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Peroxisome deficient *aP2–Pex5* knockout mice display impaired white adipocyte and muscle function concomitant with reduced adrenergic tone

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ABSTRACT

Peroxisomes are essential for intermediary lipid metabolism, but the role of these organelles has been primarily studied in the liver. We recently generated *aP2–Pex5* conditional knockout mice that due to the nonselectivity of the *aP2* promoter, not only had dysfunctional peroxisomes in the adipose tissue but also in the central and peripheral nervous system, besides some other tissues. Peroxisomes were however intact in the liver, heart, pancreas and muscle. Surprisingly, these mice not only showed dysfunctional white adipose tissue with increased fat mass and reduced lipolysis but also the skeletal muscle was affected including impaired shivering thermogenesis, reduced motor performance and increased insulin resistance. Non-shivering thermogenesis by brown adipose tissue was not altered. Strongly reduced levels of plasma adrenaline and to a lesser extent noradrenaline, impaired expression of catecholamine synthesizing enzymes in the adrenal medulla and reversal of all pathologies after administration of the β-agonist isoproterenol indicated that β-adrenergic signaling was reduced. Based on normal white adipose and muscle function in *Nestin–Pex5* and *Wnt–Pex5* knockout mice respectively, it is unlikely that peroxisome absence from the central and peripheral nervous system caused the phenotype. We conclude that peroxisomal metabolism is necessary to maintain the adrenergic tone in mice, which in turn determines metabolic homeostasis.

1. Introduction

Peroxisomes are organelles that reside in practically all mammalian cells but their function in intermediary metabolism has mainly been studied in the liver. They participate in lipid metabolism and are indispensable for the degradation of a subset of carboxylates via α- and β-oxidation [1], for the formation of conjugated bile acids from cholesterol and for the synthesis of certain polyunsaturated fatty acids (PUFAs) and ether lipids [2]. Furthermore, peroxisomes are involved in the metabolism of reactive oxygen and nitrogen species, glyoxylate and polyamine metabolism, amino acid catabolism and in a few other conversions [2].

The pathologies of peroxisomal disorders point out that peroxisomal metabolism is important for the functioning of multiple organs and tissues [3,4]. In particular, the developmental and degenerative anomalies of the nervous system in peroxisome biogenesis disorders imply that peroxisomes are essential in the brain, spinal cord and nerves. The tight connection between peroxisomes and the nervous system has been strengthened by analyzing knockout mouse models in which several of the human pathologies are mimicked [5–8]. Other tissues in which peroxisomes appear to play an essential role based on studies in patients and animal models are liver, kidney, bone, testis and eyes [3,9,10] indicating that these organelles have a broad role in tissue homeostasis. At present, our understanding of disease mechanisms as a consequence of peroxisome deficiency is rather limited.

In view of their importance in lipid metabolism, we planned to explore whether peroxisomes are essential for the functioning of adipose tissue. In fact, there are sparse reports in the literature

Abbreviations: Acox, acyl CoA oxidase; ABCD, ATP binding cassette transporter type D; BAT, brown adipose tissue; CNS, central nervous system; DBH, dopamine-β-hydroxylase; DEXA, dual-energy X-ray absorptiometry; DHA, docosahexaenoic acid; DHAPAT, dihydroxyacetone phosphate acyltransferase; GTT, glucose tolerance test; HFD, high fat diet; ITT, insulin tolerance test; PNS, peripheral nervous system; PUFA, polyunsaturated fatty acids; SNS, sympathetic nervous system; TG, triglycerides; TH, tyrosine hydroxylase; WAT, white adipose tissue.

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suggesting that peroxisomes might be necessary in the adipose tissue during the differentiation process or during adult life. It was shown that differentiating 3T3 cells rapidly acquire the ability to α-oxidize unbranched chain fatty acids [11]. Furthermore, there are indications for an active peroxisomal β-oxidation in mature WAT that can be stimulated by PPARα ligands such as bezafibrate [12]. It is also remarkable that some enzymes that are specifically involved in the metabolism of branched chain fatty acids, pristanoyl-CoA oxidase (Acox3) [1] and the acyl-CoA thioesterase Acot6, are highly expressed in WAT [13,14]. Furthermore, it was reported that the ABCD2 transporter present in the peroxisomal membrane is enriched in WAT and is up regulated during adipogenesis although it was not essential for adipocyte differentiation nor for lipid accumulation [15]. Finally, during differentiation of mouse 3T3-L1 preadipocytes into adipocytes, the activity of dihydroxyacetone phosphate acyltransferase (DHAPAT) and alkyl/acyl-CoA reductase, two peroxisomal enzymes involved in ether lipid synthesis strongly increased but the third peroxisomal enzyme of this pathway, alkyl-DHAP synthase was decreased [16]. This was not accompanied by an increase in ether lipid content, but it was shown that 40–50% of the triglycerides (TG) were synthesized via the peroxisomal acyl-dihydroxyacetone phosphate pathway instead of the expected ER localized pathway starting from glycerol-3-phosphate. More recently, it was shown that *Pex7* knockout mice that are deficient in ether lipids have reduced lipid stores in WAT and BAT that could be replenished by feeding the mice with ether lipid precursors [17]. Furthermore, it was reported that ether phospholipids are ligands of PPARγ, one of the transcription factors that drive adipocyte differentiation [18].

With regard to brown adipocytes, a potential role of peroxisomes in thermogenic metabolism was already suggested several decades ago [19–21]. Because the energy of the β-oxidation of fatty acids is not conserved as ATP, peroxisomal β-oxidation is considered to be thermogenic. During cold adaption, proliferation of peroxisomes was reported and it was suggested that this increase is mediated by up regulation of PPARγ coactivating factor 1α (PGC-1α) and is independent of PPARα [22].

In order to study the importance of peroxisomes for the functioning of brown and white adipose tissues, we recently generated conditional *aP2–Pex5*$^{-/-}$ mice. PEX5 is the import receptor of peroxisomal matrix proteins and its absence precludes formation of functional peroxisomes [23]. However, we and others noticed that the *aP2* promoter lacks specificity to drive Cre expression [24] as it also active in non-adipogenic tissues, especially in cells sharing a common lineage with adipocytes such as chondrocytes, myocytes, neurons and osteocytes [24,25]. Furthermore, it was shown that *aP2-Cre* is active in developing bone marrow [17], endothelial cells [26] and in macrophages [27]. On the other hand, we confirmed that in liver, heart, muscle and pancreas of *aP2–Pex5*$^{-/-}$ mice peroxisomes were import competent [24]. We now phenotyped these *aP2–Pex5*$^{-/-}$ mice and found besides increased fat mass and reduced lipolysis an impaired adrenergic tone which impacts on muscle function.

2. Methods

Chemicals and biochemicals were purchased from the following suppliers (unless otherwise stated): Acros (Geel, Belgium), Biosolve (Valkenswaard, the Netherlands), Eurogentec (Seraing, Belgium), Invitrogen (Merelbeke, Belgium), Sigma-Aldrich (Bornem, Belgium) and VWR (Leuven, Belgium).

2.1. Ethics statement

All animal experiments were performed in accordance with the "Guidelines for Care and Use of Experimental Animals" and fully approved by the Research Advisory Committee (Research Ethical committee) of the KU Leuven (#159/2008).

2.2. Mouse breeding, genotyping and sacrifice

Mice were housed in the KU Leuven animal facilities at a temperature of 20 °C with equal periods (12 h) of darkness and light. Animals were fed a standard rodent chow and given free access to water. Offspring was weaned at the age of 3 weeks and genotyped. Only male mice at an age of 20 weeks were used for analyses, unless otherwise stated.

For some experiments, mice were kept on a high fat diet (HFD) (TD88137, Harlan Teklad Zeist, The Netherlands) containing 42% kcal as fat, or mice were fed the standard chow fortified with 0.5% (w/w) phytol. *aP2–Pex5* knockout mice were obtained by breeding *aP2-Cre* mice (strain B6.Cg-Tg(Fabp4-Cre) 1Rev/J) purchased from The Jackson Laboratory (Bar Harbor, ME)[28] with *Pex5–loxP* mice [29] as previously described [24]. Other conditional *Pex5* knockout mice used in this study were obtained by breeding *Pex5–loxP* mice with *Nestin-Cre* mice [30] (targeting the central nervous system, CNS) or with Wnt1-Cre mice (targeting the peripheral nervous system, PNS), dorsal root ganglia, melanocytes, adrenal medulla and craniofacial mesenchyme [31]. Furthermore, tamoxifen inducible mice in which the Cre–ER™ fusion protein is under the control of the ubiquitously active CMV promoter [32] were obtained from The Jackson Laboratory. Tamoxifen was intraperitoneally (i.p.) injected in *CMV–Cre–ER™–Pex5–loxP* mice at the age of 4 weeks at a dose of 0.2 mg/g body weight. All mice received 5 injections, with one day intervals.

aP2–Pex5 knockout mice were in a mixed Swiss/C57BL/6 background but in order to minimize variation, each *aP2–Pex5*$^{-/-}$ mouse had a control counterpart from the same litter. Genotyping of the *Cre* and *Pex5* alleles was performed as previously described [10].

Mice received an overdose of sodium pentobarbital (150 mg/kg Nembutal, Abbott Laboratories, North Chicago, IL, USA) by i.p. injection before removal of organs (gonadal WAT, subcutaneous WAT, interscapular BAT, soleus muscle, adrenal medulla) or perfusion fixation.

2.3. Macroscopic analyses and physiological tests

Body weights were measured once a week and food consumption twice a week during 10 weeks. To determine adipose content, dual-energy X-ray absorptiometry (DEXA) (PIXImus™ densitometer, Lunar Corp, Madison, WI, USA) was used on sedated mice [33].

Spontaneous physical activity was evaluated by placing the mice in a separate cage equipped with a turning wheel linked to a computer to register full turning cycles in a 48 h-period. Data are expressed as number of cycles per 24 h.

Forced physical activity was tested with a treadmill (Columbus Instruments, Ohio, USA). After acclimatization by running at 10 m/min for 15 min over 3 consecutive days, forced exercise endurance was tested in a graded exercise test with an initial velocity of 6 m/min and an increase in velocity of 2 m/min every 5 min. Mice were running at a 10° incline (uphill) to induce concentric exercise in the crural musculature, which recruits primarily oxidative muscle fibers. Exhaustion was defined as the point at which mice were unable to continue running in response to a tap on the lower back.

To test thermogenic capacity of BAT, mice were housed individually at 4 °C with free access to food and water, during 2 weeks. The body temperature was measured with a rectal probe (RET-3, Physitemp Instruments, Inc., Clifton, NJ, USA) twice a day, food intake and body weight were measured every other day. To examine shivering thermogenesis, overnight fasted mice were housed individually at 4 °C during maximum of 6 h while the body temperature was measured every 2 h.

To measure the heart beating rate, mice were anesthetized by i.p. administration of 1.4 g/kg urethane (Sigma, Steinheim, Germany). Mice were placed in a supine position and body temperature was