Glycogen storage disease type 1a is associated with disturbed vitamin A metabolism and elevated serum retinol levels

Saeed, Ali; Hoogerland, Joanne A; Wessel, Hanna; Heegsma, Janette; Derks, Terry G J; Veer, Eveline; Mithieux, Gilles; Rajas, Fabienne; Oosterveer, Maaike H; Faber, Klaas Nico

Published in:
Human Molecular Genetics

DOI:
10.1093/hmg/ddz283

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

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

Publication date:
2020

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 15-05-2021
Glycogen storage disease type 1a is associated with disturbed vitamin A metabolism and elevated serum retinol levels

Ali Saeed1,2,* , Joanne A. Hoogerland3, Hanna Wessel1, Janette Heegsma1,4, Terry G.J. Derks5, Eveline van der Veer4, Gilles Mithieux6,7,8, Fabienne Rajas6,7,8, Maaike H. Oosterveer3,§ and Klaas Nico Faber1,4,§

1Department of Gastroenterology and Hepatology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands, 2Institute of Molecular Biology and Biotechnology, Bahauddin Zakariya University Multan, Pakistan, 3Department of Pediatrics, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands, 4Laboratory Medicine, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands, 5Section of Metabolic Diseases, Beatrix Children’s Hospital, Center for Liver Digestive, and Metabolic Diseases, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands, 6Institut National de la Santé et de la Recherche Médicale, U1213, Lyon F-69008, 7Université de Lyon, Lyon F-69008, France and 8Université Lyon 1, Villeurbanne F-69622, France

*To whom correspondence should be addressed at: Ali Saeed, Dept. Hepatology & Gastroenterology, University Medical Center Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands; Tel.: +31(0)503619951, +31(0)503612364; Fax: +31(0)503619306; Email: a.saeed@umcg.nl, k.n.faber@umcg.nl

Abstract

Glycogen storage disease type 1a (GSD 1a) is an inborn error of metabolism caused by mutations in the G6PC gene, encoding the catalytic subunit of glucose-6-phosphatase. Early symptoms include severe fasting intolerance, failure to thrive and hepatomegaly, biochemically associated with nonketotic hypoglycemia, fasting hyperlactidemia, hyperuricemia and hyperlipidemia. Dietary management is the cornerstone of treatment aiming at maintaining euglycemia, prevention of secondary metabolic perturbations and long-term complications, including liver (hepatocellular adenomas and carcinomas), kidney and bone disease (hypovitaminosis D and osteoporosis). As impaired vitamin A homeostasis also associates with similar symptoms and is coordinated by the liver, we here analysed whether vitamin A metabolism is affected in GSD 1a patients and liver-specific G6pc−/− knock-out mice. Serum levels of retinol and retinol binding protein...
G6pc deficiency reduces hepatic retinol levels, while retinyl palmitate is unchanged

In contrast to plasma retinol, hepatic retinol concentrations (6.59 ± 0.66 versus 11.57 ± 0.72 μg/g liver) as well as the total hepatic pool of retinol were significantly lower in L-G6pc−/− mice.
Figure 1. Serum retinol levels are increased in GSD Ia patients. Serum retinol levels were determined in GSD Ia patients (n = 22) and age- and sex-matched controls (n = 20) (A). No correlation was observed between age and sex (male: ▲ or Female: ○) in GSD Ia patients (grey symbols) nor in healthy controls (black symbols) (B).

Figure 2. G6pc deficiency in mice increases plasma retinol and reduces hepatic retinol, while retinyl palmitate remains unchanged. Ten days after tamoxifen-induced deletion of the G6pc gene in hepatocytes, L-G6pc−/− and control mice (n = 6 each group) were sacrificed and analyzed for (A) plasma retinol levels, (B) liver retinol concentrations, (C) total liver retinol pool, (D) retinol concentrations in WAT, (E) liver retinyl palmitate concentrations, (F) total liver retinyl palmitate pool and (G) retinyl palmitate concentrations in WAT.
enzyme involved in retinol esterification and lipid droplet levels sharply increased in L-
Hepatic mRNA levels of both but also for DGAT1 no clear increase in protein was detected
of active (phosphorylated) HSL (pHSL) were high in all L-
levels were unchanged compared to control mice. Still, levels
A metabolism is affected in the absence of G6pc in WAT (Fig. 2G). These results indicate that hepatic vitamin
mice, while this was variable in the control mice (Fig. 3C). Hepatic
Hepatic mRNA levels of retinol promotes its own release from the liver to the circulation
convert retinol to retinoic acids (ADH and RALDH). Moreover, enzymes that catalyze the esterification of retinol (predominantly LRAT and to a lesser extent by DGAT1), 2) enzymes that hydrolyze retinyl esters (ATGL/PNPLA2 and PNPLA3) and 3) enzymes that A-hydrolyzing enzymes in L-
levels. On the other hand, hepatic retinyl palmitate levels were
in L-
Dgat1 and Dgat2 0.02 μg/g WAT, respectively) (Fig. 2D).
RBP4 protein levels were analyzed next. RBP4 protein levels in livers and WAT of L-G6pc−/− mice were similar to control mice (Fig. 4A and B, notably, hepatic RBP4 sometimes appears as a double band in western blot analyses, as observed by others (18–20), but with unknown cause). In contrast, serum RBP4 levels in L-G6pc−/− mice were clearly elevated ~3-fold compared to control mice (Fig. 4C). Sera of GSD Ia patients also contained significantly elevated levels of RBP4 compared to age- and sex-matched healthy control (Fig. 4D). Serum retinol levels were largely in line with serum RBP4 levels in healthy controls, while such association was less evident in GSD Ia patients (Fig. 4D).

G6pc deficiency suppresses the expression of retinoic acid-responsive Cyp26a1
Next, we aimed to analyze whether G6pc deficiency may affect the production of retinoic acids in the liver. Hepatic mRNA levels of Hsd17b13, a recently identified retinol dehydrogenase (21) and all 4 retinaldehyde dehydrogenases (Raldh1–4) were hardly affected by the absence of Gsd1a in mice (Fig. 5). Only a small significant increase in Raldh2 and decrease in Raldh4 were observed in G6pc deficient mice. However, mRNA levels of the highly retinoic acid-sensitive Cyp26a1 were strongly (89%) decreased (Fig. 5) compared to control mice. Unfortunately, specific antibodies against mouse Cyp26a1 were not available to confirm the effect of G6pc deficiency at the CYP26A1 protein level.

Liver-specific G6pc deficiency in mice does not cause hepatic inflammation, nor fibrosis
Finally, we analyzed whether abnormal vitamin A metabolism in L-G6pc−/− mice leads to hepatic inflammation and/or fibrosis. Hepatic mRNA levels of markers of inflammation, e.g. Csf1, Tnfα, Nos2, Ccl2, Il6 were reduced in L-G6pc−/− mice as compared to control mice (Fig. 6A). A similar suppression of hepatic mRNA levels of markers of fibrosis, e.g. Coll1a1, Acta2, Tgf-β and Timp1, was observed in L-G6pc−/− mice as compared to control mice (Fig. 6B).

Taken together, our data show that G6pc deficiency leads to elevated serum retinol and RBP4 levels in humans and in mice. In contrast, hepatic retinol levels are reduced, most probably because of enhanced mobilization of retinol from retinyl ester stores and subsequent RBP4-mediated release from hepatocytes.

Discussion
This study shows for the first time that G6pc deficiency in human and mouse is associated with elevated levels of circulating retinol, concomitantly with an increase of circulating RBP4 levels. On the contrary, retinol levels are reduced in the liver and WAT. Tissue retinyl palmitate levels are not changed in liver-specific G6pc deficient mice, at least not within 10 days after ablation of the gene in hepatocytes. Hepatic expression profiling

Table I. GSD 1a patient general characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (F/M)</td>
<td>14/8</td>
</tr>
<tr>
<td>Age (GSD 1a patients)</td>
<td>26.1 ± 10.1</td>
</tr>
<tr>
<td>Vitamin A (serum retinol, normal 1.2–2.7 μmol/L)</td>
<td>3.0 ± 1.0</td>
</tr>
<tr>
<td>Hypervitaminosis A: serum retinol &gt; 2.7 μmol/L (no/yes)</td>
<td>12/10</td>
</tr>
<tr>
<td>Vitamin D (serum 25(OH)D; sufficient &gt; 50 nmol/L)</td>
<td>64.6 ± 27.4</td>
</tr>
<tr>
<td>Hypervitaminosis D: serum 25(OH)D &lt; 30 nmol/L (no/yes)</td>
<td>19/3</td>
</tr>
<tr>
<td>Hepatic steatosis (no/mild/severe/unknown)</td>
<td>0/12/9/1</td>
</tr>
<tr>
<td>Serum Triglycerides (normal &lt; 1.7 mmol/L)</td>
<td>12.7 ± 10.0</td>
</tr>
<tr>
<td>Serum Total Cholesterol (normal &lt; 5.2 mmol/L)</td>
<td>7.5 ± 2.8</td>
</tr>
<tr>
<td>Osteopenia/osteoporosis (no/yes)</td>
<td>15/7</td>
</tr>
<tr>
<td>Kidney disease/proteinuria (normal &lt; 1–2 g/24 h)</td>
<td>0.4 ± 0.7</td>
</tr>
<tr>
<td>Proteinuria (no/yes)</td>
<td>19/3</td>
</tr>
<tr>
<td>AST (normal &lt; 10–40 U/L)</td>
<td>55.8 ± 34.1</td>
</tr>
<tr>
<td>ALT (normal &lt; 7–56 U/L)</td>
<td>42.3 ± 25.8</td>
</tr>
<tr>
<td>Total bilirubin (normal &lt; 20.5 μmol/L)</td>
<td>5.9 ± 1.0</td>
</tr>
<tr>
<td>yGT (normal &lt; 48 U/L)</td>
<td>53.9 ± 33.1</td>
</tr>
</tbody>
</table>
suggests that metabolism of retinyl ester to retinol may promote the secretion of retinol-bound RBP4 to the circulation. 

Clinical management of GSD Ia is primarily aimed at maintaining steady circulating glucose levels by strictly controlled intake of dietary carbohydrates during day and night. Because of the impaired ability to produce glucose from glucose-6-phosphate (G6P), cellular glycogen content increases, in conjunction with elevated triglyceride storage leading to steatosis. GSD Ia is associated with hypovitaminosis D, which has been linked to the development of osteoporosis in these patients (3, 22). As fatty liver disease is associated with hypovitaminosis A (9, 10), we were interested whether GSD Ia patients may also show aberrant circulating vitamin A levels. To our surprise, we found that circulating retinol levels, as well as RBP4 levels, were significantly elevated, instead of being reduced, in GSD Ia patients and L-G6pc−/− mice. Apart from cases of excessive dietary vitamin A intake, elevated circulating retinol levels are a rare phenomenon. Serum retinol levels are, however, a sensitive measure of sharp fluctuations in dietary intake of vitamin A as early work showed that daily retinyl palmitate supplementation in a range of 0 to 36000 IU (=0–12 times the RDA [Recommended Daily Allowance]) increased serum retinol levels by only 2% (=0.04 μM) per 10000 IU vitamin A (23). On average, we observed a 28% increase in serum retinol levels in GSD Ia patients compared to healthy controls, with 45% of the patients showing levels above the normal range. Thus, even though GSD Ia patients are often prescribed multivitamin supplements, it is unlikely to cause the increased circulating retinol levels in these patients. Notably, the incidence of hypervitaminosis A in GSD Ia patients in our study was higher than that of hypovitaminosis D. Impaired kidney function, a condition associated with GSD Ia (24), may also lead to elevated serum retinol levels (25, 26). In our study GSD Ia patients did not show severe kidney disease, protein ureia was elevated only in three GSD Ia patients. Moreover, the liver-specific G6pc−/− mice also showed markedly elevated retinol levels, while these animal do not develop any kidney abnormalities, not even 15 months after ablation of the gene in the liver (15). Thus, hepatic vitamin A metabolism likely contributes to the elevated serum levels of retinol and RBP4 in GSD Ia. In contrast to blood, tissue retinol levels in the liver and WAT were reduced in L-G6pc−/− mice compared to controls, while retinyl palmitate, the main storage form of vitamin A in the liver, was not changed. Indeed, protein levels of LRAT, the main hepatic enzyme catalyzing esterification of retinol, were
Figure 4. Serum RBP4 levels are elevated in L-G6pc−/− mice and GSD Ia patients. (A–C) Ten days after tamoxifen-induced deletion of the G6pc gene in hepatocytes, L-G6pc−/− and control mice (n = 4 each group) were sacrificed and analyzed by Western blotting for RBP4 protein levels in (A) liver, (B) WAT and (C) plasma. (D) Similarly, RBP4 protein levels were analyzed in sera of age- and sex-matched healthy controls and GSD Ia patients (n = 6 each group). β-ACTIN and Ponceau S stainings are included as loading controls. Protein signal intensities were quantified and are shown to the right.

normal in L-G6pc−/− mice. Remarkably, though, Lrat mRNA levels were significantly reduced in L-G6pc−/− mice. It remains to be determined why LRAT protein levels do not follow mRNA levels. One possibility is that LRAT is a stable protein and that it takes >10 days after G6pc gene deletion to observe a significant effect of LRAT protein. Expression profiling revealed an induction of retinyl ester-hydrolyzing activity and a reduction of retinoic acid catabolism. De novo lipogenesis is increased in G6PC deficiency and activation of the carbohydrate-response-element-binding protein (ChREBP) likely contributes to this phenomenon (27, 28). Hepatic Chreb mRNA levels were indeed increased in L-G6pc−/− mice compared to controls (Supplementary Fig. S3). Notably, PNPLA3 expression is controlled by ChREBP (29) and NAFLD patients carrying the PNPLA3-145M variant show reduced circulating retinol levels and enhanced hepatic retinyl palmitate contents (30, 31). Thus, ChREBP-mediated induction of PNPLA3, together with elevated levels of ATGL and possibly pHSL, may contribute to the enhanced conversion of hepatic retinyl esters to retinol. The strong reduction in Cyp26a1 mRNA levels in L-G6pc−/− mice primarily hints to reduced production of retinoic acids, as they are potent inducers of Cyp26a1 transcription (5). Enhanced hepatic retinyl ester-hydrolysis and reduced retinoic acid catabolism are theoretically expected to lead to accumulation of retinol. However, hepatic retinol levels were actually reduced, while an increase was observed circulating retinol and RBP4 in GDS Ia patients and L-G6pc−/− mice. Enhanced retinol production in the liver promotes its own release from hepatocytes, bound to RBP4, to the circulation...
Gene expression of retinoic acid-responsive Cyp26a1 is strongly suppressed in the livers of L-G6pc−/− mice. Ten days after tamoxifen-induced deletion of the G6pc gene in hepatocytes, L-G6pc−/− and control mice (n = 6 each group) were sacrificed and analyzed by Q-PCR for hepatic mRNA levels of genes involved in the conversion of retinol to retinoic acids (Hsd1713, Raldh1, Raldh2, Raldh3, Raldh4) or catabolism of retinoic acids (Cyp26a1). Transcriptional regulation of Cyp26a1 is highly responsive to retinoic acids.

and contributes to elevated plasma levels of retinol and RBP4 (32–34). Thus, we hypothesize that an enhanced production of retinol pushes itself out of the liver and contributes to the elevated serum levels of RBP4 and retinol found in GSD Ia patients and L-G6pc−/− mice. The reduced hepatic retinol levels may be an early response to the induced deletion of the G6pc gene in this mouse model (10 days gene deletion). Future studies may include L-G6pc mice after long-term gene deletion (15) to determine whether the low hepatic retinol levels persist.

Normal serum retinol levels range from 1.2 to 2.7 μmol/L in the Dutch population (35), thus the average level detected in GSD Ia patients is just above the higher end. This will not cause acute toxicity, but chronically elevated retinol in circulation may contribute to clinical symptoms associated with GSD Ia, especially in GSD Ia patients with levels above 4–5 μmol/L. Hypervitaminosis A promotes osteoclast formation, skeleton fragility and osteoporosis due to decreased cortical bone mass and bone formation (36). Hypervitaminosis A-associated osteoporosis may already occur at twice the recommended daily allowances (RDA) of vitamin A, which will not even lead to elevated serum retinol levels (36, 37). Osteoporosis is also observed in GSD Ia patients and typically linked to hypovitaminosis D, which is analyzed in routine surveillance (1, 3). Abnormal vitamin D and A levels may synergize in aberrant bone homeostasis and may need to be monitored both to prevent this complication in GSD Ia patients. In fact, there are quite a few additional commonalities in symptoms in GSD Ia and hypervitaminosis A, like impaired growth, dizziness and irritability (38–41). Though these symptoms likely primarily result of poorly controlled blood glucose levels, it could be that chronically elevated serum retinol levels may also contribute to such symptoms. Hypervitaminosis A causes hepatic steatosis in rats (42), while vitamin A deficiency reduces hepatic lipid accumulation (43).
Figure 6. Hepatic G6pc-deficiency suppresses basal levels of inflammation and fibrosis in mice. Ten days after tamoxifen-induced deletion of the G6pc gene in hepatocytes, L-G6pc−/− and control mice (n = 6 each group) were sacrificed and analyzed by Q-PCR for hepatic mRNA levels of markers of (A) inflammation (Cd68, Tnfα, Nos2, Ccl2 and Il6) or (B) fibrosis (Col1a1, Acta2, Tgf-β and Timp3). Both markers of inflammatory and fibrosis were not increased in L-G6pc−/− livers. Instead, a significant reduction was observed for hepatic expression of Cd68, Tnfα and Timp1 in L-G6pc−/− mice compared to controls, while all other markers showed similar trends.

Increased circulating retinol has been found to reduce the risk for hepatocellular carcinoma (HCC) (44, 45). GSD Ia patients are actually at risk for the development of hepatic adenomas that may progress to HCC. The effects on retinol and tumor development in GSD Ia therefore appear counterintuitive. However, hepatic retinol levels are reduced and may promote adenoma development specifically in the liver. Here also, it is of interest what the long-term effect is of the absence of hepatic G6PC activity on vitamin A metabolism in the liver (15). It may very well be that hepatic vitamin A stores get depleted in the long-term and predispose to liver tumor development in GSD Ia. One older patient indeed showed very low-circulating retinol levels (Fig. 1B), which suggests extremely low hepatic vitamin A stores.

The hepatic pathologies and disturbed vitamin A metabolism did not induce an inflammatory or fibrotic response in livers of L-G6pc−/− mice. In fact, all tested markers for hepatic inflammation and fibrosis were suppressed to greater or lesser extent in L-G6pc−/− mice. This may also be a result of changes in hepatic retinol metabolism as vitamin A metabolites are potent controllers of hepatic inflammation and fibrosis (46, 47).

Management of hypervitaminosis A is currently limited to controlling the dietary intake of vitamin A. Given the ‘metabolic origin’ of hypervitaminosis A in GSD Ia patients, it is important to monitor circulating retinol levels and refrain from vitamin A supplementation when plasma retinol levels are close to or above normal levels. Future studies need to establish the course of vitamin A levels in the absence of G6PC activity in patients and/or mice in order to determine the necessity of management of vitamin A levels in early and late stages of disease development.

Taken together, our study shows that vitamin A metabolism is disturbed in the absence of G6PC activity in mice and GSD Ia patients, resulting in elevated circulating retinol levels. This condition may contribute to various symptoms of GSD Ia, in particular, osteoporosis, which has been linked to hypovitaminosis D in these patients so far. Vitamin A is thus a second vitamin that needs attention in the management of GSD Ia.

Materials and Methods

Patients

The study was performed in accordance with the Declaration of Helsinki and the institutional rules for studying biological rest materials. Retinol analysis was performed in serum samples from 22 genetically confirmed GSD Ia patients (male n = 9 and female n = 13), who visited the Beatrix Children’s Hospital, UMCG. Samples were randomly obtained during the day. The controls included 20 healthy, age- and sex-matched control subjects (male n = 8 and female n = 12) aged between 10 and 45 years.

Animal model

The tamoxifen-inducible hepatocyte-specific G6pc-knock-out (L-G6pc−/−) mice were used in this study as a model of the
liver-specific pathologies of GSD Ia (15). Briefly, G6pc recombinant mice with two loxp sites flank G6pc exon 3 (B.G6pcloxlox/w) were crossed with transgenic mice expressing the tamoxifen-inducible recombinase (CreERT2) under control of the serum albumin promoter to confer hepatocyte-specific expression in B6.SA(creERT2)w mutant mice. Male B6.G6pcex3lox/lox;SA(creERT2)w mice (8–12 weeks old) were injected intraperitoneally once daily with 100 μl tamoxifen (10 mg/ml, Sigma-Aldrich) for five consecutive days to obtain L-G6pc−/+ mice. All mice were sacrificed 10 days after the last tamoxifen injection. Animal experiments were performed after approval of all procedures by the Institutional Animal Care and Use Committee, University of Groningen, the Netherlands. All animals (n = 6–7) were kept in an environment with alternating dark and light cycles (07:00 p.m.–07:00 a.m.), with controlled temperature (20–24°C) and relative humidity (55% ± 15%) and ad libitum access to food and water. Prior to sacrifice, the mice were fasted from 10:00 p.m. until 08:00 a.m. the next day. Tissue and plasma samples were collected for further analysis.

Standard protocols for histochemistry and quantification of serum/tissue cholesterol, triglyceride, retinol, retinyl esters, mRNA (quantitative reverse transcription-polymerase chain reaction [qRT-PCR]), protein (Western blotting) and the corresponding statistical analyses are presented in Supplementary Material and Methods.

**Supplementary Material**

Supplementary Material is available at HMG online.

**Acknowledgements**

The authors are thankful to Trijnie Bos, Brenda Hijmans and Aycha Bleeker for providing the technical assistance in the execution of animal experiments in mice (L-G6pc−/+).

**Conflict of Interest Statement:** The authors certify that they have no affiliations with or involvement in any organization or entity with any financial or non-financial interest in the subject matter or materials described in this manuscript.

**Funding**

University of Groningen (Rosalind Franklin Fellowship to M.H.O.).

**References**


6 phosphatase mimics glycogen storage disease type 1a including development of multiple adenomas. J. Hepatol., 54, 529–537.


