PPAR-alpha dependent regulation of vanin-1 mediates hepatic lipid metabolism

Citation for published version (APA):


Document status and date:
Published: 01/08/2014

DOI:
10.1016/j.jhep.2014.04.013

Document Version:
Publisher's PDF, also known as Version of record

Document license:
Taverne

Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher’s website.
- The final author version and the galley proof are versions of the publication after peer review.
- The final published version features the final layout of the paper including the volume, issue and page numbers.

Link to publication

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the “Taverne” license above, please follow below link for the End User Agreement:
www.umlib.nl/taverne-license

Take down policy
If you believe that this document breaches copyright please contact us at:
repository@maastrichtuniversity.nl
providing details and we will investigate your claim.
Background & Aims: Peroxisome proliferator-activated receptor alpha (PPARα) is a key regulator of hepatic fat oxidation that serves as an energy source during starvation. Vanin-1 has been described as a putative PPARα target gene in liver, but its function in hepatic lipid metabolism is unknown.

Methods: We investigated the regulation of vanin-1, and total vanin activity, by PPARα in mice and humans. Furthermore, the function of vanin-1 in the development of hepatic steatosis in response to starvation was examined in Van1 deficient mice, and in rats treated with an inhibitor of vanin activity.

Results: Liver microarray analyses reveals that Vnn1 is the most prominently regulated gene after modulation of PPARα activity. In addition, activation of mouse PPARα regulates hepatic- and plasma vanin activity. In humans, consistent with regulation by PPARα, plasma vanin activity increases in all subjects after prolonged fasting, as well as after treatment with the PPARα agonist fenofibrate. In mice, absence of vanin-1 exacerbates the fasting-induced increase in hepatic triglyceride levels. Similarly, inhibition of vanin activity in rats induces accumulation of hepatic triglycerides upon fasting. Microarray analysis reveal that the absence of vanin-1 associates with gene sets involved in liver steatosis, and reduces pathways involved in oxidative stress and inflammation.

Conclusions: We show that hepatic vanin-1 is under extremely sensitive regulation by PPARα and that plasma vanin activity could serve as a readout of changes in PPARα activity in human subjects. In addition, our data propose a role for vanin-1 in regulation of hepatic TG levels during fasting.

© 2014 European Association for the Study of the Liver. Published by Elsevier B.V. All rights reserved.

Introduction

The liver is the central organ in the body’s response to starvation and switches from glucose oxidation and lipogenesis in the fed state, towards fatty acid oxidation and glucose production in the fasted state. Peroxisome proliferator-activated receptor alpha (PPARα) is a ligand-activated transcription factor that functions as the central regulator of hepatic lipid metabolism. Ligands for PPARα include endogenous fatty acids and various eicosanoids, as well as fibrates drugs used for the treatment of dyslipidemia [1]. Consistent with a crucial role of PPARα in the adaptive response to fasting, removal of the food provokes a severe metabolic phenotype in mice lacking PPARα that includes hypoglycemia, hyperthermia, hypoketonemia, and severe hepatic steatosis [2].

Over the past 20 years, multiple target genes of PPARα have been identified that play a role in different aspects of hepatic lipid metabolism. These genes encode proteins involved in different metabolic pathways, such as lipid metabolism, glucose metabolism, and inflammation. Understanding the regulation of these genes and their functions is crucial for the development of new therapeutic strategies for the treatment of metabolic diseases, including non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH).
metabolism, including uptake, binding, and oxidation of fatty acids, ketogenesis, lipid droplet biology, and lipoprotein assembly and transport [3]. In addition, PPARα has emerged as a crucial transcriptional regulator of numerous other metabolic processes including metabolism of glucose, cholesterol, bile acids, and amino acids [4–6]. Several different research groups have identified vanin-1 as an important PPARα-target gene [3,7–9] and showed that production of vanin-1 depends on PPARα activity [7]. However, despite the observations that vanin-1 is highly expressed in the liver as compared to other tissues [10,11], little is known about its actual role in hepatic lipid metabolism.

Vanin-1 is known as a glycosylphosphatidylidylinositol (GPI)-anchored enzyme with pantheinase activity catalyzing the hydrolysis of pantetheine into pantothenic acid (vitamin B5) and cysteamine. Vanin-1 is member of a larger vanin family, encoded by two mouse (Vnn1 and Vnn3) and three human (VNN1, VNN2, and VNN3) orthologous genes [12]. Functional studies indicated a role for vanins in inflammation, oxidative stress, and cell migration [10,13,14]. These effects of vanins are thought to be mediated via vanin-dependent cysteamine production [15]. By controlling the synthesis of pantothetic acid, the precursor for Coenzyme A, vanins may additionally play a role in fatty acid metabolism [15,16], although this remains to be established.

The aim of the current study was to characterize the regulation of vanin-1 and total vanin (pantheinase) activity by PPARα in mice and especially in human subjects, as well as the function of vanin-1 during fasting-induced hepatic lipid metabolism. We show that vanin activity closely reflects PPARα activity, and activation of PPARα induced by fasting or fibrate treatment enhanced plasma vanin activity in humans. Moreover, our data show that vanin activity functions as a modulator of hepatic lipid homeostasis in the fasted state.

Materials and methods

Animals

Mice and rats were housed under standard conditions and experiments were approved by the institutional ethical committee on animal care and experimentation of Wageningen University (Wageningen) or Radboud University Medical Center (Nijmegen). PPARα+/− and wild-type (WT) mice (Sv129 background; 2–3 month-old) were euthanized in the fed state vs. 6-, 12-, or 24 h of fasting, or after 5-days of 0.1% Wy14643 supplementation [17]. Vanin-1+/− (Vnn-1+/−) mice and WT littermates (C57Bl/6J background; 3–4-month old) were euthanized in the fed state vs. 24 h of fasting. Male Wistar rats (Harlan) weighing 150–200 g were given the pantheinase inhibitor RB6 [18] via drinking water (3 mg/ml) for 4 days and fasted during the final 24 h of the treatment.

Human intervention trails

The study protocols of the intervention trials have been published elsewhere [19–21], details are described in the Supplementary materials and methods. Protocols were approved by the Medical Ethical Committee of Maastricht University Medical Centre. All subjects gave their written informed consent before the start of the study.

Cell culture

Primary rat and mouse hepatocytes were isolated and cultured as described before [22]. After overnight adherence to the cell culture plate, hepatocytes were treated with various ligands for 24 h and RNA was isolated.

Vanin activity assay

Vanin (pantheinase) activity was determined in liver and plasma as described before [18,23] and in the Supplementary materials and methods. Shortly, liver homogenates and plasma samples were diluted and incubated with pantetheinate-7-amino-4-methylcoumarin (Pan-AMC; 10 μM final concentration). Fluorescence was measured using a luminescence spectrometer (LS55, Perkin Elmer).

Plasma and hepatic lipid analysis

Plasma levels of TG, total cholesterol (TC), glucose (Liquicolor, Human GmbH, Wiesbaden, Germany) and free fatty acids (FFA; NEFA-C WAKO chemicals, GmbH, Neuss Germany) were determined enzymatically according to manufacturer’s instructions. Lipids were extracted from livers as described in the Supplementary materials and methods.

RNA isolation, qPCR, and microarray analysis

Total RNA was isolated from livers and reversed transcribed for subsequent qPCR analysis. RNA from livers of fed and 24 h fasted Vnn-1+/− and WT mice was used for microarray analysis. Details of RNA isolation, primer sequences, and microarray analysis can be found in the Supplementary materials and methods section.

Statistical analysis

Data are presented as means ± SD. Statistical analyses were performed using Graphpad Prism 5.0. Statistical comparisons between 2-groups were calculated using a Student’s t test. Differences between 4-groups were tested with ANOVA followed by post hoc Bonferroni correction. For the human intervention studies, comparisons between two conditions (fed vs. fast; before vs. after fenofibrate treatment) were performed using the paired Student t test. p < 0.05 was regarded statistically significant.

Results

Hepatic vanin activity is highly regulated by PPARα

Microarray studies were performed on livers of WT mice treated with PPARα agonist WY14643 vs. control for 5-days [17], revealing that the gene encoding vanin-1 was the most significantly induced gene by WY14643 in WT mice (+90-fold; Fig. 1A). Moreover, microarray analysis of livers from 24 h fasted WT and PPARα−/− mice identified Vnn1 as the most strongly repressed gene in PPARα−/− mice in the fasted state (−712-fold; Fig. 1B). Similarly, vanin-3 mRNA was upregulated by WY14643 treatment (+1.8-fold; Fig. 1C), and downregulated in fasted PPARα−/− compared to WT mice (−9.5-fold; Fig. 1D), although to a lesser extent than vanin-1. The activation of Vnn1 expression by PPARα is a direct effect within hepatocytes, since WY14643 induced an increase of Vnn1 in primary hepatocytes of WT mice. Moreover, this increase was absent in primary hepatocytes of PPARα−/− mice (Supplementary Fig. 1A). Additionally, stimulation of human hepatocytes with the PPARα-agonist GW7647 led to a dose-dependent increase in vnn1 gene expression (Supplementary Fig. 2). Finally, stimulation of cells with PPARγ agonist Rosiglitazone (Supplementary Fig. 1B) or RXR agonists LG1069, 9cRA, and atRA (Supplementary Fig. 3) mildly induced Vnn1 expression as compared to PPARα agonists WY14643 or fenofibrate.

Using a pantheinase (vanin) activity assay that determines vanin-1 activity and potentially activity of vanin-3 [7,23], we found that PPARα not only regulates vanin-1 and vanin-3 mRNA expression, but also controls hepatic vanin enzymatic activity. Treatment of mice with WY14643 induced activation of vanin
activity (4-fold; p < 0.01; Fig. 1E). Similarly, fasting caused a time-dependent upregulation of hepatic vanin activity up to 8-fold (p < 0.001; Fig. 1F). Vanin activity was virtually undetectable in PPARα−/− mice, suggesting that hepatic vanin-1 activity, and potentially vanin-3 activity, is highly regulated by and fully dependent on PPARα activation. Subsequent analysis revealed plasma vanin activity was increased to a similar extent as hepatic vanin activity upon activation of PPARα using Wy14643 (5-fold; p < 0.001; Fig. 1G) and fasting (up to 3-fold; p < 0.001; Fig. 1H). The strong reduction in plasma vanin activity in PPARα−/− mice (Fig. 2C and D) has recently been reported [7] and shows that PPARα not only regulates hepatic vanin activity, but also strongly contributes to plasma vanin activity.

**Human plasma vanin activity increases upon PPARα activation**

Since plasma vanin activity is highly regulated by PPARα activity in mice, we evaluated whether plasma vanin activity may reflect PPARα activity in human subjects. First, plasma vanin activity was determined in subjects that were in fed state, or had fasted for 60 h. Fig. 2A shows that basal plasma vanin activity levels showed marked inter-individual variation. However, 60 h of fasting raised plasma vanin activity in each individual by at least 10%, with a mean increase of 33% (p < 0.001; Fig. 2A). To reveal whether plasma vanin activity in human subjects indeed reflects PPARα activation, vanin activity was determined in human subjects that were part of two fenofibrate intervention trials. First, vanin activity was increased significantly by fenofibrate treatment in all patients with type 2 diabetes after 3 months of treatment with the PPARα agonist fenofibrate (200 mg/day) (+23%; p < 0.01; Fig. 2B). Second, vanin activity was determined in obese subjects before and after 6 weeks of treatment with fenofibrate (200 mg/day) and placebo control. Again, vanin activity was increased upon fenofibrate treatment (+16%, p < 0.001; Fig. 2C), but not in placebo treated subjects, which actually showed a reduction in vanin activity over time (−10%, p < 0.01; Fig. 2D). These data clearly demonstrate that plasma vanin activity is
increased by PPAR\(\alpha\) activation in humans and suggest that plasma vanin activity could serve as a marker for (a change in) PPAR\(\alpha\) activity.

**Vanin-1 deficiency increases hepatic steatosis during starvation in mice**

PPAR\(\alpha\) is a critical regulator of hepatic lipid metabolism in response to starvation. The strong regulation of vanin-1 by PPAR\(\alpha\) therefore suggests a potential role of vanin-1 in hepatic lipid metabolism. To explore whether vanin-1 affects the development of fasting-induced hepatic steatosis, hepatic lipid accumulation was evaluated in WT and \(Vnn1^{-/-}\) mice. Oil-Red-O staining showed that vanin-1 deficiency caused an increased accumulation of lipids in 24 h fasted livers (Fig. 3A), which was mirrored by increased hepatic TG levels (Fig. 3B). In contrast, vanin-1 deficiency did not affect fed, or 24 h fasted plasma levels of TG (Fig. 3D), free fatty acids (FFA; Fig. 3E) or glucose levels (Supplementary Fig. 4).

**Inhibition of total vanin activity increases hepatic steatosis during starvation in rats**

We recently developed a novel pantetheine analog (RR6) that acts as a selective competitive vanin inhibitor at nanomolar concentration. This compound showed excellent pharmacodynamics in rats, but less so in mice, and caused a complete inhibition of plasma vanin activity [18]. We evaluated whether short-term inhibition of total vanin activity in rats would similarly induce fasting-induced hepatic steatosis as vanin-1 deficiency in mice. Therefore, rats were treated with RR6 in the drinking water (50 mg/kg) for 4 days and fasted for 24 h. Consistent with the effects of vanin-1 deficiency in mice, inhibition of vanin activity by RR6 treatment increased hepatic steatosis in 24 h fasted rats as determined by an increased liver weight (6.41 ± 0.18 vs. 6.88 ± 0.22; \(p < 0.001\)), Oil-Red-O staining (Fig. 3F) and TG concentrations (Fig. 3G).

**Hepatic pathways affected by vanin-1 deficiency during starvation**

To further identify the function of vanin-1 in the liver during the fasting response, we evaluated relevant pathways downstream of vanin-1 using microarray analyses in livers of 24 h fasted \(Vnn1^{-/-}\) and WT mice. These analysis revealed that 857 genes were differentially expressed (\(p < 0.05\)) in \(Vnn1^{-/-}\) mice, of which 320 were repressed and 437 increased. Since vanin-1 appears such an important target gene of PPAR\(\alpha\), and both PPAR\(\alpha\)-/- and \(Vnn1^{-/-}\) mice develop hepatic steatosis in response to fasting, we compared the microarray of \(Vnn1^{-/-}\) vs. WT mice with the microarray of PPAR\(\alpha\)-/- vs. WT mice after 24 h of fasting. Surprisingly, although 1077 genes were found to depend on PPAR\(\alpha\) and 978 genes on vanin-1, only 71 of these genes were overlapping. Indeed, the top 150 most up- and downregulated genes in \(Vnn1^{-/-}\) mice did show little overlap with their change in PPAR\(\alpha\)-/- mice (Supplementary Table 1A and B).

To investigate the function of vanin-1 in the hepatic fasting response, we evaluated pathways specifically influenced by vanin-1 deficiency. Therefore, a contrast comprising all 4 experimental groups (WT-fed compared to WT-fasted vs. \(Vnn1^{-/-}\)-fed compared to \(Vnn1^{-/-}\)-fasted) was tested for significance in the linear model to identify a robust set of genes that responded differentially to 24 h of fasting in WT and \(Vnn1^{-/-}\) mice (Fig. 4A). Ingenuity analysis revealed that gene sets affected by vanin-1 deficiency were most significantly linked to an increase in hepatic steatosis, in accordance with the observed increase in hepatic TG accumulation in \(Vnn1^{-/-}\) mice in response to fasting (Fig. 4B). In addition, gene sets involved in several other diseases or clinical pathology endpoints were significantly affected in \(Vnn1^{-/-}\) mice, such as glutathione depletion, TR/RXR activation, liver hepatitis, and oxidative stress, suggesting an impact of vanin-1 on these hepatic pathologies. For better understanding of signaling pathways that may be responsible for the changes in hepatic gene expression upon vanin-1 deficiency during the fasting response, Ingenuity upstream regulator analysis was used. These analysis predicted that transcription factors known to regulate gene sets involved in (lipid) metabolism (PPAR\(\alpha\), PPAR\(\gamma\), and KLF15), inflammation/immunity, cell differentiation and hematopoiesis (STAT3, SPI, and CBF), and oxidative stress (XBP1) were significantly changed in livers of \(Vnn1^{-/-}\) mice (Fig. 4C). Specifically, PPAR\(\alpha\), PPAR\(\gamma\), CBF, and KLF15 were predicted to be activated, while XBP1, SPI, and STAT3 were predicted...
to be inhibited. A complete list of genes affected by vanin-1 deficiency and under control of these transcription factors can be found in Fig. 4D.

Discussion

Since 2002, vanin-1 has emerged as a gene that is robustly activated in liver by PPARα agonists, such as Wy14643, fenofibrate, and clofibrate [3,8,9], and functional PPRE sites have recently been identified in the Vnn1 promoter [7]. Despite these observations, the regulation of hepatic vanin activity by PPARα and the functional implications of PPARα-dependent regulation of vanin-1 have never been evaluated. Our data reveal that vanin-1 is one of the most prominent PPARα-dependent regulated genes. We also show that vanin-3 is regulated by PPARα, although to a lesser extent, and that total hepatic vanin-1 activity, and possibly activity of vanin-3, is under strict control of PPARα. The observation that vanin-1 is membrane-bound via a GPI anchor, while vanin-3 is not, has led to the hypothesis that vanin-3 can be secreted and determines plasma vanin activity [12]. However, it has recently been revealed that vanin-1 can be secreted as a soluble Vnn1 isoform [7] and is expressed on microparticles that are secreted by hepatocytes into the circulation [24]. In humans, our data show a high inter-individual variation for plasma vanin activity, but within each subject plasma vanin activity appeared very stable over time. The VNN1 promoter contains methylation-sensitive SNPs, which makes the gene sensitive for allele-specific DNA methylation [25] and may allow for a large (epi)genetic component that determines vanin activity within a subject. We show that plasma vanin activity increased in all subjects that were fasted, or treated with fibrate drugs. Since fasting is known to increase hepatic steatosis [2], while fibrates may reduce hepatic steatosis [26], our data suggest that plasma vanin...
activity levels reflect PPARα activation rather than hepatic steato-
sis. Furthermore, the stable vanin activity levels over time imply
that plasma vanin activity could possibly be used as a marker for
(a change in) PPARα activation within a given individual. It must
be noted that in human blood, the expression of VNN2 is much
higher compared to VNN1 [10], which may explain the relatively
small increase in total plasma vanin activity in human subjects
upon fasting or specific PPARα activation, compared to mice.
Therefore, also based on our observation that the regulation of
vanin-1 by PPARα is much stronger than the regulation of
vanin-3, we suggest that an assay to specifically determine
plasma vanin-1 may even be a more sensitive read-out of PPARα
activation. In addition to the role of PPARα, levels of PPARγ are
known to be increased in a steatotic liver [27]. Noticeably,
absence of PPARα leads to an upregulation of hepatic PPARγ lev-
els in the liver upon a HFD-intervention [28]. Activation of PPARγ
by TZDs is known to reduce the levels of hepatic steatosis [27].
Part of this effect may be accomplished by direct regulation of
various hepatic PPARα-target genes by PPARγ during the pres-
ence of steatosis [28]. Hence, one may hypothesize that during
specific conditions, e.g., the presence of steatosis, enhanced hepa-
tic levels of PPARγ may regulate vnn1 expression as well.

Although vanin-1 is known to recycle pantothenic acid and
has been described as a PPARα target gene, the role of vanin
activity in hepatic metabolic processes has so far never been eval-
uated. Recent studies show strongly increased vanin-1 expression
in murine steatotic livers [29,30], prompting the authors to
hypothesize a causal role for vanin-1 in the progression of steato-
sis [30]. On the contrary, our current data show that the presence
of vanin-1 does actually protect against the development of ste-
atosis, induced by prolonged starvation. This suggests that the
previously observed relation between steatosis and vanin-1
expression may rather be explained by enhanced activation of
PPARα and subsequent transcription of its target genes in steatot-
ic livers [28].

In the current study, we use prolonged fasting to study the
development of steatosis and show that vanin-1 expression pre-
vents excessive accumulation of TGs in the liver. Whether vanin-
1 may similarly protect against fatty liver development induced
by HFD-feeding or obesity needs to be evaluated in future stud-
ies. Cysteamine, which is generated by vanin activity, is currently
under evaluation as a treatment for non-alcoholic fatty liver dis-
ease (NAFLD) in patients [31]. Since vanin-1 is the main provider
of cysteamine to tissues, and Vnn1−/− mice are known to lack
detectable amounts of cysteamine in their tissues [11], our data
would suggest that (1) the protection of vanin-1 against steatosis
may at least partly be mediated via cysteamine, and (2) activation
of vanin activity could be an alternative for cysteamine treatment
in patients with NAFLD.

Our data show no effect of vanin-1 deficiency on plasma TG,
cholesterol, FFA or glucose levels in mice. In contrast, we recently
found that inhibition of vanin activity in fasted rats increased
plasma FFA and reduced plasma cholesterol levels [18]. Since
inhibition of total vanin activity in rats also reduces vanin-3
activity, this may point towards a role of vanin-3 in the mobiliza-
tion and/or conversion of lipids. No compensatory upregulation
of hepatic vanin-3 mRNA levels in Vnn1−/− mice was observed
(Supplementary Fig. 6), but the considerable level of vanin activi-
ity in Vnn1−/− mice, as detected by Rommelaere et al. [7], sug-
gests that residual vanin-3 activity could possibly explain the
relatively mild phenotype of the Vnn1−/− mice with respect to
plasma lipid levels. Alternatively, differences between both
experiments may be explained by species differences or compen-
satory mechanisms due to life-long deficiency of vanin-1 in mice
vs. short-term inhibition of vanin activity in rats. Pantethine, the
stable disulfide of pantetheine and substrate for vanin activity,
is known as a natural compound with hypolipidemic effects [32],
which may be mediated by increased cysteamine levels [33].
Future studies in hyperlipidemic mouse models will be needed
to further elucidate whether vanin-1 or total vanin activity can
directly affect plasma lipoprotein metabolism.

We show that absence of vanin-1 in mice and inhibition of
vanin activity in rats both lead to increased TG hepatic accumu-
lation in response to starvation. Microarray analysis reveals that
this occurs despite activation of the transcriptional regulators
PPARα and KLF-15, and many of its target genes known to be
involved in FA oxidation (i.e., Abcd2, Acadm, Acot1, Acot2, Acsl5,
and Ehhadh). Besides PPARα, our analysis predicts increased activa-
tion of hepatic PPARγ activity in absence of vanin-1. Of course,
both PPARα recognizes similar DNA response elements and activa-
tion in fasted Vnn1−/− mice may result from increased availability
of PPAR ligands (i.e., fatty acids) during steatosis. In addition,
vanin-1 has shown to antagonize PPARγ activity, at least in epi-
thelial cells [13] and a similar association in liver may also under-
lie the increased hepatic PPARγ activity in absence of vanin-1.

Several genes under control of XBP-1, a main regulator of ox-
idative stress, were downregulated in Vnn1−/− mice. Vanin-1 defi-
ciency is known to enhance hepatic glutathione levels (secondary
to the lack of tissue cysteamine [34]), which is an antioxidant that
protects the tissue against reactive oxygen species. Our results are
in line with the previous observation of reduced activation of
pathways related to oxidative stress in livers of Vnn1−/− mice
[35]. Besides oxidative stress, vanin-1 deficiency affected multiple
genes involved in inflammation/immunity, predicting inhibition
of the central inflammatory transcriptional regulator STAT3. A
role for vanin-1 in immune responses has previously been re-
ported, as Vnn1−/− mice display reduced immune cell activation
during chronic intestinal inflammation, as well as a reduced pro-
imflammatory macrophage phenotype in response to C. Burnetii
[13,36]. Our data support a role for vanin-1 in the regulation of
inflammatory pathways in liver and imply that gene sets affected
by vanin-1 deficiency are involved in the development of steato-
hepatitis. Future studies in specific mouse models for non-alco-
holic steatohepatitis (NASH) development may reveal whether
vanin-1 indeed plays a role in the development of NASH.

In summary, we show that PPARα is the regulator of hepatic
vanin-1 expression and vanin activity. In addition, PPARα activity
strongly modulates plasma vanin activity, which therefore could
be used as a marker for (a change in) PPARα activity in humans.
Finally, we show that vanin activity plays an important role in the
prevention of steatosis development in response to fasting, and
actions of vanin-1 in liver are linked to changes in inflammation
and oxidative stress. This suggests that therapeutic strategies
designed to enhance vanin activity may be beneficial for the

treatment of hepatic steatosis or related diseases.

Financial support

This work was supported by a pre-seed grant of The Netherlands
Genomics Initiative, grant nr 93611013. R.S. was supported by a
grant from the Dutch Diabetes Research Foundation.
Research Article

Conflict of interest

The authors who have taken part in this study declare that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Acknowledgement

The authors thank Ivonne M.J.J. van Vlijmen-Willems for excellent technical assistance and Pedro H.H. Hermkens for his contribution to the development of RR6.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhep.2014.04.013.

References