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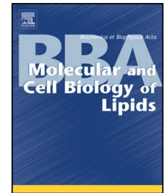
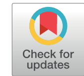


Lysosomal acid lipase regulates fatty acid channeling in brown adipose tissue to maintain thermogenesis



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ABSTRACT

Lysosomal acid lipase (LAL) is the only known enzyme, which hydrolyzes cholesteryl esters and triacylglycerols in lysosomes of multiple cells and tissues. Here, we explored the role of LAL in brown adipose tissue (BAT). LAL-deficient (Lal−/−) mice exhibit markedly reduced UCP1 expression in BAT, modified BAT morphology with accumulation of lysosomes, and mitochondrial dysfunction, consequently leading to regular hypothermic events in mice kept at room temperature. Cold exposure resulted in reduced lipid uptake into BAT, thereby aggravating dyslipidemia and causing life threatening hypothermia in Lal−/− mice. Linking LAL as a potential regulator of lipoprotein lipase activity, we found Angptl4 mRNA expression upregulated in BAT. Our data demonstrate that LAL is critical for shuttling fatty acids derived from circulating lipoproteins to BAT during cold exposure. We conclude that inhibited lysosomal lipid hydrolysis in BAT leads to impaired thermogenesis in Lal−/− mice.

1. Introduction

Lysosomal acid lipase (LAL) hydrolyzes cholesteryl esters (CE), triacylglycerols (TG), and retinyl esters within lysosomes after lipoprotein uptake or through autophagy, respectively [1–3]. In humans and mice, absence or dysfunction of LAL results in excessive lysosomal lipid accumulation, supporting the notion that LAL is responsible for lipid degradation at acidic pH in lysosomes [4–7]. In general, the disease severity in humans (collectively referred to as LAL deficiency, LAL-D) is primarily based on the absence or amount of residual LAL activity determined by LAL gene mutations. Whereas patients with total loss in LAL activity hardly survive one year of age, residual enzymatic activity is found in individuals with cholesteryl ester storage disease, a rare but still underdiagnosed condition. These patients survive beyond mid-age but develop hepatic and atherosclerotic complications, leading eventually to premature mortality [8,9]. Beside severe hepatomegaly, the phenotype of Lal-deficient (−/−) mice includes progressive loss of white adipose tissue (WAT) and brown adipose tissue (BAT) during aging [5]. Our previous study revealed reduced circulating leptin concentrations in the fed state, hypercholesterolemia, and hypoglycemia but improved glucose tolerance and insulin sensitivity in Lal−/− mice [10].

In mammals, BAT is responsible for non-shivering thermogenesis and thereby plays an important role in maintaining body temperature homeostasis, particularly in a cold environment. BAT is morphologically and functionally different from WAT due to its multilocular lipid droplets (LD), high abundance of mitochondria, increased metabolic rate, and the presence of uncoupling protein 1 (UCP1) [11,12]. UCP1 has a unique role to uncouple mitochondrial respiration from ATP production and thereby supports thermogenesis during cold exposure [13]. Additionally, the uncoupling activity of UCP1 is increased by fatty acids (FA) independently of their metabolism [14]. Cold stimulates BAT *via* β3 adrenergic receptor signaling, which triggers the utilization of FA and glucose as substrates for thermogenesis. To fuel this process, TG stored in cytosolic LD of brown adipocytes are initially catabolized *via* lipolysis mediated by the consecutive action of adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), and monoglyceride lipase [15,16]. As brown adipocytes have limited fat storage capacity, LD are

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continuously replenished by the supply of FA from the blood stream [15]. For this purpose, food intake is increased during cold and TG (in form of circulating chylomicrons) deliver sufficient FA to activated BAT [11]. In addition, WAT serves as TG depot to release FA in times of need, like during nutrient restriction or high metabolic demand *via* ATGL-mediated hydrolysis [17].

Beside cholesterol, excessive TG-rich lipoprotein (TRL) concentrations in the blood cause dyslipidemia, a risk factor for atherosclerosis and the metabolic syndrome [18,19]. New hope for hyperlipidemia treatment arose when TRL were shown to be massively cleared by activated BAT [20]. Intensive research in the last decade has revealed multiple benefits of BAT activation in reducing not only plasma TG but also cholesterol concentrations, improving glucose tolerance and insulin sensitivity, and decreasing fat depots [21,22]. Enhancing BAT activity was reported to protect from atherosclerosis development by accelerating blood cholesterol clearance in mice expressing functional apolipoprotein E and low-density lipoprotein receptor [22]. Besides lipoprotein lipase (LPL)-mediated lipolysis of TRL, an alternative uptake of entire lipoprotein particles by BAT was suggested. This process is dependent on LPL/CD36 tandem action and is possibly mediated by increased endothelial permeability or transcytosis [20]. To which extent holoparticle uptake by BAT affects thermogenesis is still unclear [23]. This type of substrate processing would require receptor-mediated endocytosis and consequently lysosomal lipolytic degradation *via* LAL [1].

Due to major metabolic adaptations caused by LAL deficiency and reduced body adiposity, we aimed to study the consequences of LAL deficiency on BAT function and thermogenesis. Here we show that Lal−/− mice have preserved weight but modified morphology and defective functionality of BAT. LAL deficiency strongly decreases UCP1 protein expression leading to transient hypothermia in mice housed at room temperature (RT). When kept at 5 °C, Lal−/− mice present life threatening cold intolerance. The current study provides evidence that LAL deficiency causes accumulation of lipid-laden lysosomes in BAT and that FA released by acid hydrolysis serve as thermogenic activators through multiple mechanisms. Importantly, cold exacerbates dyslipidemia in Lal−/− mice due to reduced FA uptake in BAT, suggesting that restoring blood lipoprotein balance depends on functional LAL.

2. Material and methods

2.1. Animals

All experiments were performed using female and male Lal−/− mice and their corresponding wild type (WT) littermates [5] on a C57BL/6 background [10] aged 20 weeks unless stated otherwise. The mice were maintained in a clean environment and a regular light-dark cycle (12 h light, 12 h dark) with unlimited access to chow diet (4% fat and 9% protein; Altromin 1324, Lage, Germany) and water. For cold exposure experiments, Lal−/− and WT mice were kept at 5 °C with food and water *ad libitum*. Core body temperature was measured using a rectal probe thermometer (Physitemp Instruments, Inc., Clifton, NJ). Blood glucose was determined using Accu-Chek® Active glucometer and glucose strips (Roche Diagnostics GmbH, Mannheim, Germany).

All animal experiments were performed in compliance with national laws and were approved by the Austrian Federal Ministry of Science, Research, and Economy, Vienna, Austria, in accordance with the European Directive 2010/63/EU.

2.2. Plasma lipid and creatine phosphokinase activity measurements

Blood was collected by facial vein puncture and plasma was prepared within 20 min. TG, total cholesterol (TC), and free cholesterol (FC) concentrations were determined in plasma from mice using enzymatic kits (DiaSys, Holzheim, Germany). Plasma free glycerol (FG) and free fatty acid (FFA) concentrations were determined using Free Glycerol Reagent (Sigma-Aldrich, St. Louis, MO) and NEFA-HR kit (Wako Life Sciences, Mountain View, CA), respectively. Lipoprotein fractions were separated from 200 μl pooled plasma from each group using fast protein liquid chromatography as previously described [24]. Creatine phosphokinase activity in plasma samples was measured using a Spotchem EZ analyzer and test strips (A. Menarini GmbH, Vienna, Austria).

2.3. Energy metabolism in vivo

Assessment of energy intake and energy expenditure was performed using a climate-controlled indirect calorimetry system (TSE PhenoMaster, TSE Systems, Bad Homburg, Germany). WT and Lal−/− mice were housed in automatic metabolic cages in a regular light-dark cycle (12 h light, 12 h dark) with free access to food and water. Weight-matched female mice were acclimatized for 48 h before experiments. During gradual cooling, the temperature was decreased from 22 °C to 5 °C over a period of 7 days. O_2 consumption, CO_2 production, and locomotor activity (using infrared sensor frames) were measured every 15 min. Carbohydrate and lipid oxidation rates were determined as described [25] and converted from mg/h into kcal/h.

2.4. Mitochondrial respiration measurement

WT and Lal−/− mice were housed at 5 °C for 4 h. Oxygen consumption rate of freshly isolated BAT was determined using a Clark electrode (Strathkelvin Instruments, Glasgow, Scotland) as previously described [26] with minor modifications. Approximately 20 mg of BAT were minced and transferred into the MT200A measurement chamber containing 1 ml of 100% air-saturated respiration buffer (2% BSA, 100 mg/dl or 450 mg/dl D-glucose, 6 mg pyruvate). Maximal respiration was measured after the addition of 25 μM carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP) to the respiration buffer. Oxygen consumption rates were normalized to tissue weight and calculated as μg O_2/min/tissue.

2.5. Determination of BAT and SM acyl-CoA and acyl-carnitine concentrations

Female mice were exposed to cold for 3 h and BAT was collected and snap-frozen in liquid nitrogen. Tissue samples were homogenized for 2 × 30 s using a tissue homogenizer (Precellys 24, Bertin Technologies, Montigny-le-Bretonneux, France). Concentrations of acyl-CoAs and carnitine esters (semi-quantitative) were measured by on-line solid phase extraction liquid chromatography-mass spectrometry as previously described [27].

2.6. Reverse transcription and quantitative real-time PCR

Two micrograms of total RNA were reverse transcribed (High Capacity cDNA Reverse Transcription Kit; Applied Biosystems, Carlsbad, CA). Quantitative real-time PCR of samples analyzed in duplicate was performed on a Roche LightCycler 480 (Roche Diagnostics, Palo Alto, CA) using the QuantiFastTM SYBR® Green PCR Kit (Qiagen, Valencia, CA). Expression profiles and associated statistical parameters were calculated using the $2^{-\Delta\Delta CT}$ method normalized to the expression of cyclophilin A as housekeeping gene. Primer sequences are listed in the supplement.

2.7. Western blotting analysis

BAT was lysed in RIPA buffer and protein concentrations were quantitated (DC™ Protein assay, Bio-Rad Laboratories, Hercules, CA). BAT lysates (40 μg protein) were separated by SDS-PAGE and transferred onto PVDF membranes. Non-specific binding sites of the membranes were blocked (5% solution of milk powder or 1% BSA in

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