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Sex differences in the effects of brown adipocyte CD47 deficiency on age-related weight change and glucose homeostasis

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Abstract

Our previous studies demonstrated that mice with global CD47 deficiency are lean and resistant to diet or aging-associated obesity and metabolic complications. This protective effect is partially through modulating brown fat function. To definitively determine the role of brown fat CD47 in age-related metabolic homeostasis, inducible brown adipocyte-specific *cd47* deficient mice were generated by crossbreeding *cd47* floxed mice with UCP1-Cre^{ERT2} mice and characterized in this study. Efficient knockdown of CD47 in brown fat was achieved in both male and female mice through tamoxifen administration. Intriguingly, our findings indicated that male mice lacking CD47 in brown fat displayed a notable reduction in body weight starting at 23 weeks of age when housed at a temperature of 22 °C, in comparison to control mice. This reduction in weight was accompanied by improved glucose tolerance. Remarkably, this phenotype persisted even when the male mice were housed under thermoneutral conditions (30 °C). Conversely, female knockout mice did not exhibit significant changes in weight throughout the study. In addition to the enhanced glucose homeostasis, brown fat CD47 deficiency in male mice also prevented age-related hypertriglyceridemia and non-alcoholic fatty liver disease. Furthermore, the brown fat tissue of male knockout mice exhibited reduced whitening, while maintaining comparable levels of thermogenic markers. This suggests the involvement of a thermogenesis-independent mechanism. Altogether, these findings highlight a sex difference in the impact of brown adipocyte CD47 deficiency on age-related weight changes and glucose homeostasis.

Keywords

Age; CD47; Brown fat; glucose homeostasis; body weight; sex-difference

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Author Contributions

SW designed the study. DL and TG performed the experiments and did the data analysis. SW wrote the manuscript.

Declaration of interests

The authors declare no competing interests.

Introduction

CD47, an integral glycoprotein cell receptor, is typically expressed at low levels on most healthy cells but becomes upregulated in aging or obese conditions [1–9]. Besides its well-established roles in immunity and self-recognition [2–4], recent research conducted in our lab has revealed a novel function of CD47 in regulating white and brown fat and its involvement in aging and diet-induced obesity, utilizing a global CD47 deficient mouse model [10–13]. Our findings indicate that young adult mice lacking CD47 globally exhibited remarkable lipid turnover in white adipose tissue and significantly increased UCP1 activity in brown fat, resulting in elevated energy expenditure and resistance to diet-induced obesity [10, 13]. In addition, aged mice lacking CD47 globally also demonstrated a lean phenotype, accompanied by increased browning of white adipose tissue and activated thermogenic activity in brown fat, thereby improving age-related metabolic disorders [11]. While these studies support the emerging role of CD47 in regulating brown fat function and energy balance, potential contributions of CD47 deletion in other organs such as the brain, muscle, or liver cells cannot be completely disregarded. Therefore, to definitively establish the impact of CD47 in brown fat on energy balance, we generated and characterized inducible brown adipocyte-specific CD47-deficient mice in this study.

Both male and female inducible brown adipocyte-specific CD47-deficient mice and control mice were characterized in this study. These mice were fed with normal low-fat diet and housed at 22 °C or 30 °C for certain time periods. The results demonstrated that only male brown adipocyte specific CD47 deficient mice exhibited an age-related weight reduction and metabolic improvement, highlighting a sex difference in the impact of brown adipocyte CD47 on age-related metabolic homeostasis.

Materials and Methods

Animal Studies

All animal procedures were approved by the University of Kentucky Institutional Animal Care and Use Committee. CD47 floxed mice ($CD47^{fl/fl}$ on C57BL6 background) were obtained from KOMP (knockout mouse project) repository at UC Davis. Mice were genotyped by PCR analysis of genomic DNA from tail of wild-type (WT, 445 bp) or $CD47^{fl/fl}$ mice for the presence of LoxP sites (658 bp) using the primers listed in the Supplementary table 1. In order to generate brown adipocyte-specific CD47 knockout mice ($CD47^{\Delta UCP1}$), $CD47^{fl/fl}$ mice were bred with UCP1-Cre^{ERT2} (generously provided by Dr. Christian Wolfrum in Switzerland [14]) for two generations. Tamoxifen induction of Cre activity was performed in 6 -week-old mice by daily intraperitoneal injection of 75 mg tamoxifen (TAM)/kg body weight for 5 consecutive days. After 2–3 weeks of final TAM injection, one set of mice (both male and female) were sacrificed to determine the gene recombination and characterization. Another set of mice were maintained on normal low-fat diet (10% kcal from fat, D12450J, Research Diets) and housed in standard cages at 22 °C in 12-hour light/ dark cycle. Body weight was monitored weekly. Glucose tolerance test (GTT) was performed in 28-week-old mice. Then mice were housed in 30 °C temperature chamber for additional 6–10 weeks.

Metabolic analysis

Echo MRI was utilized to measure mice lean mass and fat mass [10]. For mice indirect calorimetry (e.g. food intake, energy expenditure et al) analysis, Sable Promethion system was used. To collect data, mice were put in this system individually for 5 days.

Glucose tolerance test (GTT)

A glucose tolerance test was conducted on mice that were fasted for 6 hours. The test involved intraperitoneal injection of glucose (1 g/kg body weight), followed by glucose measurements using a glucometer at different time periods after the injection.

Real-time quantitative PCR

Adipose tissue or other tissues were processed to extract total RNA using the RNeasy Mini Kit (Qiagen, USA). Subsequently, the extracted RNA was reverse transcribed to cDNA using the High Capacity cDNA Reverse Transcription Kit (Invitrogen, Carlsband, CA). MyiQ Real-time PCR Thermal Cycler (Bio-Rad) and the SYBR Green PCR Master Kit (Qiagen, Valencia, CA) were utilized for performing quantitative PCR. To determine relative mRNA expression, the MyiQ system software, as previously reported [15], was employed, and the results were normalized to β-actin levels. The primer sequences used in this study can be found in Supplemental Table 1.

Blood parameter analysis

At the conclusion of the study, blood samples were collected via cardiac puncture. Plasma levels of alanine aminotransferase (ALT) were measured using an ALT assay kit from Connecticut, USA. The determination of plasma total triglyceride levels was performed enzymatically using Wako kits from Richmond, USA.

Western blotting

Brown fat tissue was homogenized and 30~50 μg of total protein was loaded onto an SDS-PAGE gel under reducing conditions and subsequently transferred onto a nitrocellulose membrane. The membrane was blocked with 3% BSA media and incubated overnight at 4°C with anti-CD47 (1:1000 dilution; Abcam, Cambridge, MA, USA) or anti-β-actin antibody (1:5000 dilution; Santa Cruz, Dallas, TX, USA), followed by incubation with horseradish peroxidase-conjugated secondary antibodies (Jackson Labs, Bar Harbor, ME, USA) and visualization using an enhanced chemiluminescence system (Pierce, Waltham, MA, USA).

Tissue Histology

Formalin fixed mice tissues (fat and liver) were embedded in paraffin, sectioned at 5 μm thickness, and subjected to standard hematoxylin and eosin-stain staining (H&E) using the service provided by COBRE Pathology Core at University of Kentucky. Additionally, liver cryo-sections were stained with Oil red O. All images were captured using a Nikon Eclipse 55i microscope.

Statistical Analysis

Prism 9 software (GraphPad Software, San Diego, CA) was utilized for statistical analysis. All data are presented as mean ± SEM. Two-tailed Student's t-test was employed to assess statistical significance between two groups. For comparisons involving multiple groups, one-way ANOVA followed by Bonferroni's multiple comparisons test or 2-way ANOVA followed by Tukey's multiple comparisons test was applied. A significance level of $p < 0.05$ was considered statistically significant.

Results and Discussion

Generation of brown adipocyte-specific CD47 deficient mice (CD47$^{\Delta UCP1}$)

Previous work from our lab suggests that CD47 is an important regulator of brown fat function and contributes to age-related obesity and metabolic disorder in a global CD47 deficient mouse model [11]. To definitively establish the impact of CD47 in brown fat on age-related metabolic disorder, we generated inducible brown adipocyte-specific CD47-deficient mice (CD47$^{\Delta UCP1}$). As shown in Fig. 1A, exons 2 and 3 of the murine CD47 gene were floxed in CD47 $^{fl/fl}$ mice. In order to generate CD47$^{\Delta UCP1}$ mice, CD47 $^{fl/fl}$ mice were crossbred with UCP1-Cre^{ERT2} mice for two generations. Mice genotyping results of loxP band and cre band were shown in Fig. 1B. After tamoxifen administration, CD47 was efficiently deleted in brown fat from both male and female mice as demonstrated by qPCR and western blotting (Fig. 1C–D). These mice were further characterized in the following studies.

Male CD47$^{\Delta UCP1}$mice but not female mice displayed an age-related reduction in body weight and fat mass, accompanied with improved glucose homeostasis and attenuated fatty liver.

Brown adipocytes are known to play a crucial role in metabolism by utilizing energy sources such as glucose and fat to generate heat. To investigate the impact of CD47 deficiency in brown adipocytes on age-related basal body weight and glucose homeostasis, we conducted experiments using male and female CD47$^{\Delta UCP1}$ and CD47 $^{fl/fl}$ mice, following tamoxifen administration, and housed them at a temperature of 22°C while being fed a low-fat diet. Body weight monitoring was initiated after a period of 2–3 weeks following the final tamoxifen injection. As shown in Fig. 2, female CD47$^{\Delta UCP1}$ and CD47 $^{fl/fl}$ mice exhibited similar age-related changes in body weight and glucose tolerance when housed at 22°C until 28 weeks of age. Subsequently, these mice were transferred to a temperature chamber set at 30°C. Notably, there were no significant differences in body weight changes, glucose tolerance, plasma triglyceride levels, or ALT levels between the knockout and control mice. These findings suggest that the deletion of CD47 in brown adipocytes does not impact age-related alterations in body weight, glucose regulation, or lipid homeostasis in female mice.

In contrast to the female mice, male CD47$^{\Delta UCP1}$ mice displayed a significant reduction in body weight starting at 23 weeks of age when housed at 22°C, compared to control mice. This decrease in body weight and fat mass was accompanied by improved glucose tolerance, as depicted in Fig. 3 A–C. Importantly, this phenotype persisted even when the male

mice were housed under thermoneutral conditions (30°C), where brown fat thermogenic activity is negligible (Fig. 3D–E). These results suggest that the reduced body weight observed in CD47$^{\Delta UCP1}$ male mice may be independent of brown fat thermogenic function. Furthermore, indirect calorimetry data indicated that food intake, energy expenditure, and total activity were comparable between CD47$^{\Delta UCP1}$ and control mice (data not shown). Additionally, apart from the enhanced glucose homeostasis (Fig. 3F), brown fat CD47 deficiency in male mice also prevented age-related hypertriglyceridemia and the development of non-alcoholic fatty liver disease (Fig. 3G–I).

Collectively, our findings demonstrate a sexual dimorphism in the effects of CD47 deficiency in brown adipocytes on age-related body weight regulation and metabolic homeostasis. While female mice were largely unaffected by CD47 deletion, male mice exhibited improved body weight control, glucose tolerance, and lipid metabolism. These results highlight the importance of considering sex-specific differences in brown adipocyte biology and its implications for metabolic regulation. Further studies are warranted to investigate the underlying mechanisms contributing to these differences such as potential sex hormone-mediated effects, differences in gene regulation or other factors that may modulate CD47 function in a sex-specific manner.

Brown fat tissue from male CD47$^{\Delta UCP1}$ mice had reduced whitening

To elucidate the potential mechanisms underlying the observed improvements in metabolic health observed in male CD47$^{\Delta UCP1}$ mice, we conducted further analysis of brown fat tissue (BAT). A slight reduction in BAT mass was observed in CD47$^{\Delta UCP1}$ mice compared to control CD47$^{fl/fl}$ mice (Fig. 4A). This suggests that CD47 deficiency may impact the overall size or composition of BAT. Furthermore, histological examination using H&E imaging revealed that brown adipocytes from CD47$^{\Delta UCP1}$ mice exhibited smaller size (Fig. 4B), indicating reduced whitening. This reduction in whitening suggests a potential alteration in lipid accumulation or metabolism within the brown adipocytes of CD47$^{\Delta UCP1}$ mice. Interestingly, the qPCR analysis demonstrated that the expression levels of key thermogenic markers such as UCP1 and PGC-1α, as well as fatty acid beta oxidation-related genes (ACOX1 or CPT1β), were comparable between CD47$^{\Delta UCP1}$ mice and control mice (Fig. 4C). These findings suggest that the improved metabolic health observed in CD47$^{\Delta UCP1}$ mice is not likely due to significant changes in BAT-mediated fatty acid metabolism and thermogenesis. Additionally, the expression of genes associated with brown fat endocrine function was found to be comparable between knockout and control mice (Fig. 4D). However, we did observe an upregulation in the expression of FGF21 in liver from male CD47$^{\Delta UCP1}$ mice (Fig. 4E). This suggests that potential alterations in glucose utilization and energy balance in CD47$^{\Delta UCP1}$ mice may contribute to their improved metabolic health [15–19]. The involved molecular mechanisms of increased FGF21 expression in liver warrant further investigation in the future.

In summary, in this study, a sexual dimorphism in the effects of CD47 deficiency in brown adipocytes on age-related metabolic homeostasis was revealed. We found that female mice were largely unaffected by CD47 deletion in brown adipocytes. However, male mice

exhibited improved body weight control, glucose tolerance, and attenuated fatty liver disease in a UCP1-independent mechanism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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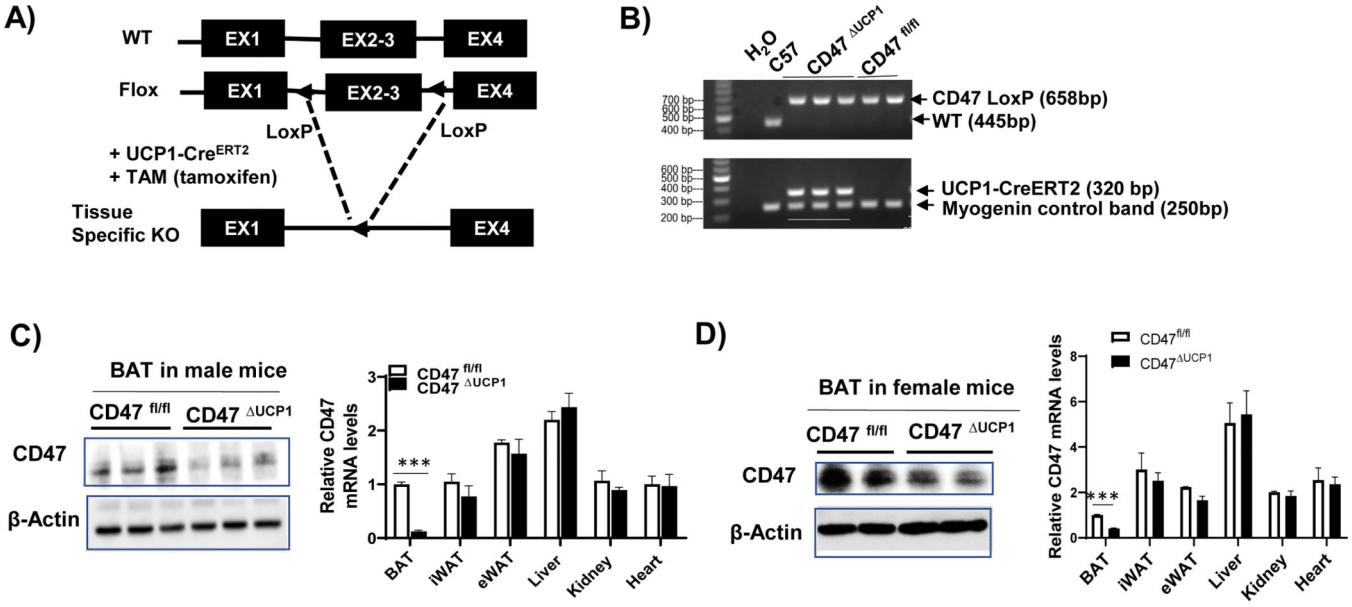

Figure 1. Generation and characterization of brown adipocyte specific CD47 knockout mice (CD47$^{\Delta UCP1}$).

A). Brown adipocyte specific CD47 gene knockout strategy; B). PCR analysis of genomic DNA from tail; To induce CD47 deletion, tamoxifen (75 mg/kg body) was intraperitoneally injected into male (C) or female (D) 6-week-old mice once a day for consecutive 5 days. After 2 weeks of tamoxifen treatment, tissues were harvested. CD47 gene expression in various tissues was determined by qPCR. CD47 protein levels in BAT were determined by immunoblotting. Data are presented as mean ± SE (n=3 mice /group), ***p<0.001.

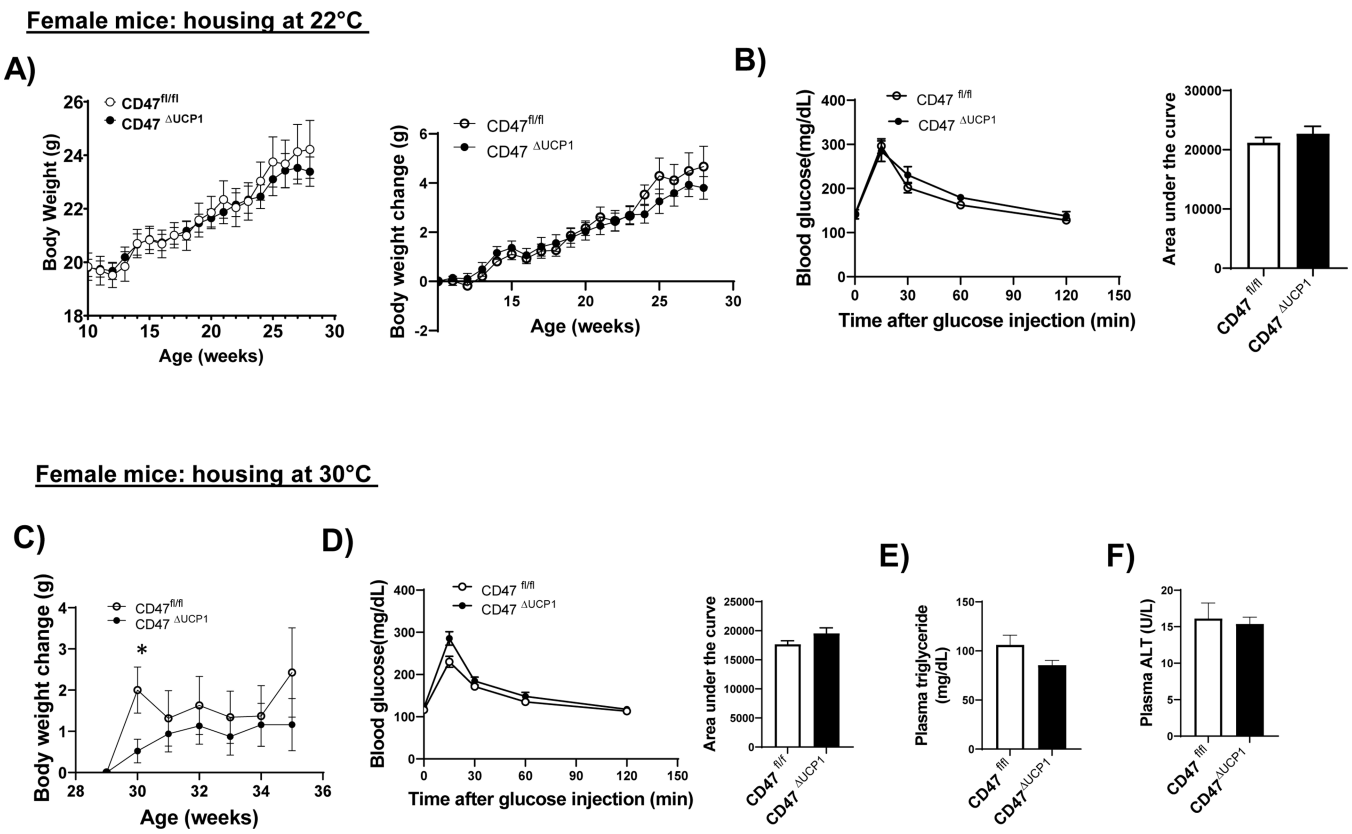

Figure 2. Brown adipocyte specific CD47 deficiency did not affect female mice body weight, glucose homeostasis, plasma triglyceride levels or ALT levels.

A-B). Female CD47$^{\Delta UCP1}$ or control CD47 $^{fl/fl}$ mice were housed at 22°C under normal low-fat diet. Body weight or body weight changes and glucose tolerance test (at 28-week-old) was determined. Then the mice were housed at 30°C (B-F). Body weight change, glucose tolerance test, plasma triglyceride level or plasma ALT levels were determined. Data are presented as mean ± SE (n=5–7 mice /group).

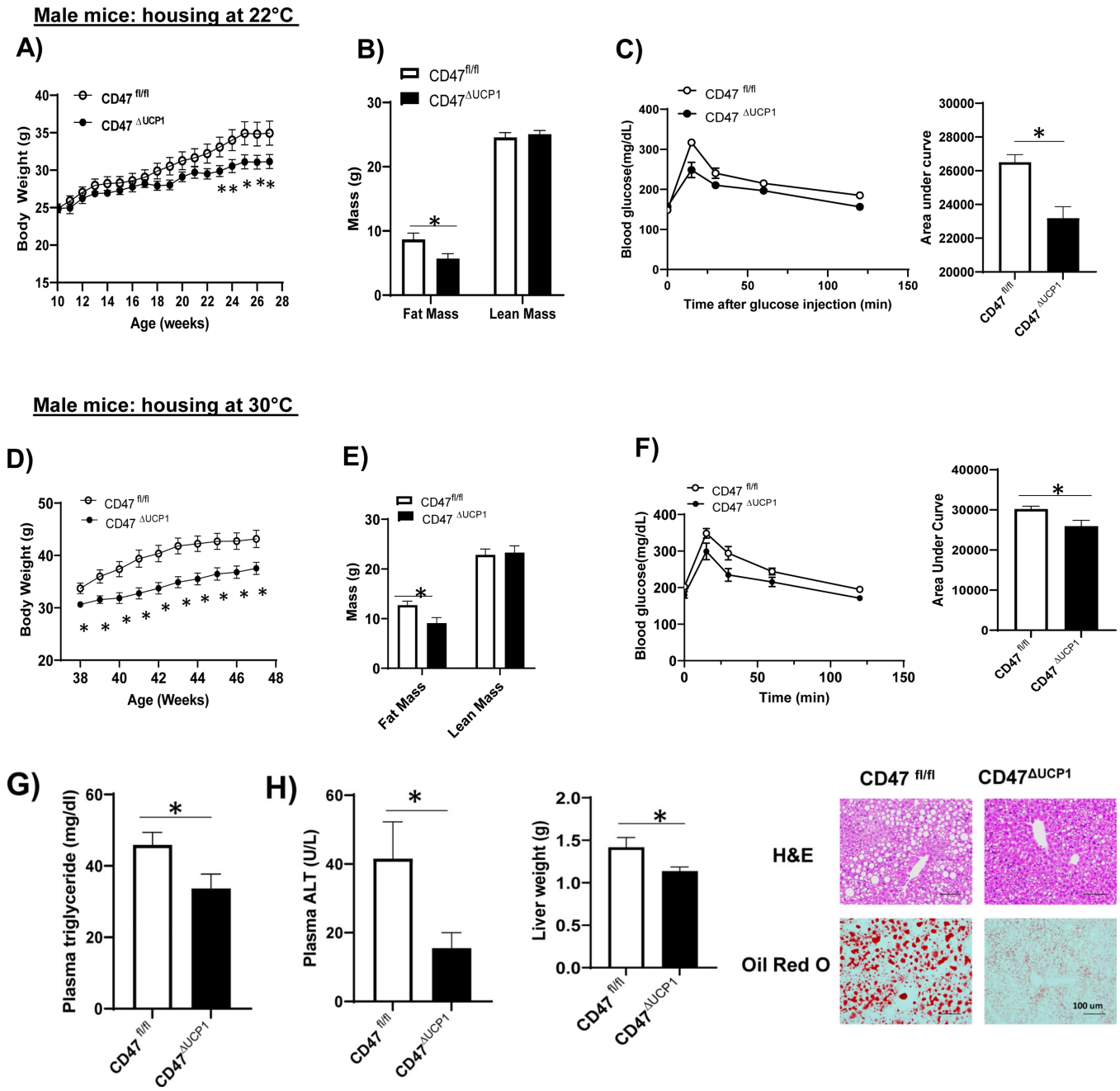

Figure 3. Male CD47$^{\Delta UCP1}$ mice displayed an age-related reduction in body weight and fat mass, accompanied with improved glucose homeostasis, attenuated plasma triglyceride levels and fatty liver disease.

A-C). Male CD47$^{\Delta UCP1}$ or control CD47 $^{fl/fl}$ mice were housed at 22°C under normal low-fat diet. Body weight, fat mass, glucose tolerance test (at 28-week-old) was determined. Then the mice were housed at 30°C (D-I). Body weight, fat mass, glucose tolerance test, plasma and liver triglyceride level, liver weight and plasma ALT levels were determined. Representative images of liver H&E staining and oil red O staining were shown. Data are presented as mean ± SE (n=5–7 mice /group). *p<0.05

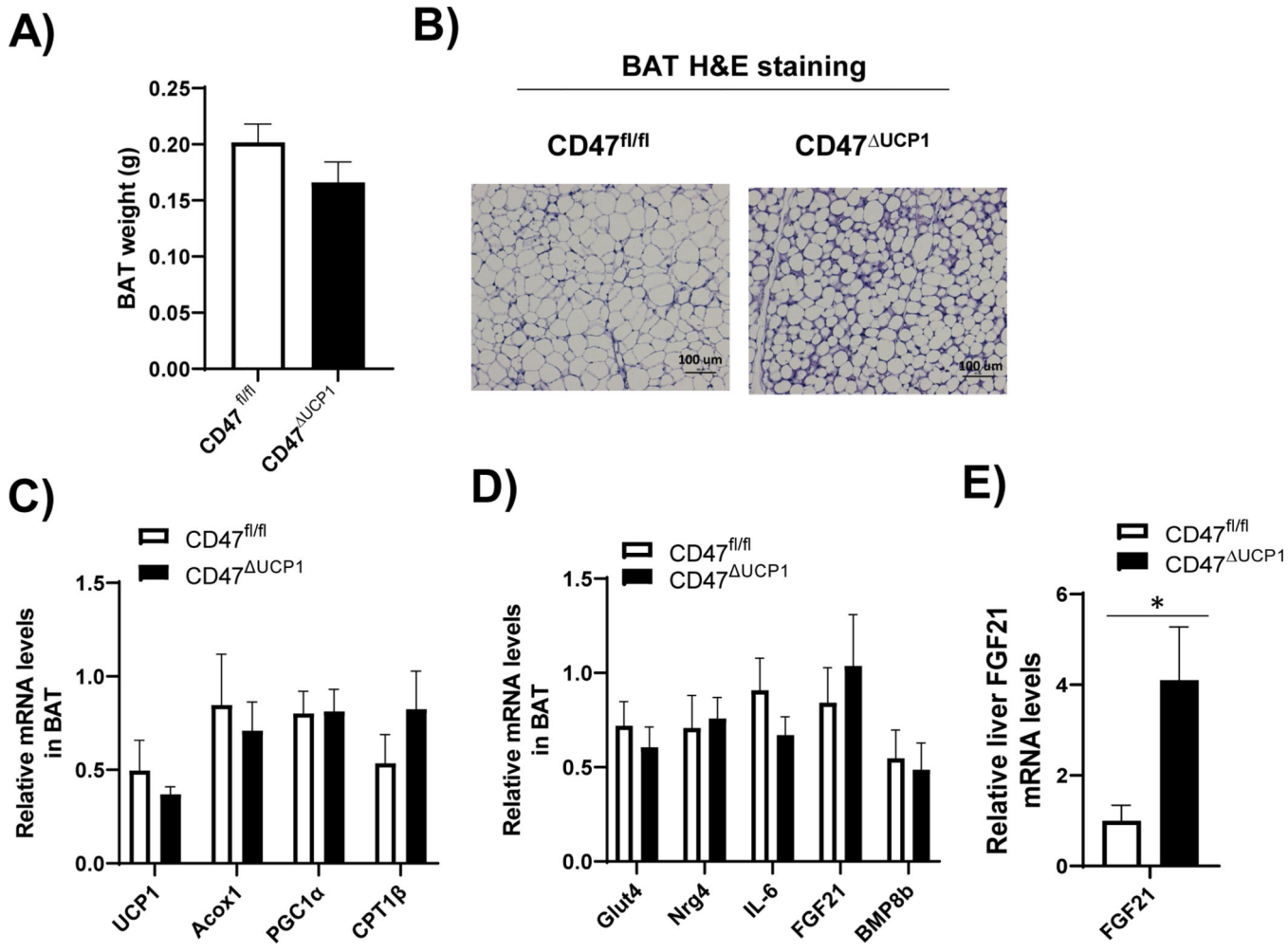

Figure 4. Brown fat tissue from male CD47$^{\Delta UCP1}$ mice had reduced whitening

A). Brown adipose tissue (BAT) mass from CD47$^{\Delta UCP1}$ and control CD47 $^{fl/fl}$ male mice (at 48-week-old); B). Representative H&E images from BAT. Scale bar represents 100 μm; C-D). qPCR of gene expression in BAT; E). qPCR of FGF21 gene expression in liver. Data are presented as mean ± SE (n=5–7 mice /group). *p<0.05