The carbohydrate response element binding protein (ChREBP) and not the liver X receptor α (LXRα) mediates elevated hepatic lipogenic gene expression in a mouse model of glycogen storage disease type 1.

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Short title: Regulation of lipogenic genes in a GSD-1 mouse model

Aldo Grefhorst[*,1,2], Marijke Schreurs[†,1], Maaike H. Oosterveer[*], Victor A. Cortés[‡], Rick Havinga[*], Andreas W. Herling[§], Dirk-Jan Reijngoud[‖], Albert K. Groen[*,‖], and Folkert Kuipers[*,‖]

[*] Department of Pediatrics, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands.
[†] Molecular Genetics Section, Department of Pathology and Medical Biology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands.
[‡] Department of Molecular Genetics, University of Texas Southwestern Medical Center at Dallas, Dallas, TX, USA
[§] Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany
[‖] Department of Laboratory Medicine, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands.

[1] These authors contributed equally to this work.
2 To whom correspondence should be addressed (a.grefhorst@med.umcg.nl)

Correspondence address: Aldo Grefhorst, Department of Pediatrics, Center for Liver Digestive and Metabolic Diseases, Room Y2147, University Medical Center Groningen, PO Box 30.001, 9700 RB, Groningen, The Netherlands. Tel.: +31-50-3611261; Fax.: +31-50-3611746; E-mail: a.grefhorst@med.umcg.nl.

Abbreviations used: ACC, acetyl-CoA carboxylase; AMPK, AMP kinase; ChoRE carbohydrate response element; ChREBP, carbohydrate response element binding protein; DNL, de novo lipogenesis; FAS, fatty acid synthase; G6P, glucose-6-phosphate; G6Pase, glucose-6-phosphatase; G6PC, glucose-6-phosphatase, catalytic subunit; G6PT, glucose-6-phosphate transporter; GK, glucokinase; GSD-1, glycogen storage disease-1; LXR, liver X receptor; Mlx, Max-like factor X; PK, pyruvate kinase; PKA, protein kinase A; PPP, pentose-5-phosphate pathway; SREBP-1c, sterol-regulatory element-binding protein-1c; TG, triglyceride.

Synopsis

Glycogen storage disease type 1 (GSD-1) is caused by an inherited defect in glucose-6-phosphatase activity, resulting in massive accumulation of hepatic glycogen content and an induction of *de novo* lipogenesis. The chlorogenic acid derivative S4048 is a pharmacological inhibitor of the glucose-6-phosphate transporter, which is part of glucose-6-phosphatase, and allows for mechanistic studies concerning metabolic defects in GSD-1. Treatment of mice with S4048 resulted in ~60% reduction of blood glucose, increased hepatic glycogen and triglyceride content, and a markedly enhanced hepatic lipogenic gene expression. In mammals, hepatic expression of lipogenic genes is regulated by the coordinated action of the transcription factors sterol-regulatory element-binding protein-1c (SREBP-1c), liver X receptor α (LXRα) and carbohydrate response element binding protein (ChREBP). Treatment of *Lxrα*$^{-/-}$ mice and *Chrebp*$^{-/-}$ mice with S4048 demonstrated that ChREBP but not LXRα mediates the induction of hepatic lipogenic gene expression in this murine model of GSD-1. Thus, ChREBP is an attractive target to alleviate derangements in lipid metabolism observed in patients with GSD-1.

Keywords

Carbohydrate response element binding protein, glucose-6-phosphate, glycogen storage disease type 1, liver X receptor, pentose-5-phosphate pathway, sterol-regulatory element-binding protein-1c.

Introduction

Patients with glycogen storage disease type 1 (GSD-1) have a defect in the glucose-6-phosphatase (G6Pase) enzyme complex that consists of the enzymes glucose-6-phosphate transporter (G6PT; SLC37A4) and the catalytic subunit of glucose-6-phosphatase (G6PC). As a result of this defect, the patients suffer from postprandial hypoglycemia. In the liver of these patients, glycogen stores are high. Besides these effects on glucose metabolism, hepatic lipid metabolism is also severely disturbed. As shown before, GSD-1 patients have an increased *de novo* lipogenesis (DNL) and suffer from hyperlipidemia [1]. The chlorogenic acid derivative S4048 is an inhibitor of G6PT and has been shown to provide a model of GSD-1 when given to laboratory animals. For instance, treatment of rats with S4048 resulted in elevated hepatic glycogen and glucose-6-phosphate (G6P) contents [2] as well as induction of hepatic lipid synthesis [3].

In fed conditions, liver and muscle store excess glucose as glycogen [4] but a certain amount of glucose enters the glycolytic pathway to yield pyruvate which is converted into acetyl-CoA, the precursor of fatty acids and triglycerides (TGs) in DNL. Acetyl-CoA carboxylase-1 (ACC1), acetyl-CoA carboxylase-2 (ACC2), and fatty acid synthase (FAS) are crucial enzymes in DNL and the hepatic expression of the genes encoding ACC1, ACC2, FAS and other lipogenic enzymes is tightly regulated by a number of transcription factors.

Important 'lipogenic' transcription factors are the sterol-response element-binding protein-1c (SREBP-1c), the liver X receptor α (LXRα), and the carbohydrate response element binding protein (ChREBP). SREBP-1c is a member of the basic-helix-loop-helix-leucine zipper (bHLHLZ) transcription factor family that induces transcription of almost all lipogenic genes [5]. LXRα has been identified as an oxysterol-activated nuclear receptor [6-8] that, after ligand-binding, heterodimerizes with the retinoid X receptor (RXR). The LXRα/RXR heterodimer binds to LXR response elements (LXREs) present in promoters of target genes and subsequently regulates the expression of multiple lipogenic genes, in part by increasing *Srebp-1c* transcription [9-12], but also by direct effects on transcription of *Fas* [13] and *Acc* [14]. Recently, it was reported that LXRα also regulates mRNA levels and activity of the third lipogenic transcription factor, ChREBP [15], whose activity is regulated by hepatic G6P and/or by the carbohydrate flux through the pentose-5-phosphate pathway (PPP).

The non-oxidative part of the PPP is in near equilibrium with glycolysis and comprises a cascade of biochemical reactions in which G6P and fructose-6-phosphate are converted into xylulose-5-phosphate, an activator of protein phosphatase 2A (PP2A), that dephosphorylates and hence activates ChREBP [16,17]. Translocation of ChREBP into the nucleus is enhanced upon dephosphorylation of Ser196 while dephosporylation of Thr666 enhances the binding activity of ChREBP to DNA [18, 19]. In the nucleus, ChREBP binds as a heterodimer with Max-like factor X (Mlx) [20] to the carbohydrate response element (ChoRE) in promoters of target genes. A recent *in vitro* study showed that hepatic G6P itself activates ChREBP transcriptional activity via a PP2A-independent route that does not require a PPP flux [21].

Remarkably, most genes regulated by ChREBP also are regulated by SREBP-1c [22], *e.g.*, *Fas* and *Acc*. Yet, expression of the gene encoding pyruvate kinase (PK) is regulated by ChREBP but not by SREBP-1c [23]. In contrast, expression of the gene encoding glucokinase (GK) is regulated by SREBP-1c but not by ChREBP [24].

Altogether, ChREBP might be considered as a switch that controls the conversion of carbohydrates into lipids. Evidence is emerging that oxysterols are not the only endogenous LXR ligands since both glucose and G6P have shown to bind and activate LXR [25]. Since both ChREBP and LXR might be activated by increased intracellular G6P concentrations, these transcription factors are likely key-players in the metabolic perturbations associated with GSD-1. We therefore determined whether ChREBP and/or LXRα are involved in the induction of lipogenic gene expression upon S4048 treatment.

Material and methods

In vivo experiments with C57BL/6J and Lxrα$^{-/-}$ mice

Male C57BL/OlaHsd mice (Harlan, Horst, The Netherlands) and Lxrα$^{-/-}$ mice and their wild-type littermates on a mixed C57BL/6J Sv129/OlaHsd background (own breeding colony) [26] were housed in a light- and temperature-controlled facility. The mice were fed a standard laboratory chow diet (RMH-B, Hope Farms BV, Woerden, The Netherlands) and had free access to water. Experimental procedures were approved by the Ethics Committees for Animal Experiments of the University of Groningen.

Mice were equipped with a permanent catheter in the right atrium of the heart [27] to infuse S4048 and the entrance of the catheter was attached to the skull. The mice were allowed a recovery period of at least five days after surgery. Mice were kept in metabolic cages during the experiment and the preliminary fasting period, allowing frequent collection of small tail blood samples under unrestrained conditions [28]. After nine hours of fasting, the mice were infused for six hours with S4048 (a generous gift of Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany) (5.5 mg S4048/ml PBS with 6% DMSO, 0.135 ml/h) or vehicle. Blood glucose concentrations were measured in a small blood sample obtained by tail bleeding every hour during the experiment. After six hours of infusion, the mice were terminated by cardiac puncture and the liver was immediately removed and freeze clamped within 30 seconds after excision.

In vivo experiments with Chrebp$^{-/-}$ mice

Male *Chrebp*$^{-/-}$ mice and their wild-type littermates [24] on a C57BL/6J background were housed in a light- and temperature-controlled facility. The mice were fed a standard laboratory chow diet (diet no. 7002, Harlan Teklad Premier Laboratory Diets, Madison, WI) and had free access to drinking water. The experiments were approved by the Institutional Animal Care and Research Advisory Committee at the University of Texas Southwestern Medical Center (Dallas, TX).

After nine hours of fasting, the mice were anesthetized with sodium pentobarbital and received a six-hour infusion with S4048 (5.5 mg S4048/ml PBS with 6% DMSO, 0.135 ml/h) or vehicle via the jugular vein as described before [29]. Blood glucose concentrations were measured in a small blood sample obtained by tail bleeding every hour during the experiment. After the infusion period, the mice were terminated by cardiac puncture and the liver was immediately removed and freeze clamped within 30 seconds after excision.

Hepatic analyses

Hepatic concentrations of TG, free cholesterol and total cholesterol were measured using commercial kits (Roche Diagnostics, Mannheim, Germany, and Wako Chemicals, Neuss, Germany) after lipid extraction according to Bligh and Dyer [30]. Hepatic glycogen and G6P contents were determined as described by Bergmeyer [31].

Gene expression

Total liver RNA was isolated using the TRI Reagent method (Sigma) according to manufacture's protocol. Integrity and concentration of RNA were determined with the Nanodrop spectrophotometer (NanoDrop™ 1000 Spectrophotometer, Thermo Scientific, Waltham, MA). cDNA was obtained using the reverse transcription procedure with Moloney Murine Leukemia Virus-RT (Sigma) with random primers

according to the protocol of the manufacturer. cDNA levels were measured in real-time quantitative PCR amplification using an ABI PRISM 7700 sequence detector (Applied Biosystems, Foster City, USA) against a calibration curve of pooled cDNA solutions. Expression levels were normalized for β-actin levels. The sequences of the primers and probes can be found on www.labpediatricsrug.nl and are deposited at RTPrimerDB (www.rtprimerdb.org).

Statistics

All values represent means ± standard deviation for the number of animals indicated. Statistical analysis was assessed by Mann-Whitney U test. Level of significance was set at $p<0.05$. Analyses were performed using SPSS for Windows software (SPSS, Chicago, IL, USA).

Results

Pharmacological inhibition of the glucose-6-phosphate transporter as a murine model of GSD-1

Previous experiments from our laboratory [2,3] showed that inhibition of G6PT with S4048 results in severe hepatic steatosis and massive hepatic glycogen stores in rats. In the present study, we treated mice with S4048. During S4048 administration, blood glucose concentrations were significantly reduced within 1 hour (Figure 1). At the end of the 6-hour infusion period, blood glucose concentrations were 64% lower in the treated mice compared to vehicle-treated mice. As expected, hepatic glycogen and G6P contents were massively elevated upon S4048 treatment, as were hepatic and plasma TG concentrations (Table 1).

In the livers of S4048-treated mice, expression of lipogenic genes such as *Fas*, *Acc1* and *Acc2* was markedly increased compared to control mice (Figure 2). Compared to control mice, hepatic mRNA levels of *Pk* and *Gk* in S4048-treated mice were increased by 400% and reduced by 70%, respectively. Taken together, this strongly suggests that S4048-induced lipogenic gene expression is related to enhanced transcriptional ChREBP activity and not to SREBP-1c activity since *Pk* gene expression is regulated by ChREBP but not by SREBP-1c [23] while *Gk* is regulated by SREBP-1c but not by ChREBP [24]. Because it has been reported that LXRα might activate ChREBP transcriptional activity [15] and that cellular G6P might be an endogenous LXR ligand [25], we explored the effects of S4048-infusion on lipogenic gene expression in *Lxrα*$^{-/-}$ and *Chrebp*$^{-/-}$ mice in comparison to their wild-type littermates.

The induction of hepatic lipogenic genes upon pharmacological inhibition of the glucose-6-phosphate transporter is not mediated by LXRα

LXRα is the major isoform that controls hepatic lipogenic gene expression and we therefore treated *Lxrα*$^{-/-}$ mice and their wild-type littermates with S4048. In both genotypes, S4048 infusion resulted in mildly increased hepatic glycogen and G6P concentrations (Table 2) and in inductions of hepatic *Pk*, *Acc1*, *Acc2* and *Fas* expression (Figure 3). Thus, S4048-induced expression of hepatic lipogenic genes is independent of LXRα. In *Lxrα*$^{-/-}$ and *Lxrα*$^{+/+}$ mice, S4048-infusion induced transcription of *G6pt*, *G6pc* and *Chrebp*. Hepatic *Gk* expression was not significantly affected upon S4048-infusion in either type of mice, although it tends to decrease upon S4048 treatment.

The induction of hepatic lipogenic genes upon pharmacological inhibition of the glucose-6-phosphate transporter is mediated by ChREBP

Next, we repeated the S4048 infusion experiments in *Chrebp*$^{-/-}$ and their wild-type littermates. In both *Chrebp*$^{-/-}$ and the wild-type mice, the infusion of S4048 resulted in enhanced liver glycogen and extremely increased intracellular G6P concentrations (Table 3). Although the S4048-infusion did not significantly affect the liver triglyceride content in the *Chrebp*$^{-/-}$ mice and their wild-type littermates, there was a tendency towards increased liver TG content upon S4048-infusion (Table 3). Expression of the lipogenic genes was increased upon S4048 in the wild-type mice but failed to reach significance for *Pk* and *Fas* due to lack of statistical power (Figure 4). Interestingly, the S4048-mediated induction of hepatic *Pk*, *Acc1* and *Fas* expression was completely abolished in the *Chrebp*$^{-/-}$ mice. In addition, the S4048-mediated induction of *Acc2* in the *Chrebp*$^{-/-}$ mice was only minor compared to the

effects of S4048 on *Acc2* in the wild-type mice (29% induction in *Chrebp*$^{-/-}$ mice *vs.* 227% induction in the *Chrebp*$^{+/+}$ mice). This, however, might be due to already higher *Acc2* expression in the vehicle infused *Chrebp*$^{-/-}$ mice compared to the vehicle infused *Chrebp*$^{+/+}$ mice. Altogether, these data show that S4048-induced hepatic lipogenic gene expression is mediated by ChREBP. Hepatic *Chrebp* and *Srebp-1c* expression were reduced in *Chrebp*$^{-/-}$ mice as compared to wild-type littermates. Finally we did not find significant differences in hepatic *Chrebp*, *Srebp-1c* and *Gk* expression in S4048-treated mice of either genotype.

Discussion

Hepatic lipid metabolism, and especially DNL, is regulated by several transcription factors, including SREBP-1c, ChREBP and LXRα. Numerous studies have addressed the respective roles of SREBP-1c and LXR in control of lipogenic gene expression [5,32-34]. Other studies have focused on the interplay between these two factors [10,14]. The role of ChREBP in control of hepatic DNL is mostly related to the adaptive induction of lipogenic genes in response to increased glucose availability [18, 21, 22, 35] and thus ChREBP has become an attractive target in the treatment of hepatic steatosis and insulin resistance [36]. Only one study addressed the role of LXR in ChREBP activation and transcription [15]. In the present work, we characterize a murine model of GSD-1. In this model, ChREBP but not LXRα mediated the induction of hepatic lipogenic gene expression. Furthermore, the phenotype depended in part on the mouse strain used. Collectively, our data point toward ChREBP as a possible target to treat a number of metabolic derangements in patients with GSD-1.

Since GSD-1 is caused by a lack of glucose-6-phosphatase activity, glucose production by glycogenolysis or gluconeogenesis is severely impaired in GSD-1 patients who, as a consequence, suffer from hypoglycemia. GSD-1 is further characterized by increased liver glycogen storage and a fatty liver. The murine model of GSD-1 we developed by short term pharmacological inhibition of the glucose-6-phosphate transporter by S4048 captured several of these characteristics. A 6 hour S4048-infusion resulted in hypoglycemia and dramatically increased hepatic glycogen and G6P contents accompanied by increased hepatic TG concentrations. Until now two mouse models of GSD-1 by genetic modification of the catalytic subunit of glucose-6-phosphatase have been published by the group of Chou [37, 38]. Mice of both these models die postnatal from very severe hypoglycemia and suffer from major disturbances in lipid metabolism such as fatty liver. Both hypoglycemia and fatty liver are also seen in our acute GSD-1 model and are apparently direct consequences of glucose-6-phosphate accumulation in the liver.

Hepatic carbohydrates regulate ChREBP transcriptional activity either by direct effects of hepatic G6P [21] or xylulose-5-phosphate via induction of the PPP due to a high G6P content [16,17]. Because our murine model of GSD-1 is characterized by massively elevated G6P contents, the first experiments already suggested ChREBP to be the most likely transcriptional regulator of the enhanced hepatic lipogenic gene expression under these conditions. The pivotal role of ChREBP was evident from studies performed in *Chrebp*$^{-/-}$ mice treated with S4048. From the results depicted in Figure 4 it is clear that ChREBP indeed mediates the effects of S4048 on hepatic lipogenic gene expression. The molecular mechanism of ChREBP activation leading to the induction of lipogenic gene expression in our model is not known. More and detailed studies are required to reveal whether PP2A-dependent and/or PP2A-independent ChREBP activation is involved in the S4048-induced lipogenic gene expression. The S4048-mediated increase of hepatic *G6pc* expression upon S4048 treatment was also dependent on ChREBP. Although the role of ChREBP in regulation of the glucose-6-phosphatase (G6Pase) enzyme complex has not been a major topic of research, Dentin *et al.* [35] showed that short hairpin RNA (shRNA) against ChREBP normalized the elevated hepatic *G6Pase* gene expression of *ob/ob* mice. The authors speculate that this is due to a normalization of hepatic insulin signaling in the shRNA-treated *ob/ob* mice. Intriguingly, SREBP-1c and ChREBP control the transcription of similar sets of genes and it is therefore hard to

distinguish effects of both transcription factors on lipogenic gene expression *in vivo*. However, *Pk* expression is regulated by ChREBP but not by SREBP-1c [23] and *Gk* expression is regulated by SREBP-1c but not by ChREBP [24]. Focusing on changes in expression of these two genes in the first experiments (Figure 2), it appears that our murine GSD-1 model is associated with enhanced hepatic transcriptional activity of ChREBP but not of SREBP-1c.

The activity of LXRα is regulated by cholesterol metabolites [6-8], the unaffected hepatic cholesterol concentrations (Table 1) already suggested that the induced hepatic lipogenic gene expression in our murine GSD-1 model was independent of LXRα. We also did not observe an induction of the LXR target gene SREBP-1c upon S4048-infusion (Figure 2), further supporting that the induction of lipogenic genes was not mediated by LXR. Accordingly, the studies with the *Lxrα*$^{-/-}$ mice clearly showed that LXRα does not mediate S4048-induced hepatic lipogenic gene expression since the effects of S4048 on hepatic gene expression levels did not differ between *Lxrα*$^{-/-}$ mice and their wild-type littermates (Figure 3). Although previous *in vitro* experiments suggested that G6P is an LXR agonist [25], the current studies clearly show that G6P does not activate LXRα *in vivo*. In addition, using *Lxrα/β* double knockout mice and ChREBP shRNA, Denechaud *et al.* [18] showed that the effects of carbohydrates on hepatic gene expression, *e.g.*, *Acc1* and *Pk*, required ChREBP but not LXR. These results are in line with the present observations showing that induced hepatic expression of *Acc1* and *Pk* upon elevated hepatic carbohydrates was absent in *Chrebp*$^{-/-}$ mice but not in *Lxrα* $^{-/-}$ mice (Figures 3 and 4).

The differential effects of S4048 on hepatic glycogen, G6P and TG concentrations between the different mouse models are striking. Already upon vehicle infusion, the *Lxrα*$^{-/-}$ mouse strain (Table 2) presented with elevated hepatic glycogen and G6P contents compared to the C57BL/OlaHsd mice (Table 1) and the *Chrebp*$^{-/-}$ mouse strain (Table 3). Previously, we already found considerably high liver glycogen and G6P concentrations in the wild-type littermates of the *Lxrα*$^{-/-}$ mouse strain [39]. Although this might lead to the conclusion that the high liver glycogen and G6P content might be due to the mixed C57BL/6J Sv129/OlaHsd background, further experiments need to be performed to investigate this in more detail. In the *Chrebp*$^{-/-}$ mice and their wild-type littermates, S4048-infusion resulted in a modest 3-fold induction of liver glycogen concentrations and a massive ~60-fold induction in liver G6P concentrations while the induction of liver TG content was severely blunted (Table 3). Although speculative, differences in infusion protocols might have resulted in these differences. The C57BL/OlaHsd mice, the *Lxrα*$^{-/-}$ mice and their wild-type littermates were infused without sedation while the *Chrebp*$^{-/-}$ mice and their wild-type littermates received the infusion under pentobarbital sedation. Despite the differences of S4048 on liver glycogen and G6P between the strains, however, the effects of S4048 on the hepatic expression of *Pk*, *Acc1*, *Acc2* and *Fas* was comparable between the first set of experiments and the wild-type littermates of the *Lxrα*$^{-/-}$ mice and *Chrebp*$^{-/-}$ mice.

In conclusion, the present study shows that ChREBP mediates the induction of lipogenic gene expression levels upon pharmacological inhibition of G6PT. Hence, increased DNL in GSD-1 patients [1] might also be due to effects on ChREBP activity and/or transcription, suggesting that ChREBP might be an interesting target for future pharmacological interventions to prevent or to treat severe hypertriglyceridemia and hepatic steatosis in these patients.

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Table 1. Hepatic triglyceride, cholesterol, glycogen, and glucose-6-phosphate (G6P) contents and plasma triglyceride and cholesterol concentrations in C57BL/OlaHsd mice after the 6 hour infusion with 30 mg/kg/h glucose-6-phosphate transporter inhibitor S4048 or vehicle. Data are mean ± S.D.; n=5; *, $p<0.05$ S4048 *vs.* vehicle.

	Vehicle	S4048
Liver triglycerides (nmol/mg liver)	25.7 ± 6.9	60.3 ± 22.6 *
Liver free cholesterol (nmol/mg liver)	8.1 ± 1.5	8.2 ± 1.6
Liver cholesterylester (nmol/mg liver)	1.9 ± 0.7	2.5 ± 1.7
Liver glycogen (nmol/mg liver)	16.1 ± 3.4	254.8 ± 85.4 *
Liver G6P (nmol/g liver)	21.4 ± 15.0	574.9 ± 133.7 *

Table 2. Hepatic triglyceride, cholesterol, glycogen, and glucose-6-phosphate (G6P) contents in *Lxrα*$^{+/+}$ and *Lxrα*$^{-/-}$ mice after the 6 hour infusion with 30 mg/kg/h glucose-6-phosphate transporter inhibitor S4048 or vehicle. Data are mean ± S.D.; n=3 (control mice); n=5 (S4048-treated mice); *, p<0.05 S4048 *vs.* vehicle.

	Lxrα$^{+/+}$ mice		*Lxrα*$^{-/-}$ mice	
	Vehicle	S4048	Vehicle	S4048
Liver triglycerides (nmol/mg liver)	18.1 ± 11.0	38.1 ± 2.5 *	24.7 ± 5.6	71.2 ± 15.3 *
Liver free cholesterol (nmol/mg liver)	7.4 ± 0.6	8.6 ± 1.4	7.2 ± 0.4	7.6 ± 0.3
Liver cholesterylester (nmol/mg liver)	1.1 ± 0.1	1.9 ± 0.4	0.9 ± 0.4	3.1 ± 0.3 *
Liver glycogen (nmol/mg liver)	78.8 ± 26.2	183.0 ± 6.1 *	101.6 ± 53.0	336.0 ± 40.1 *
Liver G6P (nmol/g liver)	265.5 ± 66.0	998.8 ± 288.9	340.7 ± 136.8	624.5 ± 50.6 *

Table 3. Hepatic triglyceride, cholesterol, glycogen, and glucose-6-phosphate (G6P) contents in *Chrebp*$^{+/+}$ and *Chrebp*$^{-/-}$ mice after the 6 hour infusion with 30 mg/kg/h glucose-6-phosphate transporter inhibitor S4048 or vehicle. Data are mean ± S.D.; n=4 (S4048-treated *Chrebp*$^{+/+}$ mice and vehicle-treated *Chrebp*$^{-/-}$ mice); n=5 (vehicle-treated *Chrebp*$^{+/+}$ mice and S4048-treated *Chrebp*$^{-/-}$ mice); *, $p<0.05$ S4048 *vs.* vehicle.

	Chrebp$^{+/+}$ mice		*Chrebp*$^{-/-}$ mice	
	Vehicle	S4048	Vehicle	S4048
Liver triglycerides (nmol/mg liver)	82 ± 55	121 ± 51	96 ± 54	135 ± 43
Liver free cholesterol (nmol/mg liver)	6.1 ± 0.5	6.7 ± 2.0	6.3 ± 0.9	6.5 ± 1.0
Liver cholesterylester (nmol/mg liver)	7.2 ± 1.3	6.0 ± 1.1	6.5 ± 0.2	5.7 ± 0.7
Liver glycogen (nmol/mg liver)	30.8 ± 12.9	91.8 ± 43.8 *	41.3 ± 8.7	108.7 ± 43.1 *
Liver G6P (nmol/g liver)	33 ± 28	2442 ± 1397 *	56 ± 20	3345 ± 522 *

Figure legends

Figure 1: Infusion of the glucose-6-phosphate transporter inhibitor S4048 results in hypoglycemia.
Relative blood glucose concentrations in C57BL/OlaHsd mice during infusion of 30 mg/kg/h glucose-6-phosphate transporter inhibitor S4048 or vehicle. Data are mean ± S.D.; n=7.

Figure 2. The glucose-6-phosphate transporter inhibitor S4048 induces hepatic lipogenic gene expression.
Changes in hepatic gene expression patterns in C57BL/OlaHsd mice upon treatment with 30 mg/kg/h glucose-6-phosphate transporter inhibitor S4048. Results were normalized to β-actin mRNA concentrations, data from untreated mice defined as '1'. Pk, pyruvate kinase; Acc, acetyl-CoA carboxylase; Fas, fatty acid synthase; G6pt, glucose-phosphate transporter; G6pc, glucose-6-phosphaase, catalytic subunit; Chrebp, carbohydrate response element binding protein; Srebp-1c, sterol-regulatory element-binding protein-1c; Gk, glucokinase. Data are mean ± S.D.; n=5; *, $p<0.05$.

Figure 3. The induction of hepatic lipogenic gene expression by the glucose-6-phosphate transporter inhibitor S4048 is not mediated by LXRα.
Changes in hepatic gene expression patterns in *Lxrα*$^{+/+}$ and *Lxrα*$^{-/-}$ mice upon treatment with 30 mg/kg/h glucose-6-phosphate transporter inhibitor S4048. Results were normalized to β-actin mRNA concentrations, data from untreated *Lxrα*$^{+/+}$ mice defined as '1'. Pk, pyruvate kinase; Acc, acetyl-CoA carboxylase; Fas, fatty acid synthase; G6pt, glucose-phosphate transporter; G6pc, glucose-6-phosphatase, catalytic subunit; Chrebp, carbohydrate response element binding protein; Srebp-1c, sterol-regulatory element-binding protein-1c; Gk, glucokinase. Data are mean ± S.D.; n=3 (control mice); n=5 (S4048-treated mice); *, $p<0.05$ S4048 *vs.* control.

Figure 4. The induction of hepatic lipogenic gene expression by the glucose-6-phosphate transporter inhibitor S4048 is mediated by ChREBP.
Changes in hepatic gene expression patterns in *Chrebp*$^{-/-}$ and *Chrebp*$^{+/+}$ mice upon treatment with 30 mg/kg/h glucose-6-phosphate transporter inhibitor S4048. Results were normalized to β-actin mRNA concentrations, data from untreated *Chrebp*$^{+/+}$ mice defined as '1'. Pk, pyruvate kinase; Acc, acetyl-CoA carboxylase; Fas, fatty acid synthase; G6pt, glucose-phosphate transporter; G6pc, glucose-6-phosphatase, catalytic subunit; Chrebp, carbohydrate response element binding protein; Srebp-1c, sterol-regulatory element-binding protein-1c; Gk, glucokinase. Data are mean ± S.D.; n=4 (S4048-treated *Chrebp*$^{+/+}$ mice and control *Chrebp*$^{-/-}$ mice); n=5 (control *Chrebp*$^{+/+}$ mice and S4048-treated *Chrebp*$^{-/-}$ mice); *, $p<0.05$ S4048 *vs.* control.

Figure 1

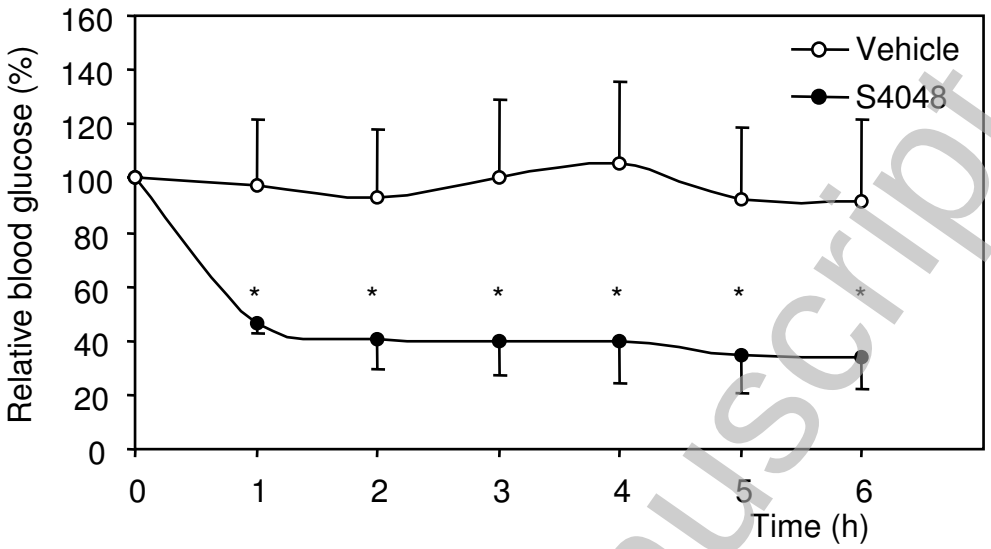

Figure 2

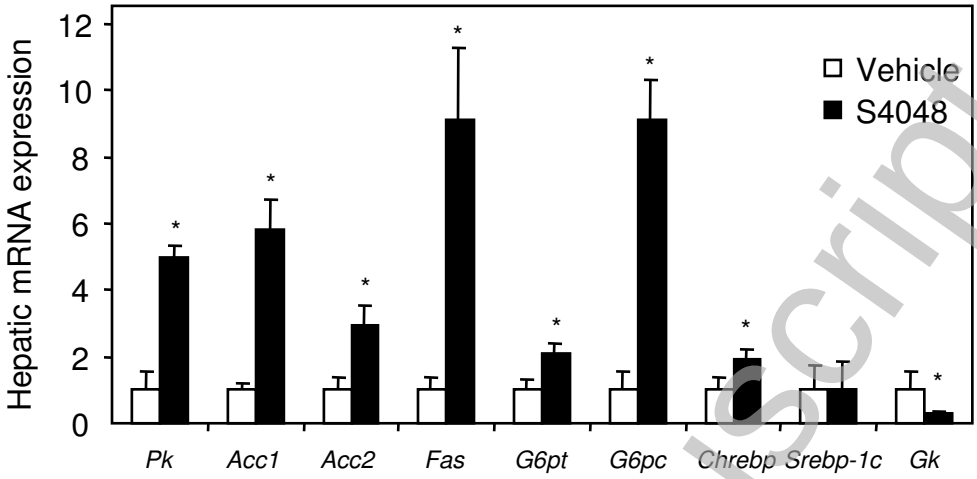

Figure 3

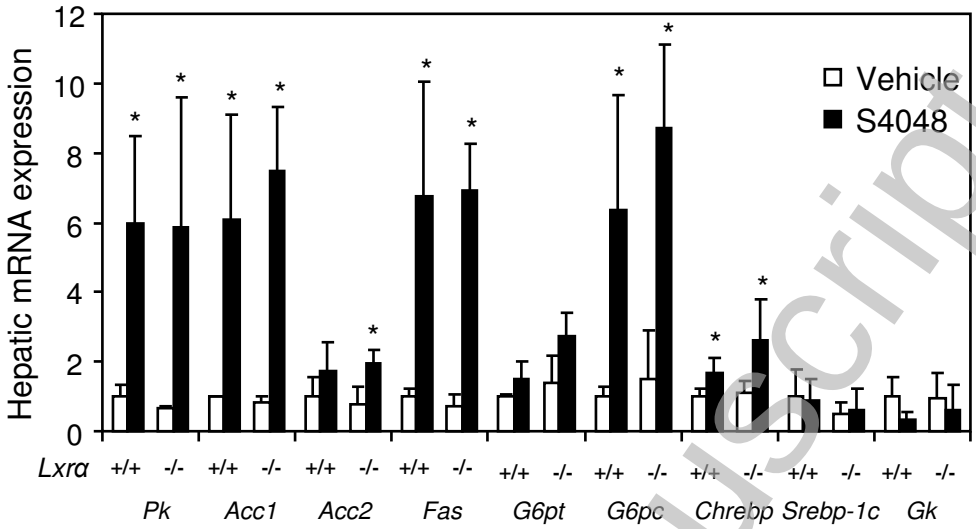

Figure 4