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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.

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*^{tm1lig}/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

(fabp4-Cre$^+$ *lpl* flox) mice compared to wild type (fabp4-Cre$^-$ *lpl* flox) mice and only SEM for fabp4-Cre$^+$ *lpl* flox mice is shown.

3. Results

3.1. Mild hypertriglyceridemia and increased de novo lipogenesis in mice with adipose-specific LPL deficiency

We used a transgenic approach with direct knockout of LPL in adipocytes using Cre recombinase under control of the *Fabp4 (aP2)* promoter. Gene expression analysis of these *Fabp4*-Cre$^+$ *lpl* flox (aLKO) mice revealed that LPL was knocked out at mRNA level by 80–95% in BAT, inguinal WAT (iWAT) and gonadal WAT (gWAT) compared to fabp4-Cre$^-$ *Lpl* flox (WT) controls (Fig. 1A). Among other organs investigated, the spleen showed the strongest decrease (70%) (Fig. 1A), probably reflecting inducible expression from the *aP2* promoter in leukocytes [29,30]. Endothelial lipase (encoded by *Lipg*), an enzyme shown to be induced and compensate for loss of adipose LPL in L0-MCK mice [31], exhibited a trend for upregulation in BAT, but not in WAT, of the aLKO mice (Fig. 1B). In line with the essential role of LPL in metabolizing TRL, plasma TG but not cholesterol levels were markedly increased in the aLKO mice (Fig. 1C). Plasma FPLC analysis revealed a significant increase in the TRL fraction (Fig. 1D, E). Thus, the mildly hypertriglyceridemic aLKO mice were different from the previously studied models which showed either reduced [2] or massively increased plasma TRL levels [32]. Given the high impact of adipose tissue LPL on TRL turnover in the postprandial phase, we performed a combined fat/glucose gavage experiment with radiolabeled tracers (^{3}H-triolein, ^{14}C-deoxyglucose, see Methods). We detected 60 min after gavage increased plasma ^{3}H radioactivity in aLKO animals (Fig. 1F), which was found in the TRL fraction (Fig. 1G). In line with the role of LPL in mediating fatty acid flux from TRL into peripheral tissues, we found that the observed increase in plasma TRL is associated with strongly decreased uptake into BAT (Fig. 1H). A compensatory higher uptake was observed in the liver and heart in aLKO

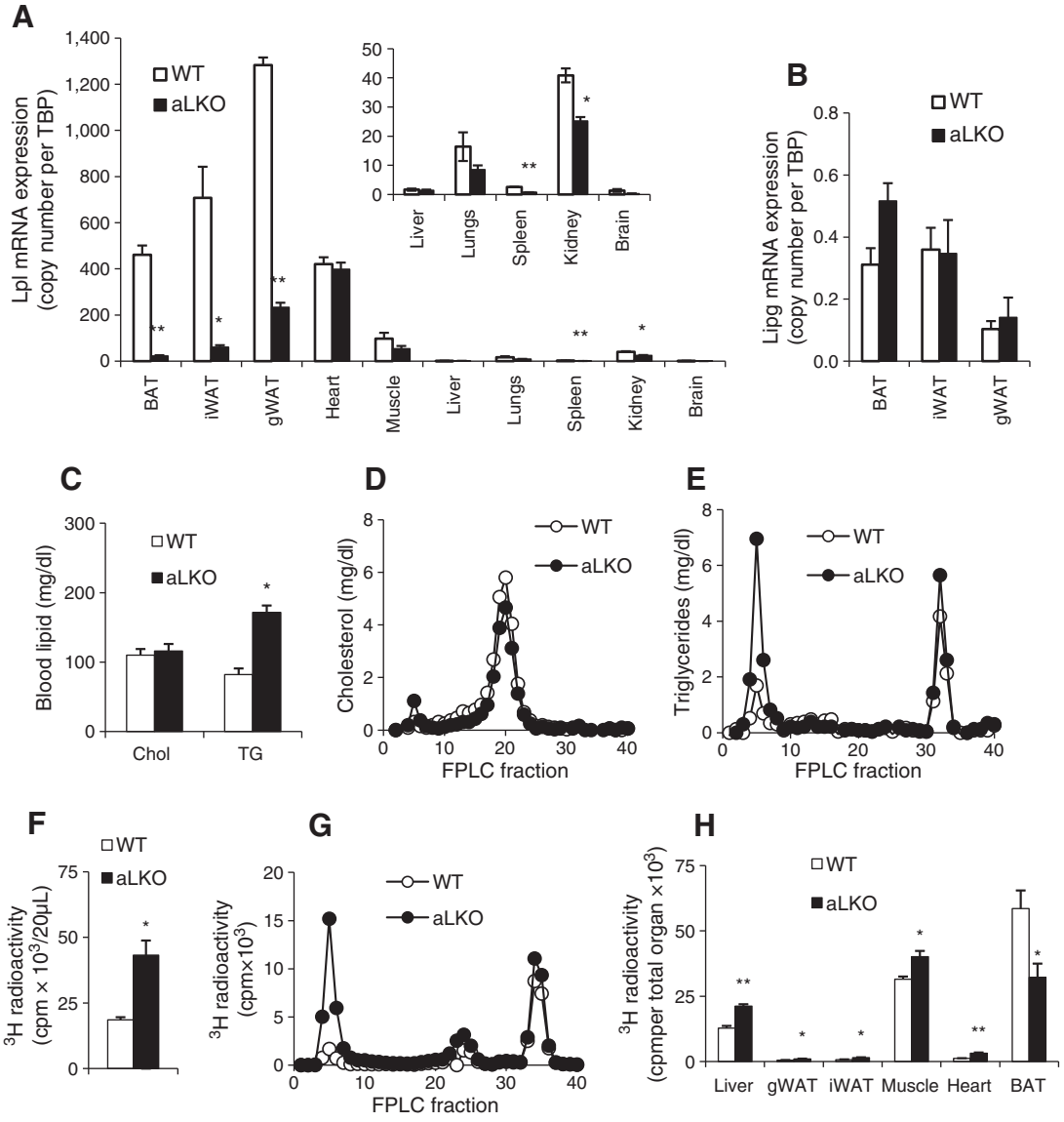

Fig. 1. Selective loss of adipocyte LPL in mice leads to mild hypertriglyceridemia. A) Lpl mRNA expression relative to housekeeper in tissues, B) Lipg mRNA expression in adipose tissues, C) total plasma cholesterol (Chol) and TG concentrations, D) plasma cholesterol, and E) TG fast performance liquid chromatography (FPLC) profiles, F) ^{3}H-radioactivity found in plasma 60 min after gavage, G) ^{3}H-radioactivity after plasma FPLC profiling and H) ^{3}H-radioactivity total organ uptake 60 min after gavage in fabp4-Cre$^+$ *lpl* flox (aLKO) and fabp4-Cre$^-$ *lpl* flox (WT) male mice on chow diet (12 weeks of age, n = 3 in A–E and n = 6 in F–H), mean ± SEM, * $p<0.05$, ** $p<0.001$.

mice, whereas — somewhat paradoxically — WAT depots are characterized by mildly increased organ uptake (Fig. 1H), possibly due to the experimental mixed meal conditions.

As lack of LPL in adipocytes influences fatty acid flux in aLKO mice, it may also lead to an altered fatty acid composition in adipose tissue. We found a substantial increase in DNL-derived C_{14} and C_{16} fatty acids in all adipose tissues investigated, iWAT, gWAT and BAT, (Fig. 2, Supplemental Fig. 1), most notably palmitoleate (C16:1n−7), myristate (C14:0) and myristoleate (C14:1n−5) whereas the relative content of linoleate (C18:2n−6) and other polyunsaturated fatty acids (PUFA) was strongly decreased. Previously, study of the adipose LPL deficiency model with chylomicronemia (GPIHBP1$^{-/-}$) found liver fatty acid changes opposite to adipose tissue, namely increased PUFAs and decreased DNL-derived fatty acids [32]. Surprisingly, in the aLKO mice hepatic fatty acid changes were similar, if weaker, compared to those found in the adipose tissue. Hepatic PUFA content was significantly decreased in livers of aLKO mice and DNL fatty acids showed a trend for increase (Fig. 2). A similar pattern was observed for plasma fatty acids (Supplemental Fig. 1).

The marked increase in DNL fatty acids indicated altered regulation of biosynthetic enzymes. In order to confirm this, we determined expression of key DNL genes in adipose tissues and liver of the mice used for fatty acid analysis. A significant increase or a trend for upregulation was observed for the genes encoding fatty acid synthase (*Fasn*), acetyl CoA carboxylase-α (*Acaca*), stearoyl CoA desaturase-1 (*Scd1*) and fatty acid elongase-6 (*Elovl6*) in BAT, iWAT and gWAT (Fig. 3A). In the GPIHBP1$^{-/-}$ mice, reciprocal regulation was observed in the liver *versus* adipose tissues [32]. In the liver of the aLKO mice, however, expression of DNL genes was not significantly different from that in WT livers (Fig. 3A), arguing that the trend for higher DNL fatty acids in the liver (Fig. 2) was due to free fatty acid flux from adipose tissue in these mice.

One important transcription factor for lipogenic genes, critical for DNL gene expression in adipose tissue [28], is carbohydrate-responsive element binding protein (ChREBP). We hypothesized that in aLKO mice induction of ChREBP would be a mechanism mediating the increased expression of DNL genes. Indeed, the mRNA of ChREBP showed a trend for increased expression in BAT and iWAT and for decreased expression in the liver of aLKO mice (Fig. 3A). We measured mRNA copy number of the recently discovered shorter isoform ChREBP-β which is a better indicator of ChREBP activity than the conventional full-length isoform ChREBP-α, because it has an auto-regulated promoter and apparently has a constitutive nuclear localization [28]. ChREBP-β mRNA correlated very strongly with *Fasn* mRNA in iWAT and in BAT (Fig. 3B), supporting a critical role of ChREBP for the regulation of *Fasn* and other DNL genes. A similar but weaker correlation was found for the mRNA of another major lipogenic transcription factor, SREBP1c (Fig. 3C), which like ChREBP-β trended to be upregulated in WAT and BAT of aLKO mice (Fig. 3A).

ChREBP senses glucose flux and is activated by intermediates of glucose metabolism [33]. In order to further explore this mechanism we measured also the expression of the glucose transporter Glut4 (*Slc2a4*), which is rate-limiting for glucose uptake in adipocytes. *Slc2a4* mRNA was increased in BAT of aLKO mice (Fig. 3A), suggesting a contribution to increased glucose flux and ChREBP activation in adipose tissues. We confirmed this hypothesis by performing an oral glucose tolerance test with ^{14}C-deoxyglucose tracer (see Methods). In line with our assumption, we find that glucose tolerance is slightly improved in aLKO mice (Fig. 3D), being associated with increased glucose uptake selectively in BAT as well as WAT depots (Fig. 3E). These results strongly support the above discussed relationship of glucose flux and DNL in aLKO mice.

3.2. Reciprocal regulation of de novo lipogenesis in iWAT and liver of aLKO mice on a high-fat diet

Next, we addressed the question whether, in the context of diet-induced obesity after high-fat diet (HFD) feeding, LPL deficiency had any impact on adipose tissue and liver DNL. First, we assessed the plasma lipoprotein profile. On HFD compared to chow diet a significant increase in the large, TG-rich HDL was observed, yet this increase was less pronounced in aLKO mice (Fig. 4A). Although total TG levels were decreased after HFD feeding, an effect we found previously in HFD-fed mice [12], we still observed higher TRL concentrations in aLKO mice compared to controls (Fig. 4B).

When we studied iWAT fatty acid patterns of HFD-fed aLKO and WT mice, we surprisingly found, in contrast to adipose tissues on chow diet, increased concentration of some PUFAs (C20:4n−6, C18:3n−3) (Supplemental Fig. 2). In order to rule out that these PUFA changes

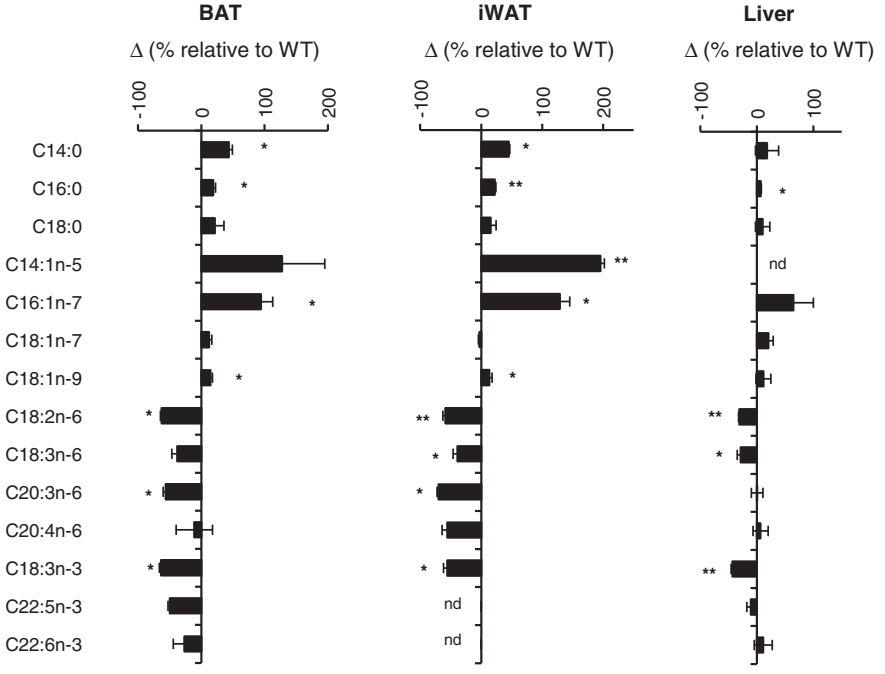

Fig. 2. Loss of adipocyte LPL remodels fatty acid patterns in adipose tissues and liver. Concentrations of individual fatty acids were determined and plotted here as % change of mean in aLKO relative to WT mice. Mean fatty acid concentrations see Supplemental Table 2. Male mice on chow diet (12 weeks of age, n = 3). nd, not detectable, * $p<0.05$, ** $p<0.001$.

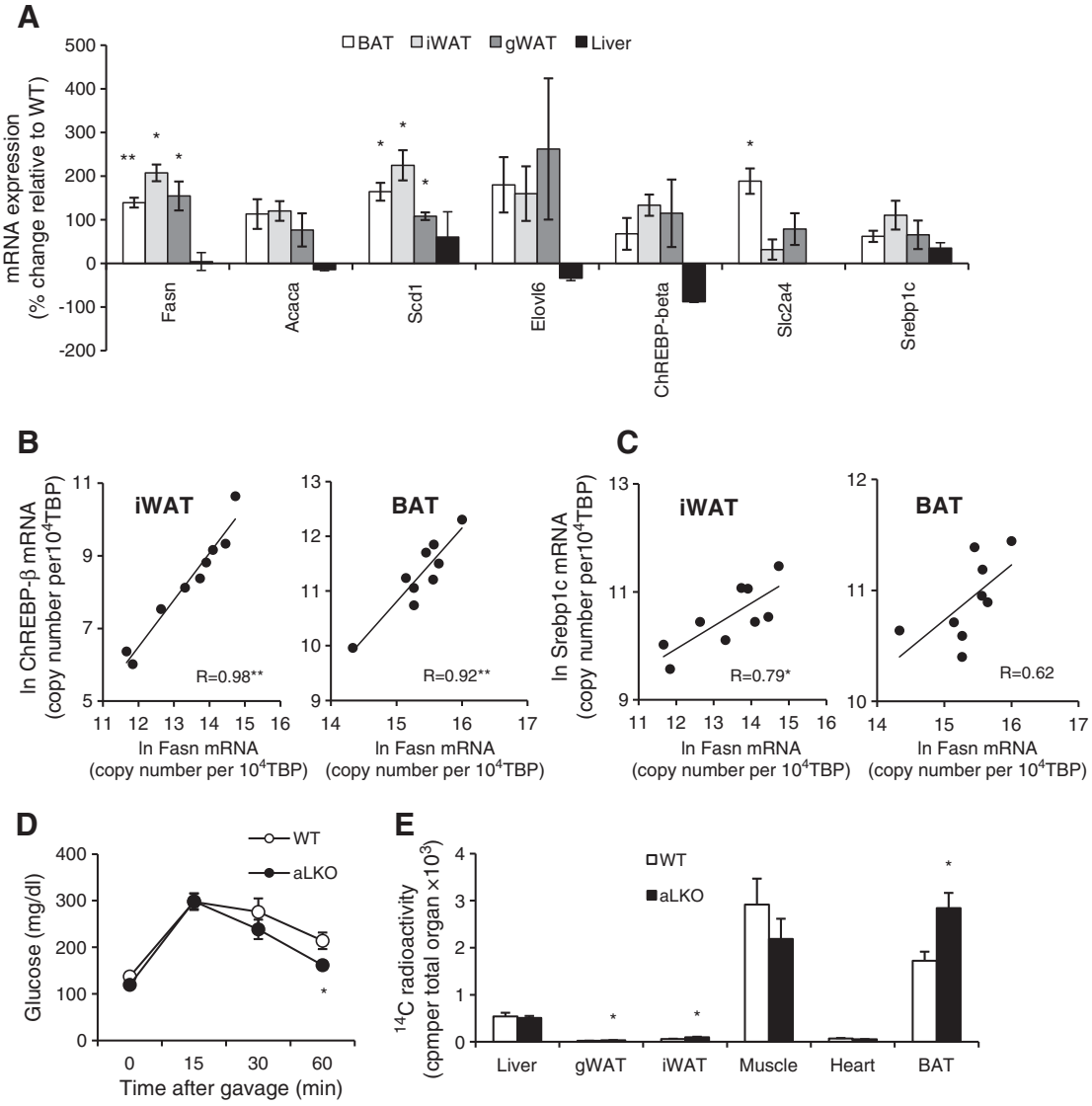

Fig. 3. Loss of adipocyte LPL causes induction of DNL genes in adipose tissues but not liver. A) Relative change of mRNA expression of DNL enzymes, the lipogenic transcription factors ChREBP-β, Srebp1c, and the glucose transporter Glut4 in male aLKO relative to WT mice (12 weeks of age, n = 3). B, C) Pearson correlation of Fasn and B) ChREBP-β and C) Srebp1c mRNA and in BAT and iWAT of male (n = 5) and female (n = 4) mice on chow diet (aLKO: n = 4, WT: n = 5, 24 weeks of age). D) Oral glucose tolerance test and E) ^{14}C-radioactivity (deoxyglucose) total organ uptake 60 min after gavage in aLKO and WT male mice on chow diet (12 weeks of age, n = 6), mean ± SEM, * $p<0.05$, ** $p<0.001$.

were caused by increased membrane phospholipid content in iWAT of aLKO mice, we subsequently focused on the major lipid class of adipose tissue and measured fatty acid patterns in the TG fractions of BAT, iWAT and liver (Fig. 4C). In BAT TG and iWAT TG of aLKO mice on HFD C_{14} and C_{16} DNL fatty acids (C14:0, C14:1n − 5 and C16:1n − 7) were significantly increased, similar to the total lipid in chow diet fed mice (Fig. 2), whereas stearate (C18:0) was strongly decreased (Fig. 4C). TG PUFA showed an opposite pattern in BAT and iWAT. They were suppressed in BAT of aLKO mice, however, in iWAT they were increased, most notably C20:4n − 6 (Fig. 4C). In the liver TG of aLKO mice, C18:0 was increased and C_{14}–C_{16} DNL fatty acids tended to be decreased (Fig. 4C). Taken together, reciprocal regulation of DNL was observed in adipose tissues and liver of aLKO mice. Surprisingly, no signs of PUFA deficiency were observed in aLKO iWAT which could be explained by high C18:2n − 6 content (11% of fatty acid) of the HFD. The expression of *Lipg*, the gene coding for endothelial lipase was unaltered in iWAT of aLKO compared to WT mice, arguing against a compensatory role of this lipase in the absence of LPL (Fig. 4D).

When we determined the expression of DNL genes we found that they were not changed in adipose tissues of aLKO mice (Fig. 4D). Interestingly, consistent with the trend for reduced DNL fatty acid content a significant decrease of DNL genes was observed in the livers of aLKO mice (Fig. 4D) which might be related to an improved steatosis phenotype. Indeed, we observed a trend for decreased TG content in livers of aLKO compared to WT mice (Fig. 4E).

3.3. HFD-fed aLKO mice exhibit less adiposity and improved plasma adipokines but not increased glucose tolerance

Previous work demonstrated reduced fat pad weights and brownish appearance of WAT depots in obese L0-MCK mice [3], suggesting that LPL deficiency induces a browning phenotype in white adipocytes. To test this hypothesis, we explored the adipose tissue phenotype in the HFD-fed aLKO mice. ALKO mice on HFD became obese, however, displayed less adiposity as indicated by reduced fat pad weights compared to WT controls (Fig. 5A, B). Consistent with reduced adiposity, plasma leptin levels were significantly decreased in aLKO compared to WT mice (Fig. 5C). Furthermore, the aLKO mice exhibited a trend for reduced fasting plasma insulin (Fig. 5D) and increased plasma adiponectin (Fig. 5E), and the ratio: adiponectin/insulin was significantly higher in

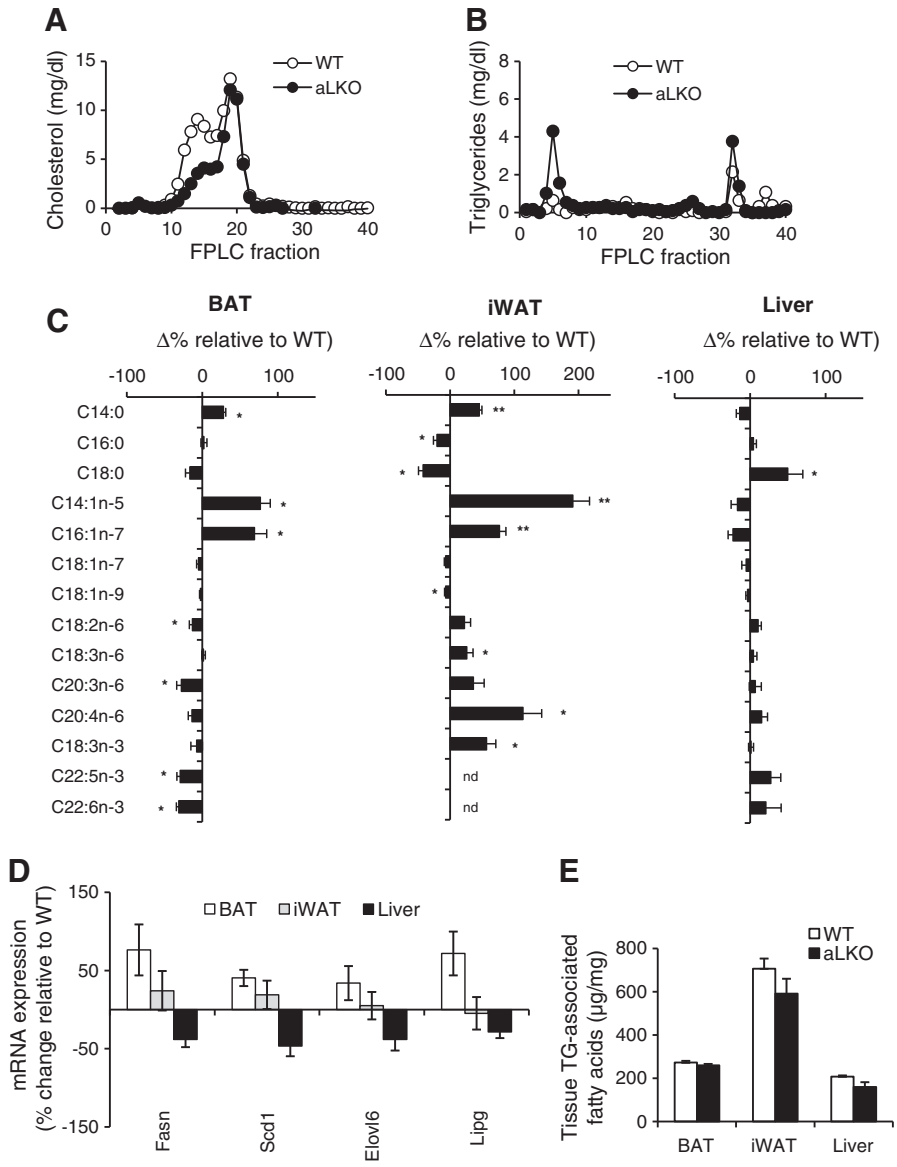

Fig. 4. Effect of adipocyte LPL deficiency on lipid metabolism in mice on HFD. A) Plasma cholesterol (Chol) and B), TG lipoprotein profiles, C) concentrations of individual fatty acids, determined in the tissue TG pools, plotted as % change of means, D) relative change of mRNA expression of DNL enzymes and Lipg, E) total tissue TG-associated fatty acids in aLKO mice and WT controls. Mean fatty acid concentrations see Supplemental Table 3. Male mice on HFD, n = 5, age 24 weeks. Mean ± SEM, nd, not detectable, * $p<0.05$, ** $p<0.001$.

aLKO mice ($p = 0.04$). Although these data indicated a mild improvement in insulin resistance, the aLKO mice were not more glucose tolerant than WT mice in an oral glucose tolerance test (Fig. 5F).

3.4. No significant alteration on inflammation or browning in adipose tissues of aLKO mice on a HFD

Hypothesizing that the strong increase in C16:1n − 7, which may have anti-inflammatory properties [34,35], induced by adipose-specific LPL deficiency translates into reduced obesity-associated inflammation we determined the expression of inflammatory genes in the liver and adipose tissues which are typically elevated in HFD-induced obesity [24,36,37]. Expression levels of the genes coding for macrophage markers *Cd68*, Cd11b (*Itgam*), F4/80 (*Emr1*), and the cytokines TNFα (*Tnf*) and IL-1β (*Il1b*) were neither in BAT, iWAT nor in the liver significantly different between aLKO and WT mice, not supporting an anti-inflammatory role of C16:1n − 7 in this context (Fig. 6A).

Next, we asked whether adipose tissue LPL deficiency of mice on HFD induced browning in iWAT and determined the expression of genes critical for this process. We observed that none of the typical browning markers tested, *Ucp1*, *Pgc1a*, *Dio2*, and *Elovl3*, were differently expressed in BAT or iWAT of aLKO *versus* WT mice (Fig. 6B), indicating no effect of adipocyte LPL loss on browning. LPL deficiency also had no impact on BAT functions when the mice were kept on a chow diet and were stimulated with the β-adrenergic agonist CL316,243 for one week to induce BAT activation and browning of WAT. Although DNL genes were significantly higher in aLKO *versus* WT mice there was no significant alteration in the expression of brown adipocyte markers in either BAT or iWAT (Fig. 6C).

4. Discussion

Here we describe the effects of adipose tissue-specific LPL deficiency on the metabolic phenotype of mice. Despite residual LPL expression, likely due to LPL expression in tissue-resident macrophages [38], significant effects on systemic lipid homeostasis were observed. First, DNL was profoundly induced in all adipose tissue types (Figs. 2–4, Supplemental Fig. 1), as previously reported for mutant mice expressing lipoprotein lipase exclusively in muscle and Gpihbp1-deficient mice lacking active lipoprotein lipase in all tissues [3,32] as well as for LPL-deficient

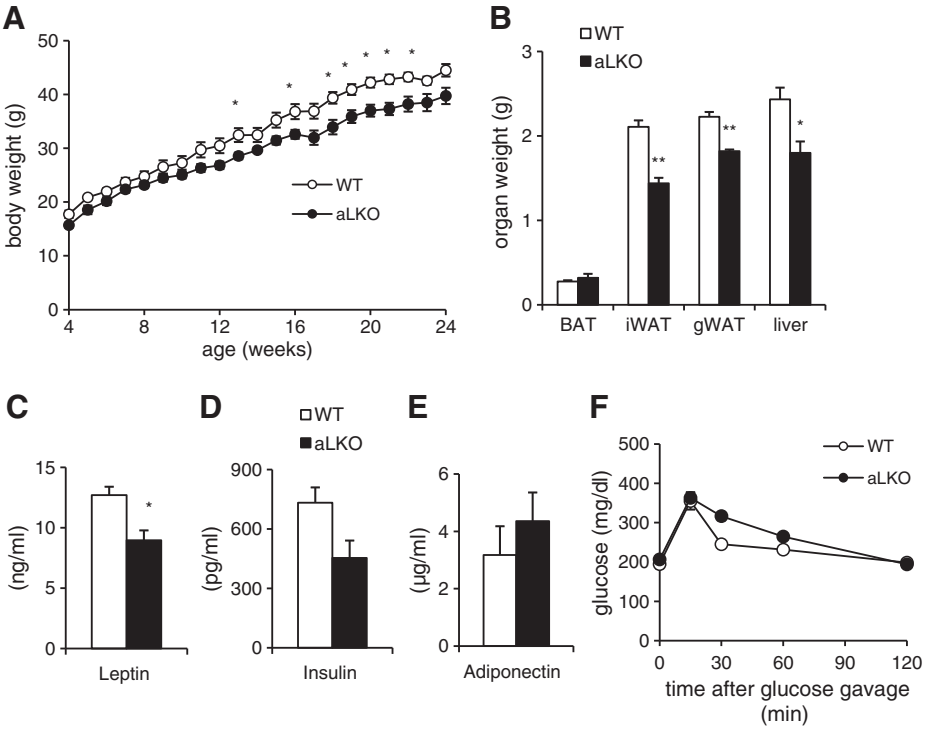

Fig. 5. Loss of adipocyte LPL results in reduced adiposity, insulinemia and improvement of adipokine profile but not glucose tolerance in mice on HFD. A) Body weight curve, B) organ weight at necropsy, C)–E) plasma leptin, insulin and adiponectin, F), oral glucose tolerance test in male aLKO and WT mice on HFD. B–F measurements at age 24 weeks, n = 5, mean ± SEM, * $p<0.05$, ** $p<0.001$.

humans [39]. Second, PUFA concentrations in adipose tissues were decreased (Fig. 2, Supplemental Fig. 1), in line with the important role of LPL in locally releasing these fatty acids, which cannot be synthesized *de novo*, from TRL. This PUFA decrease is likely one of the mechanisms causing the induction of DNL in the aLKO mice, as PUFA were previously demonstrated to suppress activity of the two major lipogenic transcription factors, SREBP1c and ChREBP [40–42]. We provide strong evidence, by coregulation and correlation of ChREBP-β [28] with Fasn (Fig. 3), that ChREBP regulates DNL in the adipose tissue and that it mediates the DNL increase by upregulation of Glut4 function and glucose uptake (Fig. 3).

Regardless of the mechanism inducing DNL in the aLKO mice, the resulting increase of the DNL-derived fatty acid C16:1n−7 has important potential implications for metabolic health. C16:1n−7 was shown in rodent models to have insulin-sensitizing as well as anti-inflammatory effects [34,43], to improve insulin resistance in rodent models upon dietary supplementation [35,44], and it was thus proposed to act as an insulin-sensitizing, so-called lipokine [43]. These beneficial effects of C16:1n−7 are in stark contrast to those of saturated fatty acids such as C16:0 and C18:0, which have well-known adverse metabolic effects when present in excess [45–47], and even to those of C18:1n−9 which can, for example, exert pro-inflammatory properties [48]. In the present study, aLKO mice showed no signs of improved HFD-induced inflammation in WAT or liver despite a putatively beneficial adipose tissue fatty acid profile, namely a massive increase in C16:1n−7 *versus* a decrease in C16:0 and C18:0, (Fig. 6). Also, they exhibited only a trend for improved insulin sensitivity, as indicated by higher adiponectin and lower insulin plasma levels (Fig. 5). Consequently, our data do not directly support the C16:1n−7 lipokine hypothesis. However, a beneficial role of DNL fatty acids cannot be ruled out either. It needs to be taken into account that the aLKO mice cannot efficiently store exogenous fatty acids in WAT, potentially leading to lipotoxcicity in other organs, especially on HFD [49]. Under those circumstances a mild improvement in insulin resistance, and especially, the absence of liver steatosis despite plasma hypertriglyceridemia (Fig. 5) could also be interpreted as a compensatory effect of the DNL-dominated fatty acid profile of LPL-deficient adipose tissues.

An attenuated adiposity after high-fat diet feeding might also relate to alterations in food intake. In this context, alterations in hypothalamic availability of circulating fatty acids species have been linked to central metabolic rate control in mice lacking LPL in neurons [50]. In our study we have only anecdotally measured 24 h food intake and found a non-significant trend towards reduced food intake (data not shown), potentially contributing to reduced adiposity in aLKO mice. However, further studies are needed to clarify whether certain fatty acid species, in particular such derived from DNL, impact on energy expenditure in aLKO mice thorough the central nervous system.

Previous studies observed a strong adipose tissue phenotype of LPL deficiency, namely reduced fat pads with brownish appearance in L0-MCK mice on an obese ob/ob background [3]. Those data suggested that fat pad size was not only decreased because of defective fatty acid delivery but also due to browning of WAT. As this hypothesis was not tested, and as increased DNL is known to be important in BAT of cold exposed mice [20,22,23] and may therefore favor brown adipocyte activation or differentiation, we characterized the BAT-like phenotype of adipose tissues. No significant effect of LPL deficiency on the expression of four BAT activation markers was found either under metabolic stress induced by a HFD or after induction of WAT browning through the beta3-adrenergic agonist CL316,243 (Fig. 6). Importantly, these data clearly show that a marked induction of DNL in adipose tissue *per se* does not lead to increased brown adipocyte activation or induction in WAT. Thus, while DNL appears to be essential for proper brown adipocyte function, as previously shown by dysfunctional activation after blocking DNL induction by Dio2 knockout [20], it is not sufficient to nudge adipocytes toward a paucilocular, Ucp1-positive phenotype. Another aspect of browning potentially influenced by LPL deficiency is synthesis of C20:4n−6-derived eicosanoids through cyclooxygenase-2 (COX-2), shown recently to mediate browning of WAT [51]. Although LPL is important for shuttling C20:4n−6 or its precursors into adipose tissue, we surprisingly found not a decrease but an increase of C20:4n−6 in iWAT of HFD-fed

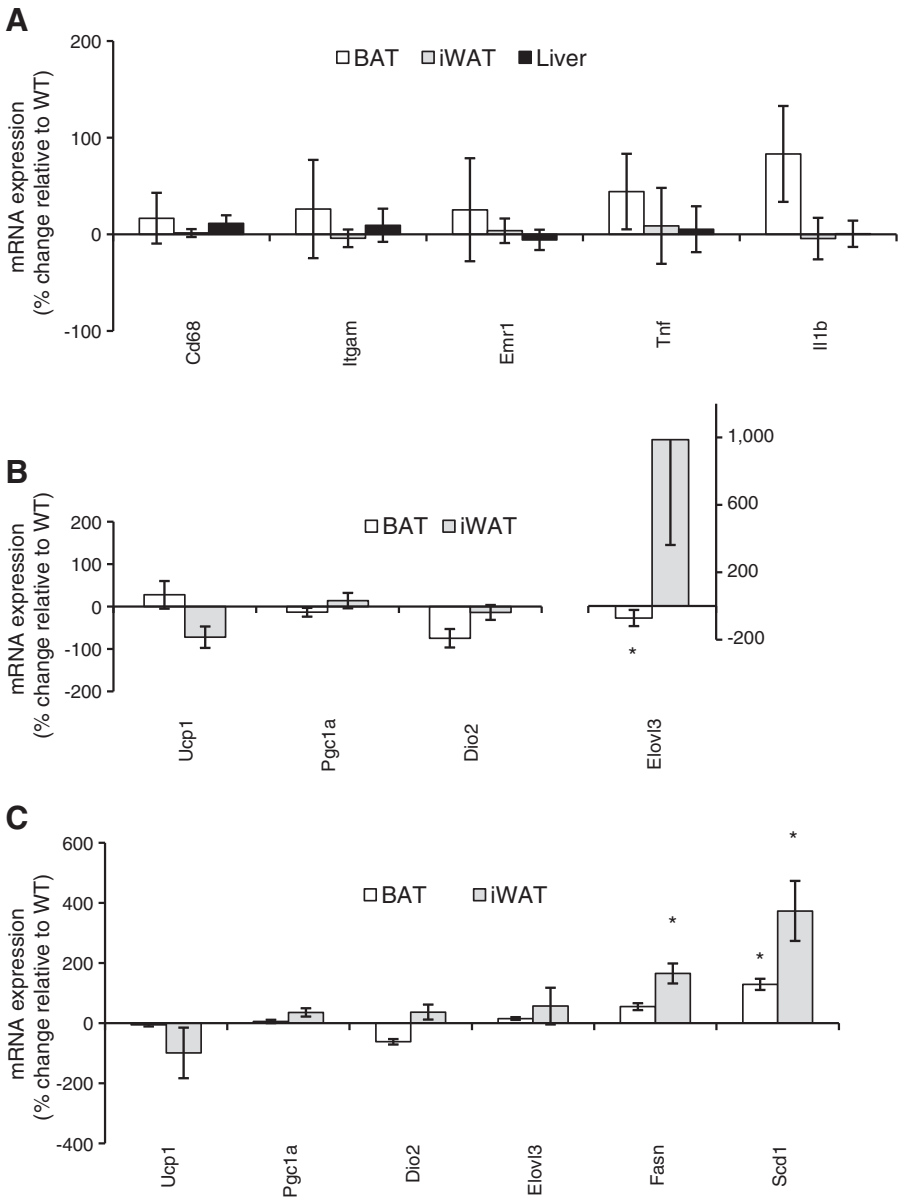

Fig. 6. No effect of adipocyte-selective LPL deficiency on inflammatory or brown adipocyte markers in mice on HFD or after beta-adrenergic stimulation. Relative change of mRNA expression of A) inflammatory genes and B) brown adipocyte markers in adipose tissues and livers of male aLKO mice on HFD compared to WT mice (age 24 weeks, n = 5). C) Relative change of mRNA expression of brown adipocyte markers in adipose tissues of aLKO treated with CL316,243 for one week compared to WT mice (chow diet, 24 weeks of age, n = 5–6). * $p<0.05$, ** $p<0.001$.

aLKO mice (Fig. 4). This finding suggests compensatory mechanisms maintaining adipocyte C20:4n − 6 levels in LPL deficiency and indicates that, at least on HFD, sufficient C20:4n − 6 is present for potential COX-2-mediated effects on browning.

Taken together, adipose tissue-specific knockout of LPL leads to a metabolically favorable fatty acid profile containing high amounts of C16:1n − 7 along with improved glucose tolerance and probably glucose utilization. Although this does not lead to an overall healthy phenotype in diet-induced obesity, it may relieve lipotoxicity stress caused by the reduced capacity of aLKO mice to store superfluous fatty acids in WAT. The compensatory mechanisms apparently include neither reduced inflammation nor BAT recruitment nor BAT activation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbalip.2012.11.011.

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