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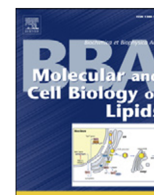

Effects of adipocyte lipoprotein lipase on *de novo* lipogenesis and white adipose tissue browning ☆

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ABSTRACT

Efficient storage of dietary and endogenous fatty acids is a prerequisite for a healthy adipose tissue function. Lipoprotein lipase (LPL) is the master regulator of fatty acid uptake from triglyceride-rich lipoproteins. In addition to LPL-mediated fatty acid uptake, adipocytes are able to synthesize fatty acids from non-lipid precursor, a process called *de novo* lipogenesis (DNL). As the physiological relevance of fatty acid uptake *versus* DNL for brown and white adipocyte function remains unclear, we studied the role of adipocyte LPL using adipocyte-specific LPL knockout animals (aLKO). ALKO mice displayed a profound increase in DNL-fatty acids, especially palmitoleate and myristoleate in brown adipose tissue (BAT) and white adipose tissue (WAT) depots while essential dietary fatty acids were markedly decreased. Consequently, we found increased expression in adipose tissues of genes encoding DNL enzymes (*Fasn*, *Scd1*, and *Elovl6*) as well as the lipogenic transcription factor carbohydrate response element binding protein-β. In a high-fat diet (HFD) study aLKO mice were characterized by reduced adiposity and improved plasma insulin and adipokines. However, neither glucose tolerance nor inflammatory markers were ameliorated in aLKO mice compared to controls. No signs of increased BAT activation or WAT browning were detected in aLKO mice either on HFD or after 1 week of β3-adrenergic stimulation using CL316,243. We conclude that despite a profound increase in DNL-derived fatty acids, proposed to be metabolically favorable, aLKO mice are not protected from metabolic disease *per se*. In addition, induction of DNL alone is not sufficient to promote browning of WAT. This article is part of a Special Issue entitled Brown and White Fat: From Signaling to Disease.

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1. Introduction

Lipoprotein lipase (LPL) is an enzyme essential for the degradation of triglyceride-rich lipoproteins (TRL) in the intraluminal space of capillaries. LPL is highly expressed in its major tissues of action, heart, skeletal muscle and adipose tissue. Complete knockout of LPL is perinatally lethal in mice [1]. In order to study the role of LPL in lipid metabolism, mice with transgenic LPL expression in the skeletal muscle on a LPL knockout background (L0-MCK) were generated [2,3]. Mice deficient for glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (GPIHBP1$^{-/-}$), a protein essential for intraluminal localization of LPL in blood vessels [4,5], are characterized by strongly reduced intraluminal levels of LPL, presumably in all metabolically active tissues. The L0-MCK mice have reduced plasma triglycerides (TG), due to deposition of fatty acids into the muscle, whereas the GPIHBP1$^{-/-}$ mice have highly elevated plasma TG reminiscent of human hyperchylomicronemia [6], likely due to the systemically impaired LPL function. These studies, as well as studies using mice with transgenic overexpression or knockout of physiological LPL modulators (reviewed in [7]), have demonstrated that LPL not only regulates plasma TG concentration but that it is also quantitatively important for deposition of fatty acids in adipose tissues. Mice with increased LPL activity have higher fat mass and are more insulin resistant [8] whereas the opposite is true for mice with decreased adipose LPL [9], suggesting a functional link of adipose tissue LPL to metabolic disease.

Abbreviations: Acaca, acetyl CoA carboxylase-α; aLKO, adipocyte LPL knockout; BAT, brown adipose tissue; Dio2, type 2 thyroid hormone deiodinase; ChREBP, carbohydrate response element binding protein; COX-2, cyclooxygenase-2; DNL, *de novo* lipogenesis; Elovl, fatty acid elongase; Fasn, fatty acid synthase; FPLC, fast performance liquid chromatography; Glut4, glucose transporter 4; LPL, lipoprotein lipase; GPIHBP1, glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1; HFD, high-fat diet; Pgc1a, peroxisome proliferator activator receptors gamma coactivator-1-α; PUFA, polyunsaturated fatty acids; Scd1, stearoyl CoA desaturase-1; SEM, standard error of mean; SREBP1c, sterol regulatory element binding protein 1c; Tbp, TATA box binding protein; TG, triglycerides; TRL, triglyceride-rich lipoproteins; WT, wild type; WAT, white adipose tissue; iWAT, inguinal WAT; gWAT, gonadal WAT; Ucp1, uncoupling protein 1

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Excessive accumulation of lipids in white adipose tissue (WAT) accompanied by chronic low grade inflammation and insulin resistance is a hallmark of unhealthy obesity [10]. Conversely, brown adipose tissue (BAT) activated by cold has positive effects on metabolic disease by burning excessive calories [11]. BAT was recently shown to efficiently reduce plasma TG by taking up considerable amounts of TRL [12]. In addition to activating BAT already present, cold exposure or beta-adrenergic stimulation mimicking cold leads to conversion of large white adipocytes into smaller, paucilocular or beige or brite (brown-in-white) adipocytes with numerous lipid droplets and rich in mitochondria containing uncoupling protein 1 (Ucp1) [13,14]. This "browning" of WAT thus leads to the development of adipose tissue morphologically intermediate between WAT and BAT. Previous research has elucidated many of the key signaling pathways inducing the remodeling of WAT. Proximally, sympathetic activation, subsequent beta-adrenergic signaling [15], as well as other recently discovered signals play a major role [16,17]. Distally, transcriptional mechanisms involving peroxisome proliferator activator receptors (PPAR) and PPAR-γ coactivator-1-α (Pgc1a) play a critical role in reprogramming expression toward a brown adipocyte-specific profile [11,19,18]. Another critical mechanism is local activation of thyroid hormone by type 2 deiodinase (Dio2) [20]. The fatty acid elongase Elovl3 is a lipid metabolism enzyme highly induced during BAT activation and browning of WAT. Although it is not essential for surviving cold exposure, this enzyme is apparently important for efficient brown adipocyte remodeling during BAT activation, possibly through regulation of lipid droplet formation [21].

Brownish WAT is believed to have, at least to a substantial extent, the beneficial physiological properties of BAT and, therefore, understanding the conditions promoting its development is an important task. *De novo* lipogenesis (DNL), the *de novo* synthesis of fatty acids from non-lipid substrates, is a lipid metabolic pathway tightly linked to cell growth and expansion of intracellular membranes. It was shown to be a prominent metabolic pathway in BAT [22,23] and it appears to be important for BAT activation [20]. DNL may therefore also play a critical role in the remodeling of WAT during browning. Previously, the L0-MCK mice were found to have reduced adiposity and to exhibit a brownish appearance of white fat pads [3]. Furthermore these mice were reported to have increased WAT DNL, resulting in elevated concentration of DNL-derived fatty acids. In this paper, we set out to study the effect of selective adipocyte LPL-deficiency on lipid metabolism and on adipose tissue phenotype. Specifically, we asked whether adiposity was reduced in these mice and whether they showed signs of WAT browning as indicated by altered expression of key BAT genes.

2. Methods

2.1. Mouse treatments

All experiments were performed with approval from the Animal Welfare Officers at University Medical Center Hamburg-Eppendorf and Behörde für Soziales, Familie, Gesundheit und Verbraucherschutz Hamburg, Germany. Mice were bred and housed in the animal facility of University Medical Center Hamburg-Eppendorf at 22 °C with *ad libitum* access to standard laboratory chow diet (Lasvendi). Mice carrying floxed alleles of Lpl (B6.129S4-*Lpl*^{tm1Ijg}/J) as well as Fabp-Cre (B6.Cg-Tg(Fabp4-cre)1Rev/J) were purchased from Jackson Laboratory (http://www.jax.org). For all experiments littermates were used. The diet-induced obesity model was conducted by feeding a high-fat diet (Bio-Serv F3282, 35 wt.% lard) *ad libitum* for 20 weeks beginning at 4 weeks of age as described [24]. Ucp1-positive adipocyte development was induced by subcutaneous injection of CL316,243 (1 μg per g body weight in 0.9 w/v % NaCl; Tocris) for 7 days. All tissue and blood collections were performed after a 4 h daytime fast. Mice were anesthetized with a lethal dose of Ketamine/Xylazine, blood was withdrawn transcardially with syringes containing 0.5 M EDTA and animals were perfused with PBS containing 10 U/ml heparin. Organs were harvested and immediately conserved in TRIzol® reagent (Invitrogen) or snap-frozen in liquid N_2 and stored at −80 °C for further processing.

2.2. Plasma parameters

Plasma TG and cholesterol were determined using commercial kits (Roche) that were adapted to microtiter plates. For FPLC pooled plasma was separated using S6-superose columns (GE Healthcare) and lipid levels were analyzed in each fraction as described above. Oral glucose tolerance tests were performed by oral administration of glucose (1 mg per g body weight) after a 4 h fasting period. Blood glucose levels were measured at indicated time points using AccuCheck Aviva sticks (Roche). Oral glucose and fat tolerance test was combined by gavage of a mixture of liposomes (2 mg TRL lipids) containing 12 kBq [9,10-^{3}H (N)]-triolein/g body weight and glucose (1 mg/g body weight) traced with 0.62 kBq 2-deoxy-D-[^{14}C]-glucose/g body weight in H_2O as described [12]. Plasma insulin was measured using a commercially available rat/mouse insulin assay kit (Chrystal Chem). Adiponectin and leptin were determined using ELISAs from R&D Systems.

2.3. Lipid and fatty acid analysis

Total hepatic TG and cholesterol levels were quantified as described previously [25]. Tissue extracts for gas chromatography were prepared as described [26] with a solvent amount of 60 μl/mg tissue (iWAT) or 20 μl/mg (liver). TG was separated on silica gel 60 plates: 25 μl of extract was spotted and developed with an eluent containing hexane, diethylether and acetic acid (80:20:1.5). Visualization of lipid bands was performed with primuline (5 mg in 100 ml acetone:water = 80:20). Fatty acid methyl esters were prepared from 25 μl extract (total fatty acids) of the scratched bands without further extraction based on the method of Lepage and Roy [27], by adding 1 ml methanol/toluene (4:1), 100 μl heptadecanoic acid (200 μg/ml in methanol/toluene, 4:1), 100 μl acetylcloride and heating in a capped tube for 1 h at 100 °C. After cooling to room temperature 3 ml of 6% sodium carbonate was added. The mixture was centrifuged (1800 *g* 5 min). 30 μl of the the upper layer was diluted with 120 μl toluene and transferred to auto sampler vials. Gas chromatography analyses were performed using an HP 5890 gas chromatograph (Hewlett Packard) equipped with flame ionization detectors (Stationary phase: DB-225 30 m × 0.25 mm id., film thickness 0.25 μm; Agilent, Böblingen). Peak identification and quantification were performed by comparing retention times respectively peak areas to standard chromatograms. All calculations are based on fatty acid methyl esters values and concentration of individual fatty acids was calculated as % of total fatty acids.

2.4. Expression analysis

Total RNA was isolated and purified from liver or WAT specimens using NucleoSpin RNA II kit (Macherey & Nagel). Complementary DNA was synthesized using SuperScript® III Reverse Transcriptase (Invitrogen). Quantitative real-time PCR reactions for indicated genes were performed on a 7900HT sequence detection system (Applied Biosystems) using TaqMan Assay-on-Demand primer sets (Supplemental Table 1) supplied by Applied Biosystems and selected to recognize RefSeq sequences and a maximum of Genbank ESTs. ChREBP-β expression was determined using a custom-made TaqMan assay based on the Genbank mRNA sequence deposited by Herman et al. [28]: Forward: 5′-AGCCCG ACGCCATCTG-3′, reverse: 5′-TTGAGGCCTTTGAAGTTCTTCCA-3′, reporter: 5′-CCAGCTTGCCACTGAGC-3′. Gene expression was calculated as copy number per copies of the house keeper gene TATA binding protein (Tbp).

2.5. Statistics

Student's *t*-test was used for pairwise comparison of groups. Changes are calculated and plotted as % change of mean of knockout

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