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Research paper

# Modulation of the hepatic fatty acid pool in peroxisomal 3-ketoacyl-CoA thiolase B-null mice exposed to the selective PPARalpha agonist Wy14,643☆

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ABSTRACT

The peroxisomal 3-ketoacyl-CoA thiolase B (Thb) gene was previously identified as a direct target gene of PPARalpha, a nuclear hormone receptor activated by hypolipidemic fibrate drugs. To better understand the role of ThB in hepatic lipid metabolism in mice, Sv129 wild-type and Thb null mice were fed or not the selective PPARalpha agonist Wy14,643 (Wy).

Here, it is shown that in contrast to some other mouse models deficient for peroxisomal enzymes, the hepatic PPARalpha signaling cascade in Thb null mice was normal under regular conditions. It is of interest that the hypotriglyceridemic action of Wy was reduced in Thb null mice underlining the conclusion that neither thiolase A nor SCPx/SCP2 thiolase can fully substitute for ThB *in vivo*. Moreover, a significant increased in the expression of lipogenic genes such as Stearoyl CoA Desaturase-1 (SCD1) was observed in Thb null mice fed Wy. Elevation of Scd1 mRNA and protein levels led to higher SCD1 activity, through a molecular mechanism that is probably SREBP1 independent. In agreement with higher SCD1, enrichment of liver mono-unsaturated fatty acids of the n-7 and n-9 series was found in Thb null mice fed Wy.

Overall, we show that the reduced peroxisomal β-oxidation of fat observed in Thb null mice fed Wy is associated with enhanced hepatic lipogenesis, through the combined elevation of microsomal SCD1 protein and activity. Ultimately, not only the amount but also the quality of the hepatic fatty acid pool is modulated upon the deletion of Thb.



## 1. Introduction

Under normal physiologic conditions, mitochondrial β-oxidation is quantitatively the most important pathway for fatty acid catabolism. Besides mitochondrial β-oxidation ((80% of long-chain fatty acids (LCFA) up to 22 carbons)), β-oxidation of long-chain (for about 20%) and that of very-long chain fatty acids (VLCFA, >22 atoms of carbon) occurs within the peroxisome [1]. Similar to mitochondrial β-oxidation, peroxisomal β-oxidation can be divided into different steps, involving multiple enzymes that sequentially act. Some of these enzymes (fatty acyl-CoA oxidase, peroxisomal D-3-hydroxyacyl-CoA hydratase/D-3-hydroxyacyl-CoA dehydrogenase ((D-PBE)) and peroxisomal 3-ketoacyl-CoA thiolase) are considered to be more prone to oxidize straight-chain fatty acyl-CoAs. In rodents, two closely related genes, namely Thiolase a (*Tha*) and Thiolase b (*Thb*) have been cloned and characterized, while in humans only a single

*Abbreviations:* ThB, peroxisomal 3-ketoacyl-CoA thiolase B; ThA, peroxisomal 3-ketoacyl-CoA thiolase A; ACOX-I, peroxisomal acyl-CoA oxidase-I; PPARα, Peroxisome Proliferator-Activated Receptor alpha; LCFAs, Long-Chain Fatty Acids; VLCFAs, Very-Long-Chain Fatty Acids; SFAs, Saturated Fatty Acids; MUFAs, Mono-Unsaturated Fatty Acids; PUFAs, Poly-Unsaturated Fatty Acids; SREBP, Sterol Response Element Binding Protein; TG, Triglycerides; Wy, Wy14,643; FAO, Fatty acid oxidation.

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gene encoding for a peroxisomal 3-oxoacyl-CoA thiolase has been reported so far [2–4]. An infant girl with a deficiency in peroxisomal 3-ketoacyl-CoA thiolase has been initially reported [5]. However, close reinvestigation of this patient identified the true defect at the level of d-bifunctional protein [6]. Thus, whether human patients with alterations at the level of the peroxisomal 3-ketoacyl-CoA thiolase exist still remains an open question.

Other and/or similar peroxisomal enzymes such as branched-chain fatty acyl-CoA oxidase, D-PBE and sterol carrier protein2/3-ketoacyl-CoA thiolase (SCP2/SCPx) are rather devoted to the oxidation of branched-chain fatty acyl-CoAs.

Besides LCFAs and VLCFAs, 2-methyl-branched-chain, dicarboxylic fatty acids, eicosanoids and bile acids derivatives have been shown to be also metabolized within the peroxisomal matrix, extending the importance of the peroxisome. In agreement with the critical role of the peroxisome in lipid metabolism, absence of a single enzyme of the peroxisomal β-oxidation system has been shown to be associated with serious plasma lipid disorders, as shown by the marked elevation of plasma VLCFAs levels in patients suffering from *Acox-1* deficiency [7]. Ablation of the *Acox-1* gene in mice also revealed the critical role of this enzyme in lipid metabolism, since *Acox-1* null mice suffered from a pronounced fatty liver which was associated with the constitutive activation of prototypical PPARα target genes and a spontaneous peroxisome proliferation [8]. Besides *Acox-1*, other mouse models deficient for a single peroxisomal enzyme have been established over the past few years. It was recently found that mice deficient for *Mfp-2* displayed a coordinated induction of the transcription factors PPARα and SREBP2 [9]. In turn, the expression of typical SREBP2 and PPARα target genes was markedly induced in the liver of *Mfp-2*$^{-/-}$ mice, leading to decreased levels of plasma triglycerides (TG) and free fatty acids (FFA) in adult mice. Others studies indicate that lack of *Scp-x thiolase* gene (Sterol Carrier Protein) only was enough to decrease ability of the mutant mice to metabolize branched-chain lipids [10]. With respect to the double loss of Sterol Carrier Protein X/Sterol Carrier Protein-2, null mice displayed alteration in the peroxisomal β-oxidation of 2-methyl-branched chain fatty acids [11]. Further investigations also led to the finding that SCPx/SCP2 thiolase was involved in the conversion of cholesterol into bile acids [12].

A deficient mouse model for ThB was previously generated and partially characterized, yet the *in vivo* role of ThB still remains ill defined [13]. In order to close in on the potential *in vivo* relevance of ThB, we studied WT and *Thb*$^{-/-}$ mice fed or not the potent PPARα agonist Wy14,643 (Wy). The nuclear hormone receptor PPARα plays a pivotal role in fatty acid handling by up-regulating the expression of numerous genes involved in mitochondrial and peroxisomal oxidation [14]. Here, exposure of mice to Wy is expected to markedly stimulate peroxisomal FAO and *Thb* mRNA levels in WT mice and not or less in *Thb*$^{-/-}$ mice, thus helping in the identification and characterization of the roles of ThB *in vivo*.

Our main finding is that exposure of *Thb*$^{-/-}$ mice to Wy leads to a significant elevation of the hepatic content of MUFA n-7 and n-9 fatty acids, secondary to the induction of the microsomal SCD1 enzyme, through a likely SREBP1 independent manner.

## 2. Materials and methods

### 2.1. Animal experiments

All mice were on a pure-bred Sv129 genetic background. Male animals were kept in normal cages with food and water *ad libitum*. Mice were routinely fed a commercial and standard pellet diet (UAR A03-10 pellets from Usine d'Alimentation Rationnelle, Epinay sur Orge, France, 3.2 kcal/g) consisting (by mass) about 5.1% of fat (±0.89% of C16:0, ±0.09% of C16:1n-7, ±0.45% of C18:0, ±1.06% of C18:1n-9, ±1.53% of C18:2n-9 and traces of C18:3n-9). At the time of sacrifice, animals were around 4–5 months of age. Male mice in the fasted state were deprived of food for 6 h starting at 4:0 pm. Blood was collected *via* cardiac or orbital puncture into EDTA tubes. Tissues were excised, weighted and immediately frozen in liquid nitrogen before being stocked at −80 °C. The animal experiments were approved by the animal experimentation committee of the University of Burgundy (protocol number *n*°1904) and were performed according to the European Union guidelines for animal care.

### 2.2. Chemicals

Wy14,643 (Wy) was obtained from ChemSyn Laboratories (Lenexa, Kanasa). SYBR green was from Eurogentec. Standards for fatty acids measurement by HPLC were from Sigma or Larodan. Pentafluorobenzyl bromide, N,N′-diisopropylethylamine, potassium hydroxide and BHT were from Sigma. All solvents were HPLC grade.

### 2.3. Histology and neutral fat staining

Haematoxylin and Eosin staining of liver sections were done using standard protocols. Histology was examined on frozen sections after Oil Red O staining for neutral lipid using standard procedures.

### 2.4. Liver triglycerides

Total lipids from liver were extracted with chloroform/methanol (2/1 v/v) according to the method of Folch. Glyceryl triheptadecanoate as an internal standard was added for quantification. Triglycerides were separated by thin layer chromatography (TLC) using hexane/ethylique ether/acetic acid (90/30/1 v/v/v) as solvent. The triglycerides were then scaped and extracted from silica gel with chloroform/methanol (9/1 v/v). The total fatty acids of triglycerides were methylated and quantified by Gas Chromatography (GC) using a Chrompack CP 9002 Gas Chromatograph equipped with a Varian FactorFour VF-23 ms capillary column (30 m × 0,32 mm).

### 2.5. Liver and plasma fatty acid profile

10 mg of liver (or 50 μl of plasma) was saponified with 1 ml of ethanolic potassium hydroxide (final concentration 0.6 N) containing 10 μg of heptadecanoic acid and 250 ng of tricosanoic acid as internal standards and 50 mg/L of butylated hydroxytoluene. The tubes were incubated at 56 °C for 45 min. After incubation, fatty acids were extracted by adding 1 ml of 1.2 M HCl and 2 ml of hexane to each tube. The tubes were shaken at room temperature for 5 mn, centrifuged and the organic phase was evaporated to dryness under nitrogen and derivatized to pentafluorobenzyl esters with 100 μl of acetonitrile, 5 μl of pentafluorobenzyl bromide and 5 μl of diisopropylethylamine at room temperature for 30 min. One ml of water was added and derivatives were extracted with 2 ml of hexane. After centrifugation, the organic phase was evaporated and 100 μL of hexane were added. One μl was further injected in the split mode. Quantification of liver and plasma fatty acid esters was performed using a HP7890A Gas Chromatograph equipped with an HP7683 Injector and a HP5975C Mass Selective Detector (Agilent Technologies). Chromatography was performed using an HP-5MS fused silica capillary column (30 m × 0.25 mm inner diameter, 0.25 μm film thickness, Agilent Technologies). The GC–MS conditions were as follows: carrier gas, helium at a flow-rate of 1.1 ml/min; injector temperature, 250 °C, split mode; oven temperature 140 °C, increased at 5 °C/min to 300 °C, and held for 10 min. The mass spectrometer was operated under negative chemical ionization mode with methane as reactant gaz. The ion source temperature and the quadrupole temperature were 150 °C and 106 °C respectively. A SIM program was used for mass spectrometry with [M − 181] (−) ions as quantification.