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Research paper

Disturbances in cholesterol, bile acid and glucose metabolism in peroxisomal 3-ketoacylCoA thiolase B deficient mice fed diets containing high or low fat contents

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ABSTRACT

The peroxisomal 3-ketoacyl-CoA thiolase B (ThB) catalyzes the thiolytic cleavage of straight chain 3-ketoacyl-CoAs. Up to now, the ability of ThB to interfere with lipid metabolism was studied in mice fed a laboratory chow enriched or not with the synthetic agonist Wy14,643, a pharmacological activator of the nuclear hormone receptor PPARα. The aim of the present study was therefore to determine whether ThB could play a role in obesity and lipid metabolism when mice are chronically fed a synthetic High Fat Diet (HFD) or a Low Fat Diet (LFD) as a control diet. To investigate this possibility, wild-type (WT) mice and mice deficient for Thb ($Thb^{-/-}$) were subjected to either a synthetic LFD or a HFD for 25 weeks, and their responses were compared. First, when fed a normal regulatory laboratory chow, $Thb^{-/-}$ mice displayed growth retardation as well as a severe reduction in the plasma level of Growth Hormone (GH) and Insulin Growth Factor-I (IGF-I), suggesting alterations in the GH/IGF-1 pathway. When fed the synthetic diets, the corrected energy intake to body mass was significantly higher in $Thb^{-/-}$ mice, yet those mice were protected from HFD-induced adiposity. Importantly, $Thb^{-/-}$ mice also suffered from hypoglycemia, exhibited reduction in liver glycogen stores and circulating insulin levels under the LFD and the HFD. *Thb* deficiency was also associated with higher levels of plasma HDL (High Density Lipoproteins) cholesterol and increased liver content of cholesterol under both the LFD and the HFD. As shown by the plasma lathosterol to cholesterol ratio, a surrogate marker for cholesterol biosynthesis, whole body cholesterol *de novo* synthesis was increased in $Thb^{-/-}$ mice. By comparing liver RNA from WT mice and $Thb^{-/-}$ mice using oligonucleotide microarray and RT-qPCR, a coordinated decrease in the expression of critical cholesterol synthesizing genes and an increased expression of genes involved in bile

Abbreviations: ThA, 3-ketoacyl-CoA thiolase A; ThB, 3-ketoacyl-CoA thiolase B; FAO, fatty acid oxidation; ACOX1, acyl-CoA-oxidase 1; MFP1, peroxisomal multifunctional enzyme type 1; MFP2, peroxisomal multifunctional enzyme type 2; WAT, white adipose tissue; LFD, Low Fat Diet; HFD, High Fat Diet; GH, Growth Hormone; IGF-I, Insulin Growth Factor-I; IGFBP-3, insulin-like growth factor-binding protein-3; PPARα, peroxisome proliferator-activated receptor alpha; Wy, Wy14,643; WT, wild-type; KO, knock-out; HDL, high density lipoproteins; SR-BI, scavenger receptor class B member 1; SREBP, sterol regulatory element-binding protein; RXR, retinoid X receptor.

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acid synthesis (*Cyp7a1*, *Cyp17a1*, *Akr1d1*) were observed in *Thb*$^{-/-}$ mice. In parallel, the elevation of the lathosterol to cholesterol ratio as well as the increased expression of cholesterol synthesizing genes were observed in the kidney of *Thb*$^{-/-}$ mice fed the LFD and the HFD. Overall, the data indicate that ThB is not fully interchangeable with the thiolase A isoform. The present study also reveals that modulating the expression of the peroxisomal ThB enzyme can largely reverberate not only throughout fatty acid metabolism but also cholesterol, bile acid and glucose metabolism.

1. Introduction

Adipose tissue lipolysis generates free fatty acids that are taken up from the blood plasma by the liver where they are activated into their fatty acyl-CoA derivatives. The activated fatty acyl-CoAs are subsequently imported into mitochondria or peroxisomes for degradation to acetyl-coenzyme A (acetyl-CoA) *via* the β-oxidation process. Whereas the mitochondrion oxidizes short-, medium- and mostly long-chain fatty acids, the peroxisome oxidizes some long-chain but mostly very long chain fatty acids. The peroxisome is also involved in the α-oxidation of very-long-straight-chain or branched-chain acyl-CoAs (reviewed in Ref. [1]). At the biochemical level, the peroxisomal β-oxidation of straight-chain acyl-CoAs starts with a reaction catalyzed by the ACOX1 (acyl-CoA oxidase 1) enzyme that is the first and rate-limiting enzyme of the pathway. The step orchestrated by ACOX1 is followed by two enzymatic reactions carried out by the MFP1 or MFP2. The fourth and last step of the process is catalyzed by the peroxisomal 3-ketoacyl-CoA thiolases. In humans, only a single corresponding gene (peroxisomal 3-acetyl-CoA acetyltransferase-1 also known as peroxisomal 3-oxoacyl-CoA thiolase or acyl-CoA:acetyl-CoA-acyltransferase, ACAA1, EC 2.3.1.16) has been identified [2,3] and no isolated peroxisomal deficiency at the level of the peroxisomal 3-ketoacyl-CoA thiolase, has not been reported yet [2,3]. To date, two closely related but differentially regulated peroxisomal 3-ketoacyl-CoA thiolases isoforms (thiolase A, ThA, Acaa1a and thiolase B, ThB, Acaa1b, EC:2.3.1.16) have been identified in rodents [4,5]. It is still not clear whether these two proteins stem from a unique and original ancestral gene. In agreement with their very high degree of nucleotide sequence identity (97%), the mature forms of ThA and ThB differ in only nine amino acids in rats [4–6]. Given the large amino acid sequence identity (96%) between ThA and ThB, major overlaps in the enzymatic activities of ThA and ThB were found [4,7]. As a consequence, both enzymes share virtually the same substrate specificity *in vitro* that includes very-long-straight-chain 3-oxoacyl-CoAs [7]. The cleavage of 2-methyl-branched as well as straight-chain 3-ketoacyl-CoA esters is under the dependence of a third thiolase isoform (SCP-2/3-ketoacyl-CoA thiolase, SCPx) previously characterized in both humans and rodents [8–10].

In addition to fatty acid β-oxidation, the peroxisome is involved in other aspects of lipid metabolism ranging from synthesis of bile acids, plasmalogens, cholesterol and isoprenoids. Consistent with this notion, different studies have shed light on the existence of an alternative pathway for cholesterol synthesis in the peroxisomal matrix, with a specific 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase that displays functional and structural properties different from the endoplasmic reticulum HMG-CoA reductase [11–13].

In order to investigate the physiological role of the *3-ketoacylCoA thiolase B* (ThB) *in vivo*, a mouse model deficient for *Thb* (*Thb*$^{-/-}$) has been partly characterized in our laboratory [14]. At the molecular level, *Thb* was identified as a direct target gene of the peroxisome proliferator-activated receptor alpha (PPARα, NR1C1) in the liver [15]. PPARα forms a heterodimer with the Retinoid X Receptor (RXR) and following the physical binding of PPARα/RXRα by the synthetic agonist Wy14,643 (Wy), the mRNA expression of a large arrays of genes ranging from lipid, amino-acid, glycerol and glycogen metabolism as well as inflammation control is upregulated ([16–23]). A crucial piece of evidence that liver *Thb* is functionally regulated by the nuclear receptor PPARα is the observation that some hepatic fatty acid contents are different between WT and *Thb*$^{-/-}$ mice exposed to the PPARα agonist Wy [21,24]. Follow-up investigations also revealed that ThB could play an indirect role in the control of PPARα mediated upregulation of Sterol Regulatory-Element-Binding-Protein-2 (SREBP-2) target genes in the liver of mice fed with Wy [25]. This result further demonstrates that the deletion of a single peroxisomal activity such as ThB is sufficient to impact the transcription of biosynthetic cholesterol genes in the liver. Combined, these previous data support the notion that despite the similarities between ThA and ThB, ThB displays a unique biochemical function that deserves further characterization.

In addition to be the molecular target of the fibrate drug Wy, PPARα also mediates the effects of a High Fat Diet (HFD) on hepatic gene expression [26]. As *Thb* is a PPARα target gene, we expected that chronic HFD could shed light on the putative consequences of the deletion of *Thb* in mouse in a more physiological context, i.e., HFD-induced obesity and insulin-resistance.

The recent demonstration that resistance to diet-induced obesity is accompanied by a marked increase in peroxisomal β-oxidation has been instrumental in advancing our thoughts about the origin of oxidative changes during obesity [27,28]. Using two strains of mice resistant (A/J) or sensitive (C57Bl/6) to diet-induced hepatosteatosis and obesity, it was found that 10 peroxisomal oxidative genes were specifically upregulated in A/J mice leading to a significant increase in peroxisomal β-oxidation [27]. It was thereby hypothesized that this peroxisomal fatty acid β-oxidation could partly prevent diet-induced hepatosteatosis and obesity.

The aim of the present study was therefore to evaluate the role of ThB and its ability to potentially interfere with HFD-induced lipid metabolism disorders. To explore the function of ThB in this aspect, WT mice and *Thb*$^{-/-}$ mice were chronically fed a synthetic High-Fat Diet (HFD) and a Low-Fat Diet (LFD) as control. Our results indicate that *Thb* deletion in mouse induces alterations not only in fatty acid metabolism but also in carbohydrate, cholesterol and bile acid metabolism, extending the function of ThB to other unexpected metabolic pathways.

2. Materials and methods

2.1. Animal experiments and ethical considerations

Only male mice on a pure-bred *Sv129* genetic background have been used and previously described [14]. Male mice were kept in normal cages with food and water *ad libitum*, unless clearly indicated. In absence of dietary challenge, mice were routinely fed a standard commercial pellet diet (UAR A03-10 pellets from Usine d'Alimentation Rationnelle, Epinay sur Orge, SAFE, France, 3.2 kcal/g) consisting (by mass) of about 5.1% fat (C16:0 ± 0.89%; C16:1 n-7 ± 0.09%; C18:0 ± 0.45%; C18:1 n-9 ± 1.06%, C18:2 n-9 ± 1.53% and