Hanks'/HEPES buffer containing 0.05% (w/v) collagenase (type IV, Sigma) and 1 mM $CaCl_2$. Hepatocytes were isolated after mincing the liver in Hanks' buffer containing 0.3% BSA, filtering through nylon gauze and centrifugation for three times 10 min at 50 × g. The pellets consisted of pure (>99%) hepatocytes (PC) as judged by light microscopy. The supernatants were centrifuged for 10 min at 500 × g in order to harvest the non-parenchymal cells. By the modified method as described previously [11], liver endothelial cells (EC) and Kupffer cells (KC) were isolated at 4°C by density-gradient centrifugation and centrifugal elutriation (3250 rev/min at 26 ml/min for endothelial cells and 65 ml/min for Kupffer cells). The purity of the cell preparations was checked by peroxidase staining, which showed that Kupffer and endothelial cells were >80% and >95% pure, respectively.

Serum lipid analyses
Serum concentrations of total cholesterol and triglycerides were determined using enzymatic colorimetric assays (Roche Diagnostics). The cholesterol and triglyceride distribution over the different lipoproteins in serum was analysed by fractionation of 30 µl serum of each mouse using a Superose 6 column (3.2×30 mm, Smart-system, Pharmacia). Total cholesterol and triglyceride content of the effluent was determined using enzymatic colorimetric assays (Roche Diagnostics).

Analysis of gene expression by real-time quantitative PCR
Quantitative gene expression analysis on perfused organs was performed as described [12]. In short, total RNA was isolated according to Chomczynski and Sacchi [13] and reverse transcribed using RevertAid™ reverse transcriptase. Gene expression analysis was performed using real-time SYBR Green technology (Eurogentec). Beta-actin was used as the standard housekeeping gene. Relative gene expression numbers of were calculated by subtracting the threshold cycle number (Ct) of the gene of interest from the Ct of beta-actin and raising 2 to the power of this difference.

Microarray analysis
The Mouse Genome Survey Arrays used in the study contained 33.012 different probes representing 26.514 genes, which included transcripts from the public domain as well as from the Celera library. Total RNA from hepatocytes from LDLR-/- mice fed a regular chow diet or Western-type diet for 2, 4, or 6 weeks ad libitum was isolated according to Chomczynski and Sacchi [13]. Double stranded cDNA was prepared from 2 µg of total RNA. An in vitro transcription (IVT) reaction was used to synthesize 50-100 µg of UTP-digoxigenin-labeled cRNA. Equal amounts of cRNA (10 µg) from 2 pooled RNA samples of 2 mice (total of 4 mice) per time point

was hybridized to Mouse Genome Survey Arrays for 16 hours at 55°C. Subsequently, an alkalic phosphatase-linked digoxigenin antibody was incubated with the array and the phosphatase activity was initiated to start the chemiluminescent signal. The chemiluminescent (cRNA) and fluorescent (spot backgound) signals of the cRNA and standard controls spots were scanned for 5 and 25 seconds using an AB1700 Chemiluminescence Analyzer (Applied Biosystems). Using the software supplied with the AB1700 apparatus, the spot chemiluminescent signal was normalized over the fluorescent signal of the same spot (using the standard control signals) to obtain the normalized signal value that was used for further analysis. In the analysis, the median value of the normalized signal of two independent arrays for each time point was calculated as an indication for the relative gene expression number at that time point. Profile similarity search was performed using a Euclidean distance-based comparison.

Statistical analysis

The significance of differences in relative gene expression numbers between fasted or ad libitum fed mice and between mice on a chow or a Western-type diet was calculated using a two-tailed unpaired Student's t-test on the differences in Ct (Ct β-actin – Ct PNPLA3). The difference in Ct values was tested for normality using Graphpad Instat 3 software (Graphpad Instat Software, San Diego, CA). The significance of differences in serum lipid levels between the different groups of mice was tested using analysis of variance and the Student-Newman-Keuls multicomparison test (Graphpad Instat Software, San Diego, CA). Probability values less than 0.05 were considered significant.

RESULTS

The relative expression level of PNPLA3 in livers from wild-type C57BL/6 mice and low-density lipoprotein receptor knockout (LDLR-/-) mice, an established diet-induced atherosclerosis and NASH mouse model [14,15], under ad libitum feeding conditions was determined to gain insight in the basal role for hepatic PNPLA3. The mRNA expression of PNPLA3 in livers of regular chow-fed C57BL/6 mice and LDLR-/- mice as measured with quantitative real-time PCR was extremely low, since the threshold cycle for the fluorescent signal in the real-time quantitative PCR was rather high (Ct=30-32). In parallel, Baulande et al. showed that PNPLA3 mRNA was virtually undetectable in livers from SWISS mice under ad libitum feeding conditions [16]. As a result, the hepatic expression level of PNPLA3 was 50- to 1000-fold lower as compared to the level of expression detected in (cardiac) muscle, brown and white adipose tissue isolated from LDLR-/- mice (Fig.1), suggesting that PNPLA3 does not play a major role in liver metabolism under basal feeding conditions.

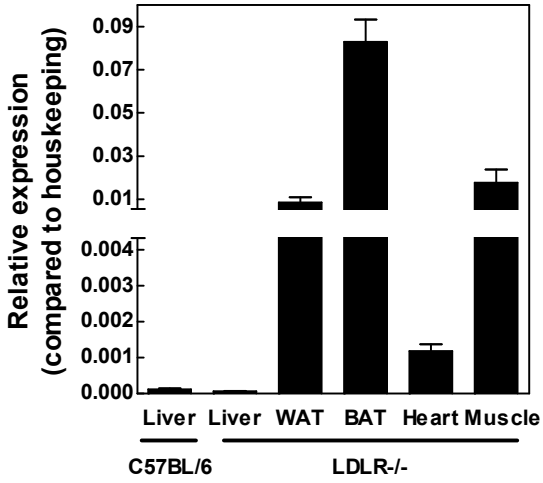

Fig. 1. Relative mRNA levels of PNPLA3 in livers and other metabolic tissues from C57BL/6 wild-type mice and LDL receptor knockout (LDLR-/-) mice fed a regular chow diet. Values represent means+SEM (n=5).

The human association studies suggested that PNPLA3 expression in liver is positively related to obesity and liver fat content [7]. Previously, it has been shown that feeding a Western-type diet containing fat and cholesterol not only stimulates atherosclerotic lesion development, but also induces obesity and fatty liver development in LDLR-/- mice [17]. We therefore determined the effect of Western-type diet feeding on the expression of PNPLA3 in livers of wild-type and LDLR-/- mice. Two weeks of feeding a Western-type diet containing 0.25% 9w/w) cholesterol and 15% (w/w) high fat, which for humans compares to an unhealthy high fat diet [18], induced an increase in plasma total cholesterol and triglyceride levels in C57BL/6 and LDLR-/- mice (Fig.2A). In LDLR-/- mice, the increase in plasma cholesterol could be primarily attributed to a rise in the level of the pro-atherogenic lipoproteins very-low-density lipoprotein (VLDL) and LDL, while in C57BL/6 mice Western-type diet feeding led to an increase in the plasma level of both pro- (VLDL/LDL) and anti-atherogenic high-density lipoproteins (HDL)(Fig.2B). Importantly, an increase in dietary lipid levels was associated with a significant 23-fold stimulation ($P<0.001$) of the relative expression level of PNPLA3 in livers of both C57BL/6 and LDLR-/- mice (Fig.3). The marked induction of its expression upon Western-type diet feeding suggests that PNPLA3 probably does play an important role in hepatic lipid metabolism under conditions of lipid excess. Furthermore, it thus seems that hepatic PNPLA3 levels in mice are positively related to plasma/liver fat content, similarly as observed in the human situation [7].

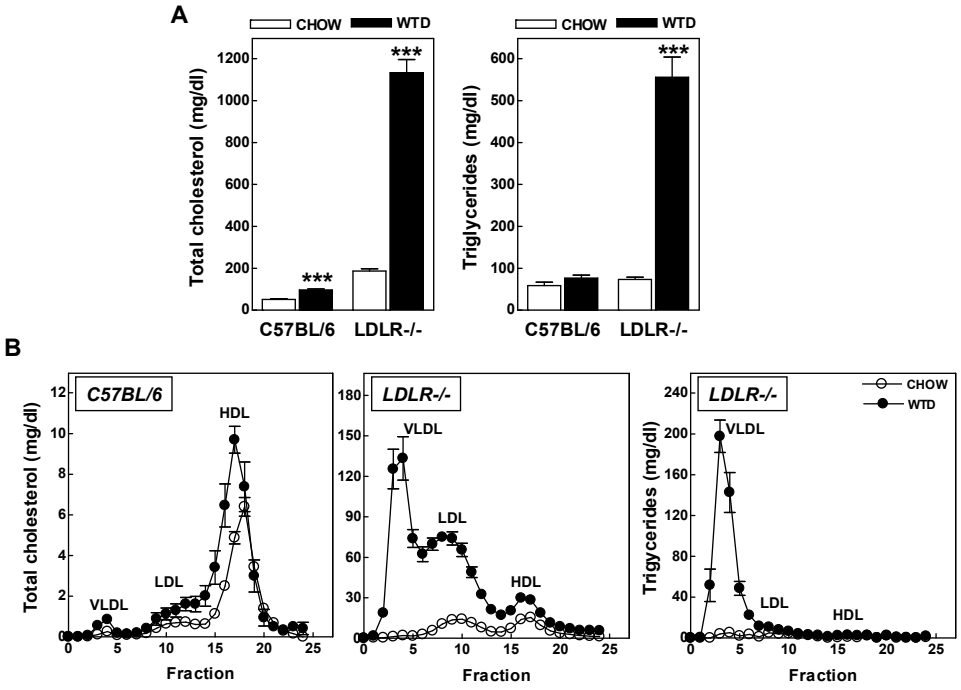

Fig. 2. A) Plasma total cholesterol and triglyceride levels, and B) plasma lipoprotein cholesterol and triglyceride distribution in C57BL/6 wild-type mice and LDL receptor knockout (LDLR-/-) mice fed a chow diet (white bars / open circles) or an atherogenic Western-type diet (WTD; black bars / closed circles) for two weeks. Fractions 2-6 represent VLDL, fractions 7-13 represent LDL, and fractions 14-20 represent HDL, respectively. Values represent means±SEM (n=5). *** P<0.001 compared to chow diet values.

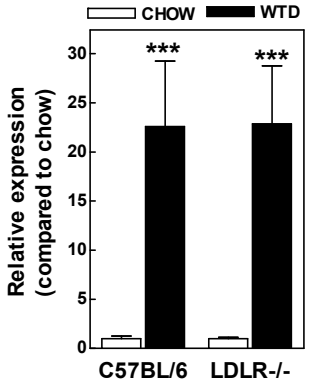

Fig. 3. Relative mRNA levels of PNPLA3 in livers from C57BL/6 wild-type mice and LDL receptor knockout (LDLR-/-) mice fed a chow diet (white bars) or an atherogenic Western-type diet (WTD; black bars) for two weeks. Values are expressed as fold compared to the chow diet and represent means+SEM of 5 mice. *** P<0.001 compared to chow diet values.

In accordance with a possibly important role for PNPLA3 in the protection against tissue lipid excess, several studies have shown that adipose tissue

PNPLA3 levels become virtually undetectable upon food restriction (i.e. external lipid depletion) [16,19,20]. To investigate whether external lipid depletion does also induce changes in hepatic PNPLA3 levels, we determined the effect fasting on PNPLA3 mRNA levels in livers and white adipose tissue of chow-fed (basal conditions) and Western-type diet-fed (lipid excess). Hepatic PNPLA3 expression was decreased by fasting in chow-fed C57BL/6 mice, while it actually increased in chow-fed LDLR-/- mice (Fig.4A). Strikingly, the 23-fold increase in the PNPLA3 expression in liver upon feeding the Western-type diet was completely abolished after a subsequent 16 hour fasting period, resulting in a relative hepatic expression level of PNPLA3 in fasted Western-type diet-fed mice identical to that of chow-fed animals (Fig.4A). As anticipated, fasting induced a ~90% decrease in white adipose tissue PNPLA3 mRNA expression of mice that were fed either the regular chow or the atherogenic Western-type diet (Fig.4B). Combined, these data show that fasting induces a similar response in PNPLA3 expression in fatty liver and white adipose tissue, suggesting that PNPLA3 may have a comparable function in adipose tissue and livers that exhibit an adipocyte-like (i.e. fatty) phenotype.

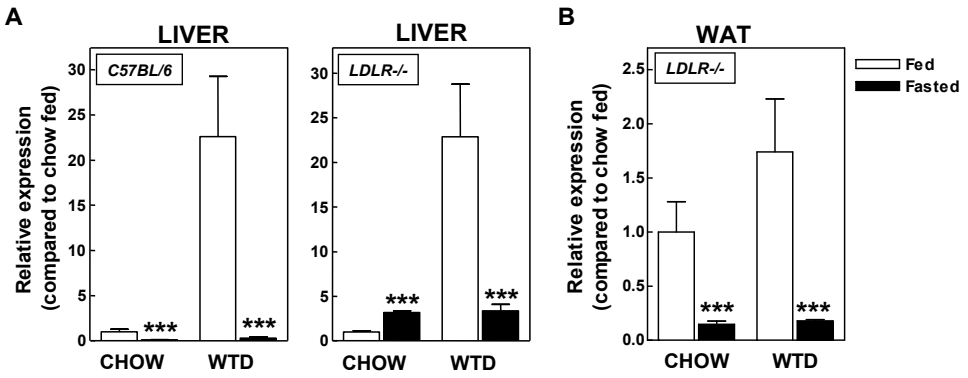

Fig. 4. A) Relative mRNA levels of PNPLA3 in livers from C57BL/6 wild-type mice and/or LDL receptor knockout mice (LDLR-/-) that were fed a chow diet (CHOW) or an atherogenic Western-type diet (WTD) for two weeks ad libitum (Fed) or that were subsequently subjected to 16h of fasting (Fasted). B) Relative mRNA levels of PNPLA3 in white adipose tissues (WAT) from LDLR-/- mice. Values represent means+SEM of 5 mice. *** P<0.001 compared to ad libitum fed values.

The liver consists of several different cell types which each play a distinct role in hepatic metabolism. In earlier experiments we have observed that for studies on the function of genes it is important to take their intra-hepatic cellular localization into account [12,21]. Feeding mice a Western-type diet enriched in cholesterol induces hepatic inflammation [15], ultimately leading to an increased number of macrophages within the liver. To exclude that the rise in hepatic PNPLA3 expression upon Western-type diet feeding could be attributed to an influx of monocytes in response the hepatic inflammation, we examined the relative PNPLA3 expression level in isolated hepatocytes, liver endothelial, and Kupffer cells (tissue macrophages). Importantly, expression of PNPLA3 was detected (although at low levels; Ct=31) in hepatocytes from C57BL/6 mice, while it was absent (Ct>35) in both liver endothelial and Kupffer cells (Fig.5). This suggests that effects measured in total liver PNPLA3 expression actually represent changes in the relative expression of PNPLA3 specifically in hepatocytes.

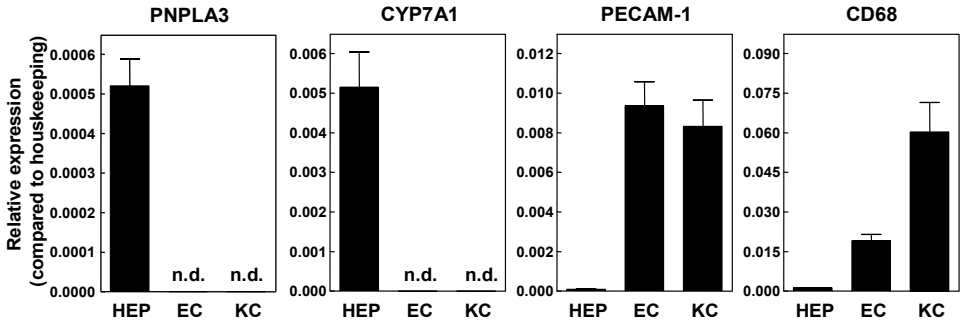

Fig. 5. Relative mRNA levels of PNPLA3 in hepatocytes (HEP), liver endothelial (EC), and Kupffer cells (KC). For comparison, the relative expression levels of the hepatocytes-specific gene cholesterol 7alpha-hydroxylase (CYP7A1), and the respective non-parenchymal and macrophage markers platelet endothelial cell adhesion molecule-1 (PECAM-1) and CD68 are also indicated. Values represent means+SEM of 4-6 different cell isolations. n.d., not detectable.

Previously, we have performed microarray-based gene expression profiling of hepatocytes isolated from livers of LDLR-/- mice to identify genes involved hepatic lipid metabolism specifically under conditions of high lipid pressure [22]. In our original analysis we used a quite stringent signal-to-noise ratio threshold to identify genes as being expressed. Because PNPLA3 did not reach the required signal-to-noise threshold value in normal fed LDLR-/- mice, our primary analysis suggested that PNPLA3 is not expressed in isolated hepatocytes. Re-analysis of our original data-set without the signal-to-noise limitation showed that out of a total of 27.857 transcripts detected PNPLA3 was the most highly upregulated one upon feeding mice the atherogenic Western-type diet. Verification by real-time quantitative PCR showed a very similar expression profile as observed in the microarray with a marked ~50-fold higher PNPLA3 expression in hepatocytes after two weeks of Western-type diet feeding, which remained elevated until the end of the feeding period (6 weeks)(Fig.6A).

To gain insight in the possible role for PNPLA3 in hepatocytes, we performed profile similarity analysis on the microarray data in which we compared the time-dependent expression profile of PNPLA3 to that of all transcripts detected (Fig.6B). In Table 1 the 10 genes whose time-dependent expression profile closely resembled that of PNPLA3 in hepatocytes are shown, including fatty acid binding protein 5 (FABP5), several unassigned FABP5-like proteins, malic enzyme 1 (ME1), stearoyl-CoA desaturase 1 (SCD1), and SPOT14. Interestingly, the expression of glucose-6-phosphate dehydrogenase 2 (G6PD2) and PNPLA3 followed the most similar pattern. As observed for PNPLA3, the expression of G6PD2 was extremely low under basal feeding conditions, while it was markedly induced by the Western-type diet. As a result, the relative expression level of PNPLA3 and G6PD2 in hepatocytes of LDL receptor knockout mice after different times of Western-type diet feeding was significantly correlated (R=0.96; P=0.038; Fig.6C). No expression of G6PD2 was observed in livers of C57BL/6 wild-type mice on a chow or Western-type diet (Ct>35), suggesting that in contrast to in the LDL receptor knockout mice the expression of G6PD2 in C57BL/6 does not reach a detectable level even under conditions of lipid excess. However, real-time PCR profiling of other genes that

were subject to coordinate regulation with PNPLA3 in hepatocytes of LDL receptor knockout mice showed that feeding the Western-type diet led to a significant stimulation of the relative expression level of FABP5 (3.1-fold; P=0.032), ME1 (2.6-fold; P=0.046), SCD1 (5.4-fold; P=0.002), and SPOT14 (3.1-fold; P=0.046) in livers of C57BL/6 wild-type mice (Fig.7). It thus seems that the expression of PNPLA3 in liver of both LDL receptor knockout mice and C57BL/6 wild-type mice is coordinately regulated with that of key lipogenic genes.

Table 1. Ten genes whose time-dependent expression profile was most comparable to that of PNPLA3 in hepatocytes of LDL receptor knockout mice.

Celera ID	Gene Name	Fold change on Western-type diet compared with chow diet		
		2 Weeks	4 Weeks	6 Weeks
mCG121722	Patatin-like phospholipase domain containing 3 (PNPLA3)	47.7	38.0	72.1
mCG50373	Glucose-6-phosphate dehydrogenase 2 (G6PD2)	15.6	10.6	16.8
mCG5289	Fatty acid binding protein (unassigned)	16.6	10.4	11.9
mCG22653	Fatty acid binding protein (unassigned)	16.5	10.6	11.1
mCG16174	PDZK1 interacting protein 1 (PDZK1IP1)	10.2	8.4	11.4
mCG22278	Fatty acid binding protein (unassigned)	19.3	7.4	7.7
mCG9729	Fatty acid binding protein (unassigned)	14.3	9.2	7.2
mCG7050	Thyroid hormone responsive SPOT14 homolog (THRSP)	16.5	8.1	6.9
mCG11880	Malic enzyme 1 (ME1)	8.1	8.4	10.6
mCG131749	Stearoyl-Coenzyme A desaturase 1 (SCD1)	7.3	8.1	10.6
mCG1638	Fatty acid binding protein 5, epidermal (FABP5)	22.3	5.9	5.5

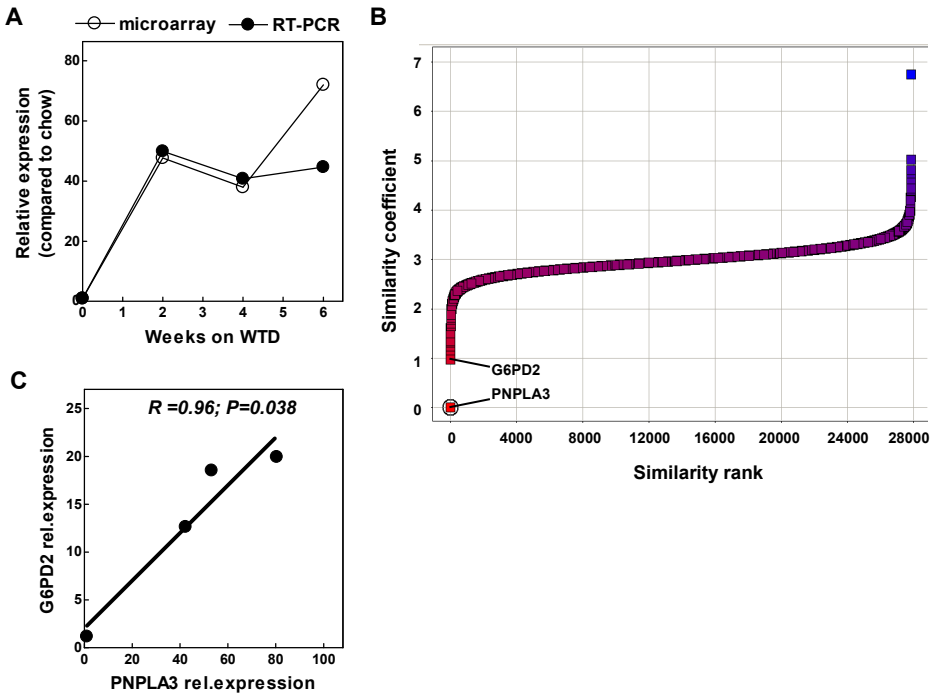

Fig. 6. A) Real-time quantitative PCR validation (closed circles) of the Western-type diet (WTD)-induced changes in the expression of PNPLA3 in hepatocytes from LDL receptor knockout mice, as observed with microarray analysis (open circles). B) Graphical representation of the results from the profile similarity analysis on the 27.857 transcripts present on the microarray. A higher similarity coefficient indicates a lower degree of profile comparison with the input gene PNPLA3. Marked dots represent PNPLA3 and glucose-6-phoshate dehydrogenase 2 (G6PD2), respectively. C) Correlation of the relative mRNA levels of PNPLA3 and G6PD2 in hepatocytes from LDL receptor knockout mice. Values are significantly correlated (P=0.038; R=0.96) and are relative to the expression on chow diet.

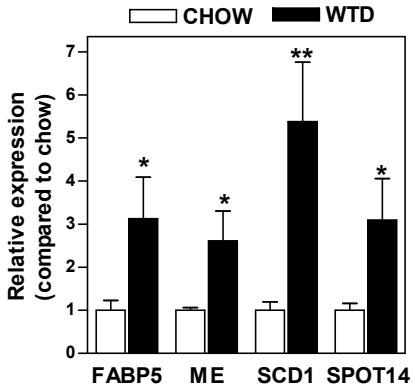

Fig. 7. Relative mRNA levels in livers of C57BL/6 wild-type mice that were fed a chow diet (CHOW) or an atherogenic Western-type diet (WTD) for two weeks ad libitum of genes showing a coordinate regulation with PNPLA3 in hepatocytes of LDL receptor knockout mice. Values represent means+SEM of 5 mice and are relative to the expression on chow diet. * P<0.05 and ** P<0.01 compared to chow diet values.

DISCUSSION

Non-alcoholic fatty liver disease (NAFLD), the presence of hepatic steatosis due to non-alcoholic causes, is a commonly observed phenomenon in Western societies. Although factors such as obesity, diabetes, and insulin resistance promote the accumulation of triglycerides in the liver, it has become clear that genetic variation is also a prominent risk factor for the development of NAFLD. Recent association studies have identified PNPLA3 as a possible important player in liver lipid metabolism as variations in the PNPLA3 gene are linked to an increased susceptibility for NAFLD [6-8]. However, no data are present on the exact role of PNPLA3 within the liver. In the current study, we have shown that PNPLA3 is expressed in the primary metabolic cell type in the liver, namely the hepatocytes, but not in liver endothelial cells or tissue macrophages (Kupffer cells). The relatively low hepatic expression of PNPLA3 upon chow diet feeding argues against a major role for this protein under basal conditions. As PNPLA3 among 27.857 transcripts is the most highly induced gene in hepatocytes upon exposure to exogenous lipid (i.e. upon Western-type diet feeding) we anticipate, however, that it does play a major role in liver under conditions of high lipid exposure.

A microarray profile similarity search indicated that the Western-type diet-induced change in the expression profiles of PNPLA3 and glucose-6-phosphate dehydrogenase (G6PD) type 2 was highly similar in hepatocytes of LDL receptor knockout mice. G6PD is a cytosolic enzyme that takes part in the pentose phosphate pathway. It transforms glucose-6-phosphate into 6-phosphoglucono-δ-lactone and NADP to NADPH that is subsequently used for the biosynthesis of fatty acids, making it a key enzyme involved in the synthesis of triglycerides from glucose (lipogenesis) [23,24]. As the PNPLA3 expression pattern follows that of G6PD2 it is suggested that PNPLA3 may also play an important role in hepatocyte lipogenesis. Importantly, other genes that had a comparable time-dependent expression profile as observed for PNPLA3 in hepatocytes in LDL receptor knockout mice and that are also significantly upregulated in livers of wild-type mice upon feeding a Western-type diet, i.e. SPOT14 and malic enzyme, are also established mediators of lipogenesis [24]. In agreement with a related function for PNPLA3 and G6PD2 in lipid metabolism, they respond similarly to changes in metabolic conditions. Moldes et al. have shown that PNPLA3 expression is significantly stimulated in adipose tissue upon infusion of insulin [25]. In parallel, Kastrouni et al. detected an enhanced activity of G6PD in adipose tissue upon administration of insulin, which is related to an increased transcription rate of the G6PD gene [26]. G6PD expression in liver and brain is significantly decreased upon fasting [27]. In accordance with the functional overlap between G6PD and PNPLA3, in the current study we observed that fasting also significantly diminished the expression level of PNPLA3 in adipose tissue and livers from Western-type diet-fed mice. Combined, these findings suggest that the mRNA expression level of PNPLA3 and G6PD is tightly controlled by the same upstream regulator. The lipogenic transcription factor sterol regulatory element binding protein 1 (SREBP-1) has been identified as a possible regulator of G6PD expression in liver [24,28]. It will therefore be interesting to study whether SREBP can also act as a transcriptional regulator of hepatic PNPLA3 expression.

In conclusion, we have shown that, in mice, the NAFLD-related gene PNPLA3 is expressed in hepatocytes within the liver and that its relative expression level is

highly influenced by the intra-hepatic lipid status. Furthermore, our studies suggest that the function of PNPLA3 in liver is related to lipogenesis.

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