의학석사 학위논문

The metabolic effects of Retinol-Binding Protein 4 in brown adipocytes

갈색지방에서 Retinol Binding Protein 4의 역할

2021년 8월

서울대학교 대학원

분자의학 및 바이오제약 학과

하 은 선

The metabolic effects of Retinol- Binding Protein 4 in brown adipocytes

지도 교수 박 경 수

이 논문을 의학석사 학위논문으로 제출함

2021년 5월

서울대학교 대학원

분자의학 및 바이오제약 학과

하 은 선

하은선의 의학석사 학위논문을 인준함

2021년 7월

위 원 장 **김 태 유**

부위원장 **박 경 수**

위 원 **박 영 주**

Abstract

The metabolic effects of Retinol-Binding Protein in brown adipocytes

Eun Sun Ha

Molecular Medicine and Biopharmaceutical Science

Seoul National University Graduate School

Obesity is defined as a condition in which fat accumulation exceeds the normal ranges to an extent that it may present health risks. The fundamental cause of obesity is a long-term energy imbalance between energy consumption and expenditure. Contrary to the conventional notion that the role of adipose tissue is to store energy, brown adipose tissue (BAT) is known for its capability to utilize energy through thermogenesis when stimulated by cold. In relation to the thermogenic programming of adipose tissues, retinoid metabolism is an important factor to be considered. More specifically, retinol-binding protein 4 (RBP4), which is a retinol carrier protein in the serum, is well known for its effects in the adipose tissues. RBP4 is highly expressed in the liver and adipose tissues, and it has been reported that RBP4 can act as an adipokine in the white adipose tissue (WAT). In WAT, RBP4 induces insulin resistance and hepatic steatosis by increasing lipolysis through elevation of TNFα. However, RBP4 expression in BAT is significantly increased along with uncoupling protein 1 when thermogenesis is activated. Moreover, it was found that RBP4 is

necessary for the cold-mediated adipose tissue browning and thermogenesis, but much is yet unknown about the role of RBP4 in BAT and thermogenesis.

Therefore, this study aims to analyze the metabolic effects of RBP4 in the brown fat. To investigate the role of RBP4 in BAT, brown adipocyte-specific human RPB4 transgenic mice (UCP1-hRBP4 mice) were generated. I have found that these transgenic mice are lower in body weight and have lower fat mass compared to the littermate controls. Moreover, the RBP4 overexpression in brown adipose tissue leads to a moderate improvement in glucose tolerance, and an increase in energy expenditure as well as core body temperature. Furthermore, fatty acid oxidation was substantially increased in the BAT. In summary, the metabolic phenotypes of RBP4 overexpression in BAT suggest that RBP4 could be involved in the activation of the brown fat, and further study is required to understand the molecular mechanisms.

Keywords: Retinol-Binding Protein 4, Brown adipocyte, Fatty acid oxidation, Browning, Obesity

Student Number: 2019-24521

Table of Contents

List of Abbreviations

ATRA: All-Trans Retinoic Acid

cAMP: Cyclic adenosine monophosphate

DIO2: Type II iodothyronine deiodinase

ELOVL3: Elongation of very long chain fatty acids

GLUT4: Glucose transporter type 4

HSL: Hormone-sensitive lipase

LPL: Lipoprotein lipase

PGC1α: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha

PKA: Protein kinase A

PPAR: Peroxisome proliferator-activated receptor

PRDM16: Histone-lysine N-methyltransferase

RAR: Retinoic acid receptors

RARE: Retinoic acid-response element

RXR: Retinoid X receptors

RBP4: Retinol binding protein 4

UCP1: Uncoupling protein 1

List of Figure and Tables

Introduction

The search for a novel alternative to treating obesity

Obesity, as the major risk factor for noncommunicable diseases such as type 2 diabetes and cardiovascular diseases, is now considered a global problem in the 21st century [1]. Obesity results from a long-term energy imbalance in energy consumption and expenditure, which can be due to a combination of increased ingestion of high energy food, low physical activity, and impairment in energy metabolism [2]. The current anti-obesity medications are mostly aimed at promoting satiety by stimulating the release of compounds such as serotonin and dopamine [3]. However, since the actions of these compounds are sympathomimetic, only short-term use is approved and the side effects of long-term use are yet controversial. Therefore, the search for an alternative approach to address the energy imbalance in obesity through methods that could direct the metabolic flux towards energy expenditure continued.

Brown adipose tissue

Brown adipose tissue (BAT) was already identified as the organ responsible for non-shivering thermogenesis in 1961 [4]. In infants and rodents, BAT depots are found in the interscapular, axillary, and perirenal regions. It was first assumed that BAT depots are only present in infants that are incapable to shiver to maintain body temperatures, and in animals that needed to hibernate [5]. However, along with the

development of fluorodeoxyglucose positron emission tomography (FDG-PET) in late 2000, it was revealed that functionally active brown fats were present even in adult humans [33].

Thereupon, BAT received renewed attention for its potential to combat obesity. Despite its small size, a mere 50g of BAT is potent enough to utilize almost up to 20% of basal caloric needs in humans when it is maximally stimulated [6]. Unlike the white adipose tissue, which stores excess energy in the form of triglycerides, brown adipose tissue is capable to convert the energy in triglyceride and dissipate it as heat by activating the sympathetic nervous system upon exposure to cold [7]. The brown adipocyte's specialized function to regulate energy expenditure and maintain the body temperature is mediated by a mitochondrial transmembrane protein known as uncoupling protein 1 (UCP1). UCP1 uncouples oxidative phosphorylation from the production of ATP and stimulates energy to dissipate as heat and provokes oxidation of fatty acids [8]. Upon initiation by cold stimuli or excess caloric intake, norepinephrine is released, which activates the ß-adrenoreceptors of the brown adipocytes. This is followed by a consecutive increase in the intracellular cAMP level and the activation of the PKA pathway. Thereupon, lipolysis is increased, and the subsequent increased free fatty acid levels act as the prime activators of UCP1 [5].

Retinoid metabolism

Retinoid metabolism is another key metabolic process in the regulation of energy balance [9]. Retinoid is a term for the derivatives of vitamin A, which is primarily stored in the liver as retinyl esters. The retinol-binding protein 4 (RBP4) transports the retinol to target tissues by binding to the receptors stimulated by retinoic acid 6 (STRA6), which are plasma membrane receptors on the cell membrane. Intracellular retinol is then reversibly oxidized to retinaldehyde by alcohol- and retinol dehydrogenases, which is further irreversibly oxidized to retinoic acid by retinaldehyde dehydrogenase [10]. All-*trans* retinoic acid (ATRA) can modulate numerous metabolic processes, mainly by gene regulation through signaling retinoic acid receptor (RAR) and retinoid X receptor (RXR) nuclear receptors. As concerned to the transcriptional control of the BAT and thermogenesis, it was first reported in 1995 that ATRA induced UCP1 expression. It was found that retinoic acid-response element (RARE) is sited in the upstream of the UCP1 gene promoter region. The RAR and RXR bind to the RARE to regulate the *Ucp1* expression in brown adipocytes [11].

Retinol-Binding Protein 4

RBP4's physiological role extends far beyond transporting retinol: RBP4 can also act as both adipokine and batokine [12]. RBP4 belongs to the lipocalin family and its expression is the highest in the liver where retinol is stored, followed by a robust expression in all adipose tissues.

Barbara Khan and her team have first proposed the causal relationship between RBP4 and adipocytes in the development of insulin resistance and type 2 diabetes. Literature supports that along with the development of insulin resistance, glucose transporter 4 (GLUT4) expression is downregulated in adipocytes, and RBP4 level in the serum is elevated in the absence of GLUT4 in the adipose tissue. Moreover, in the adipose tissue-specific GLUT4 knock-out mice, RBP4 expression was selectively increased in the adipose tissue, and the treatment with rosiglitazone reduced the RBP4 expression levels [13]. In their subsequent study, they showed that RBP4 induced inflammation through the activation of CD4-positive T cells and macrophages, and that the resulting cytokines such as TNF-α stimulated lipolysis, which established the role of RBP4 as an adipokine [20]. These findings were consistent with the study by Lee et al. that revealed the correlation between the expression of RBP4 in adipocytes and the development of hepatic steatosis. In their study, the adipocyte-specific overexpression of RBP4 increased the expression of lipases including hormone sensitive lipase (HSL) and adipocyte triglyceride lipase (ATGL). It was suggested that the cause of increased lipolysis was adipose inflammation, as supported by the increase in both mRNA and protein levels of TNFα. The increase in hydrolysis of triglycerides in adipose tissue increased the mobilization of free fatty acids from adipocytes to the liver, resulting in hepatic steatosis [14].

RBP4 in brown adipose tissue

Compared to the findings reported in the white adipocytes, much less is known about the role of RBP4 in brown adipocytes. Contrary to the insulin resistance and inflammation promoting features of RBP4 in white fat, RBP4 in brown fat is prospected to be quite the opposite. Notably, Rosell et al. reported that activation of the brown fat via signaling of the ß-adrenergic pathway, either by exposure to 4°C cold or treatment with norepinephrine and cyclic AMP, significantly increased RBP4 expression levels [15]. Moreover, in a study of RBP4-deficient mice, thermogenesis and browning of the white adipose tissue was significantly impaired, which suggested the importance of retinol transport in oxidative metabolism [10].

Objective of the study

The previous findings of the dynamics of RBP4 during cold exposure suggest the correlation between RBP4 and the activity of the brown adipose tissue. However, the role of RBP4 in BAT and its metabolic significance remains unclear. Therefore, I have hypothesized that RBP4 stimulates BAT activation by affecting the downstream of the ß-adrenergic pathway (Figure 1). Thus, this study aims to define the role of RBP4 in the brown adipose tissue, and investigate the metabolic effects by using a mouse model that specifically overexpresses human RBP4 in BAT (UCP1-hRBP4 mice).

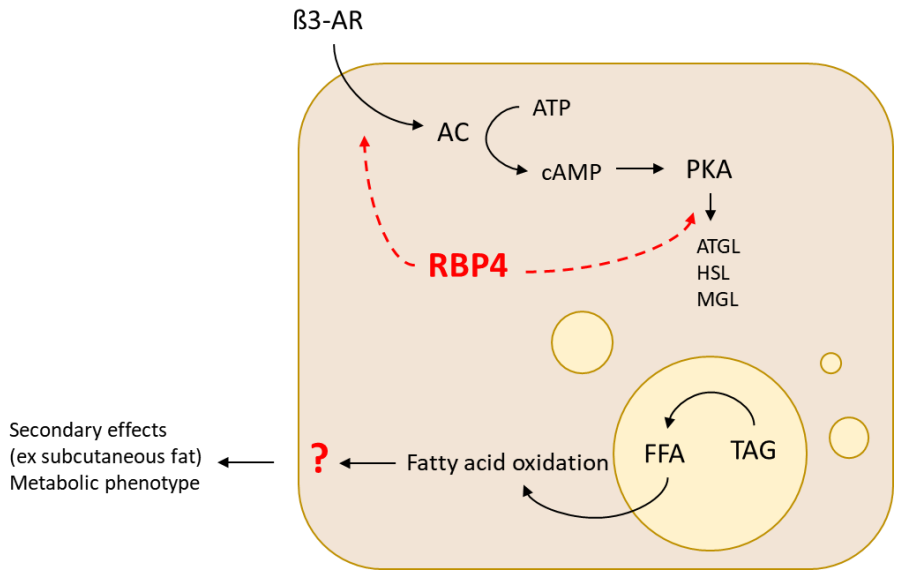

Figure 1. Illustrative representation of the hypothesis for the effects of RBP4 in brown adipocytes.

Materials and Methods

Animals

All animal experiments were conducted under the approval of Institutional Animal Care and Use Committee (IACUC) of the Seoul National University. Transgenic mice with human RBP4 complementary DNA cloned into pROSA26-1 vector (Addgene, Cambridge, USA) was bred with UCP1-Cre mice to generate brown adipose tissue-specific hRBP4 transgenic mice. Littermates lacking the UCP1-Cre transgene were used as control mice in this study, and both genotypes were fed with a chow diet.

Core body temperature and cold exposure

Core body temperature was measured using a rectal thermometer, and measurements were made before noon. Cold exposure was performed at 4°C for up to 8 hours and rectal temperature was measured at 0,1,2,4,8-hour terms. No prior starvation was conducted, and food and water were provided *ad libitum* throughout the experiment.

Body composition analysis

Whole body composition analysis in the percentage of lean, fat, and fluid were obtained by using the Minispec LF50 (Bruker, Billerica, USA). Tissue to bodyweight composition analysis was performed by finding the percentage of the tissue to body weight.

Intraperitoneal injection glucose tolerance test

After 16 hours of starvation, mice were intraperitoneally injected with 20% D-Glucose (2 g/kg body weight). Blood glucose levels were measured using the one-touch ultra-blood glucose meter (Life scan, Philadelphia, USA) by collecting blood via the tail at 0,15,30,45,60,120 time points.

Western blot Analysis

Mice tissue were harvested and homogenized in liquid nitrogen by using mortar and pestle. RIPA lysis buffer (0,5M Tris-HCl pH 7.4, 1.5M NaCl, 2.5% deoxycholic acid, 10% Np-40, 10mM EDTA) (Merck Millipore, Burlington, USA) containing protease inhibitor cocktail (Sigma, St. Louis, USA) was used to extract tissue protein. After 10 minutes of centrifugation, supernatants were collected and 30µg of protein were loaded in vertical SDS-PAGE gel for gel electrophoresis. The separated proteins were transferred onto a nitrocellulose membrane and were blocked in 5% BSA for 1 hour. The resulting membranes were incubated in the following antibodies overnight at 4°C: human RBP4 and mouse RBP4 (Sino Biological Inc, Philadelphia, USA. 1:1000). ß-actin (MBL, Woburn, USA. 1:1000).

Plasma and Tissue Triglyceride and free fatty acid level

Colorimetric assays were performed to quantify triglyceride (Cayman Chemical, Ann Arbor, USA) and free fatty acid levels (Biovision, Milpitas, USA).

Tissue histology

Harvested mice tissues were fixed in 4% formaldehyde, embedded in paraffin, and sectioned. Sections were stained using hematoxylin and eosin stain (H&E).

Metabolic study CLAMS

Metabolic study was conducted for 5 consecutive days using the Comprehensive Lab Animal Monitoring System (CLAMS, Columbus Instruments, Columbus, USA). Of the 5 days, only 3-day worth of data in between the periods were utilized for analysis.

RNA preparation and Real-Time PCR

Total RNA from tissue and cell was isolated by using TRIzol (Invitrogen, Waltham, USA). cDNA was synthesized with 1μg or 2μg of RNA, 5μl of reaction buffer, 2.5μl of 100mM DTT, 1.25μl of 10mM dNTP mix, 0.5μl of Oligo dT, 0.25μl of RNase inhibitor, 1μl of RTase (Invitrogen). mRNA expression level was evaluated with the SYBR Green Master Mix (Takara, Otsu, Japan) using the ABI 7500 real-time PCR system (Applied Biosystems, Waltham, USA). The primer sequences are as shown in Table 1.

Measurement of Fatty acid oxidation

Brown adipose tissue was homogenized in ice cold mitochondrial isolation buffer (250 mmol/L sucrose, 10mmol/L Tris-HCl, and 1mmol/L EDTA). After 2 hours of incubation with 0.2mmol/L [1-14C] palmitate, $^{14}CO_2$ and ^{14}C-labeled acid-soluble metabolites were quantified by the liquid scintillation counter. Radioactivity of each lysate was normalized by protein quantification.

Enzyme-Linked Immunosorbent Assay (ELISA)

Commercially available ELISA kits were used to measure adiponectin, leptin (CSB-E07272m, CSB-E04650m, Cusabio, Wuhan, China), and RBP4 (ab195459, Abcam, Cambridge, USA) levels in serum according to the manufactures' procedure.

Statistics

Statistical analysis of data is shown by using GraphPad Prism 8 (GraphPad Software, San Diego, USA). Data are presented as means ± standard errors of the means (SEM). Statistical significance was evaluated with the 2-tailed Student t-test. A *P* value less than 0.05 was deemed significant.

Table 1. Sequences of primers used for Real-Time PCR

Gene	Forward primer sequences	Reverse primer sequences
RBP4(human)	GAGGGCCTCTTTCTGCAGGACAA	GGGTCCTCGGTGTCTGTGA
Rbp4	CTGGGAAGTGT TGCAGACAT	CAGAGCGAAGGTGTCGTAGT
36b4	GCTTCGTGTTCACCAAGGAGGA	GTCCTAGACCAGTGTTCTGAGC
Adrb3	AGGCACAGGAATGCCACTCCAA	GCTTAGCCACAACGAACACTCG
Acc1	GGATGACAGGCTTGCAGCTAT	TTTGTGCAACTAGGAACGTAAGTCG
Acsl1	ATCAGGCTGCTTATGGACGACC	CCAACAGCCATCGCTTCAAGGA
Atgl	GGAACCAAAGGACCTGATGACC	ACATCAGGCAGCCACTCCAACA
Cd36	GGACATTGAGATTCTTTTCCTCTG	GCAAAGGCATTGGCTGGAAGAAC
Cpt1b	ATGTATCGCCGCAAACTGGACC	CTCTGAGAGGTGCTGTAGCAAG
Cpt2	GATGGCTGAGTGCTCCAAATACC	GCTGCCAGATACCGTAGAGCAA
Cidea	GGTGGACACAGAGGAGTTCTTTC	CGAAGGTGACTCTGGCTATTCC
Cox7a	AAACCGTGTGGCAGAGAAGCAG	CCCAAGCAGTATAAGCAGTAGGC
Cox8b	TATCCTGCGGCTGCTCCAA	CGACTATGGCTGAGATCCCCAC
Dgat1	GAGTCTATCACTCCAGTGGG	GGCGGCACCACAGGTTGACA
Dgat2	CTGTGCTCTACTTCACCTGGCT	CTGGATGGGAAAGTAGTCTCGG
Dio2	GGTGGTCAACTTTGGTTCAGCC	AAGTCAGCCACCGAGGAGAACT
Elovl3	GTGCTTTGCCATCTACACGGATG	ATGAGTGGACGCTTACGCAGGA
Fas	GTTGCCCGAGTCAGAGAACCTACA	CTTTCCAGACCGCTTGGGTAATCCAT

Fatp1	TGCCACAGATCGGCGAGTTCTA	AGTGGCTCCATCGTGTCCTCAT
Fatp4	GACTTCTCCAGCCGTTTCCACA	CAAAGGACAGGATGCGGCTATTG
Hsl	GCTGGGCTGTCAAGCACTGT	GTAACT GGGTAGGCTGCCAT
Lpl	ATCAACTGGATGGAGGAGGAGTTT	TTGGTCAGACTTCCTGCTACGC
Mcad	AGGATGACGGAGCAGCCAATGA	GCCGTTGATAACATACTCGTCAC
Nrf1	GGCAACAGTAGCCACATTGGCT	GTCTGGATGGTCATTTCACCGC
Pgc1a	GAATCAAGCCACTACAGACACCG	CATCCCTCTTGAGCCTTTCGTG
Prdm16	ATCCACAGCACGGTGAAGCCAT	ACATCTGCCCACAGTCCTTGCA
Tfam	GAGGCAAAGGATGATTCGGCTC	CGAATCCTATCATCTTTAGCAAGC
Ucp1	GCTTTGCCTCACTCAGGATTGG	CCAATGAACACTGCCACACCTC

Results

Thermogenic activation of the brown adipose tissue upregulates UCP1 and RBP4 gene expression

It was confirmed that after 3 hours of cold exposure at 4°C as well as intraperitoneal injection with a β3-adrenergic receptor agonist (CL 316,243), UCP1 is upregulated in BAT of the C57BL/6 mice as compared to the group housed at room temperature (Figure 2A, C). UCP1 expression was also increased in subcutaneous fat after cold exposure, but the changes were not significant. Along with UCP1, a significant increase of RBP4 mRNA levels was observed in BAT whereas no effects were seen in WAT (Figure 2B, D).

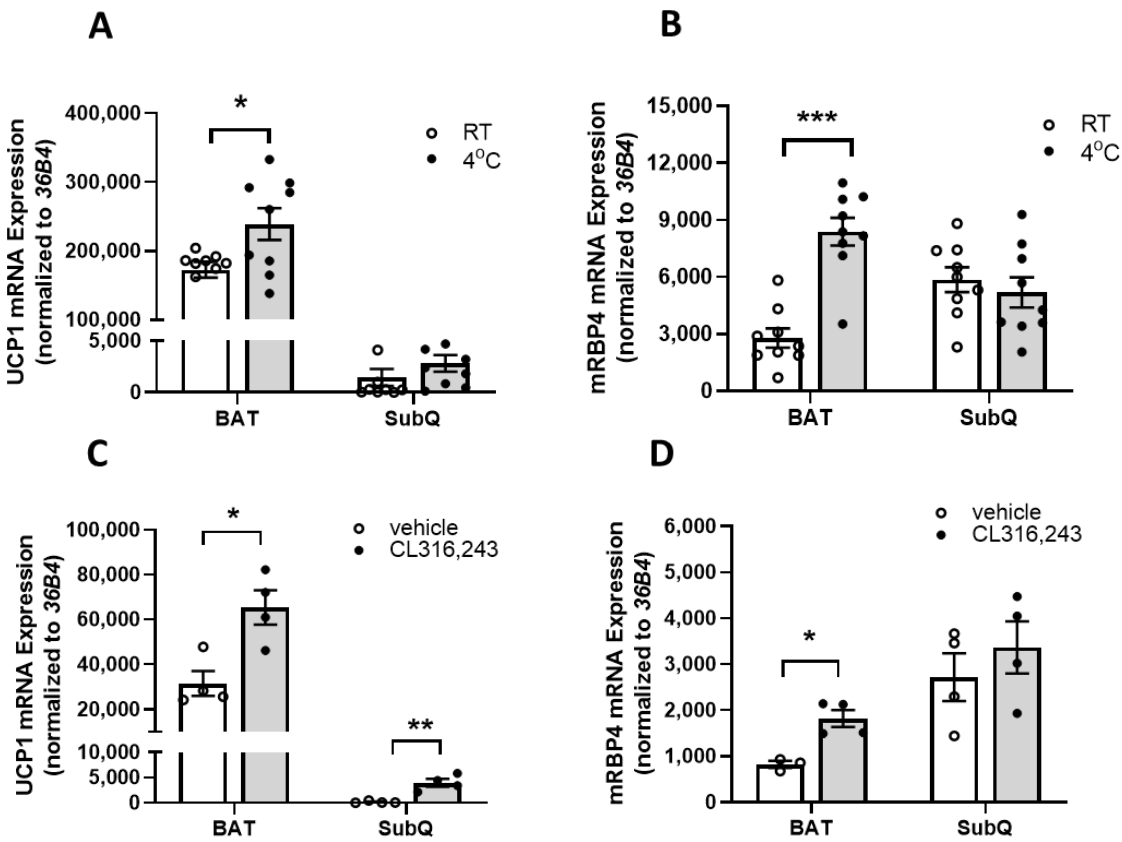

Figure 2. m RNA expression of UCP1 and mouse RBP4 in brown and subcutaneous fat tissues after thermogenic stimulations.

C57BL/6 mice were exposed to 4°C ambient temperature for 3 hours (A, B), or intraperitoneally injection with 1mg/kg of CL 316,243 (C, D). *UCP1* (A, C) and mouse RBP4 mRNA (B, D) expression levels in brown adipose tissue (BAT) and subcutaneous adipose tissue (SubQ) were determined, and values were normalized to the *36b4* reference gene. (n=9 per group). *, $p<0.05$, and ***, $p<0.001$ vs. C57BL/6 RT and vehicle.

Generation and characterization of the UCP1-hRBP4 mice

The significant increase of RBP4 expression in BAT during cold exposure (Figure 2B), along with the findings of the previous studies of RBP4 in white adipose tissue which suggested its function as an adipokine [12,13,20], led us to further investigate the role of RBP4 in BAT.

To study the role of RBP4 in BAT, brown adipocyte-specific human RBP4 transgenic mice were generated. ROSA 26 knock-in strategy was adopted, which allows for the expression of a bicistronic message, encoding both human RBP4 transgene and EGFP, after removal of a *lox*P-flanked *neo*^{r}stop cassette (Figure 3A). The knock-in mice were bred with UCP1-cre mice. To verify transgene expression, tissue extracts were analyzed by western blotting for hRBP4 expression. Liver and adipose tissues are sites where RBP4 is normally synthesized, but Figure 3B shows that transgene expression is observed only in BAT, and not in subcutaneous and visceral fats, or the liver of UCP1-hRBP4 mice. The overexpression of human RBP4 in BAT of UCP1-hRBP4 mice compared to its littermate controls was further confirmed by quantitative RT-PCR analysis, which showed 140 times increase in fold change (Figure 3C), whereas endogenous mouse RBP4 expression remained unchanged (Figure 3D). This resulted in a slight elevation in total RPB4 (mRBP4+hRBP4) mRNA expression in BAT of UCP1-hRBP4 mice.

The phenotypic characteristics are as follows in these transgenic mice. Figure 4A shows that UCP1-hRBP4 mice are significantly lower in body weight compared to the control group. When tissue weight was measured and normalized to body weight, visceral and brown fats were significantly lower than those of the controls, whereas subcutaneous and liver showed no difference in weight (Figure 4B). This was further confirmed by body composition analysis using minispec LF50, which showed that fat mass is significantly lower while lean mass is substantially higher in the UCP1-hRBP4 mice (Figure 4C).

UCP1-hRBP4 mice are more glucose tolerant

Glucose homeostasis is determined by the rate of insulin release and the sensitivity of the insulin targeted tissues [17]. To assess the glucose homeostasis in UCP1-hRBP4 mice, glucose and insulin tolerance tests were conducted. In a glucose tolerance test performed by intraperitoneal injection, I have observed an improved glucose tolerance in the UCP1-hRBP4 mice (Figure 5A). The starting point at time zero and the blood glucose level peak at 15 min point were similar but the glucose level was significantly cleared faster at the 90-minute time point. In addition to the absolute blood glucose measurement on a time course, the area under the curve, which is a calculation of the whole glucose excursion after injection, was also statistically lower than the control group (Figure 5B). However, no difference between the genotypes was observed in the insulin tolerance test (Figure 5C).

RBP4 in BAT enhances whole-body energy balance

The UCP1-hRBP4 mice and their littermate controls were individually housed in metabolic cages (Comprehensive Lab Animal Monitoring System) for three days. Cumulative food intake did not differ between control and UCP1-hRBP4 on a chow diet, and the physical activities within the cage during this period were similar in both groups (Figure 6F, G).

The basal rate of oxygen consumption as well as carbon dioxide production in UCP1-hRBP4 mice were substantially increased in the light condition (Figure 6A, B). Accordingly, heat production was significantly higher in the transgenic mice (Figure 6C), which positively correlated with higher core body temperature compared to the controls (Figure 6G). The respiratory quotient (RER) is comparable between the two genotypes (Figure 6D).

Fasting plasma parameters, as well as lipogenesis and lipolysis factors, are not significantly altered except for the plasma leptin levels.

No statistical difference was observed in the plasma RBP4, free fatty acid, triglycerides, and adiponectin levels between UCP1-hRBP4 and control mice (Figure 7A-D). The only difference observed in the plasma parameters was leptin levels, which are significantly decreased in the UCP1-hRPB4 group (Figure 7D).

Brown fat TG levels in the fasted state, as well as the representative H&E staining of BAT, were comparable between genotypes (Figure 8A, B). Since the lipid metabolism in tissue is determined by the rate of lipolysis and lipogenesis, expression levels of adipose triglyceride lipase, hormone-sensitive lipase, and lipoprotein lipase (*Atgl, Hsl, Lpl*) which are associated with lipolysis, as well as genes related to fatty acid uptake, including cluster of differentiation 36, fatty acid transporter 1 and 4 (*Cd36, Fatp1, Fatp4*) were screened, and no changes were found except for an increase in expression of *Fatp1* (Figure 8C). Also, there were no changes in the expression of genes associate to lipogenesis such as diacylglycerol acyltransferases, fatty acid synthetase, acetyl-CoA carboxylase (*Dgat1, Dgat2, Fasn, Acc1*) (Figure 8D).

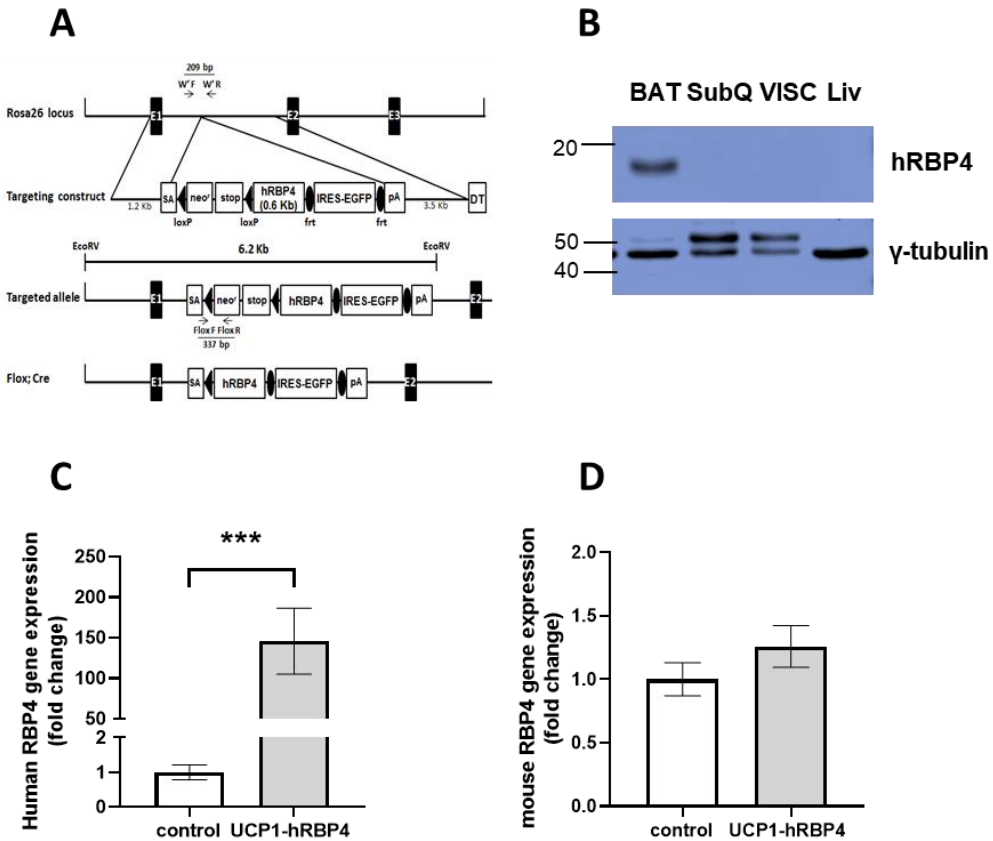

Figure 3. The UCP1-hRBP4 transgenic mouse model construct and validation of specific human RBP4 overexpression.

(A) Targeting strategy of the endogenous ROSA26 hRBP4 knock-in mice bred with *Ucp1*-cre mice. (B) Western blot analysis showing human RBP4 protein expression in brown adipose tissue, but not in subcutaneous, visceral adipose tissues and liver of UCP1-hRBP4 mice. (C) Human and (D) mouse RBP4 mRNA expressions in BAT of UCP1-hRBP4 mice were determined by qPCR (n=5-10). ***, $p<0.001$ vs. control. Data are presented as mean ± SEM.

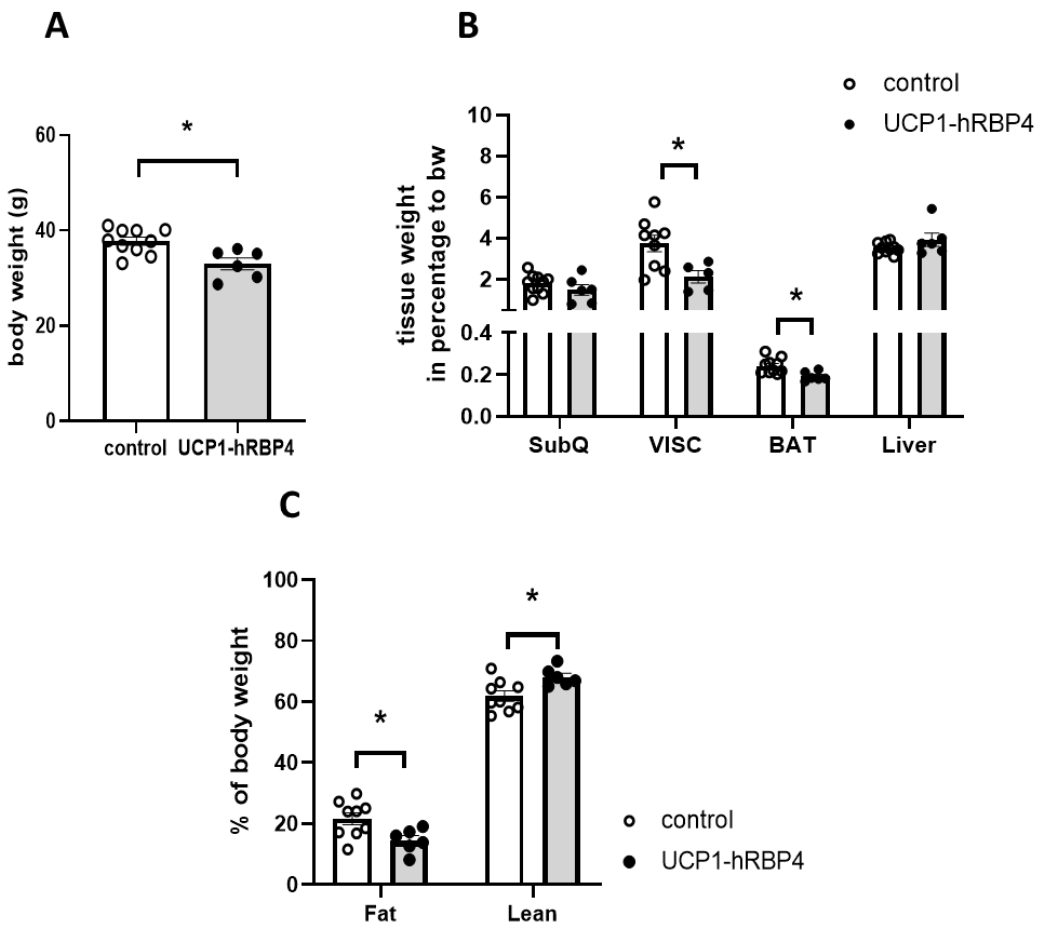

Figure 4. Bodyweight, tissue weight, and body composition of the UCP1-hRBP4 mice.

(A) Mouse body weight in grams. (B) Body composition of UCP1-hRBP4 and littermate control mice. Weight of subcutaneous (SubQ), visceral (VISC), brown fat (BAT), and liver normalized to body weight. (C) Fat and lean mass in percentage to body weight as measured by minispec. (n=5-10). *, $p<0.05$ vs. control. Data are presented as mean ± SEM.

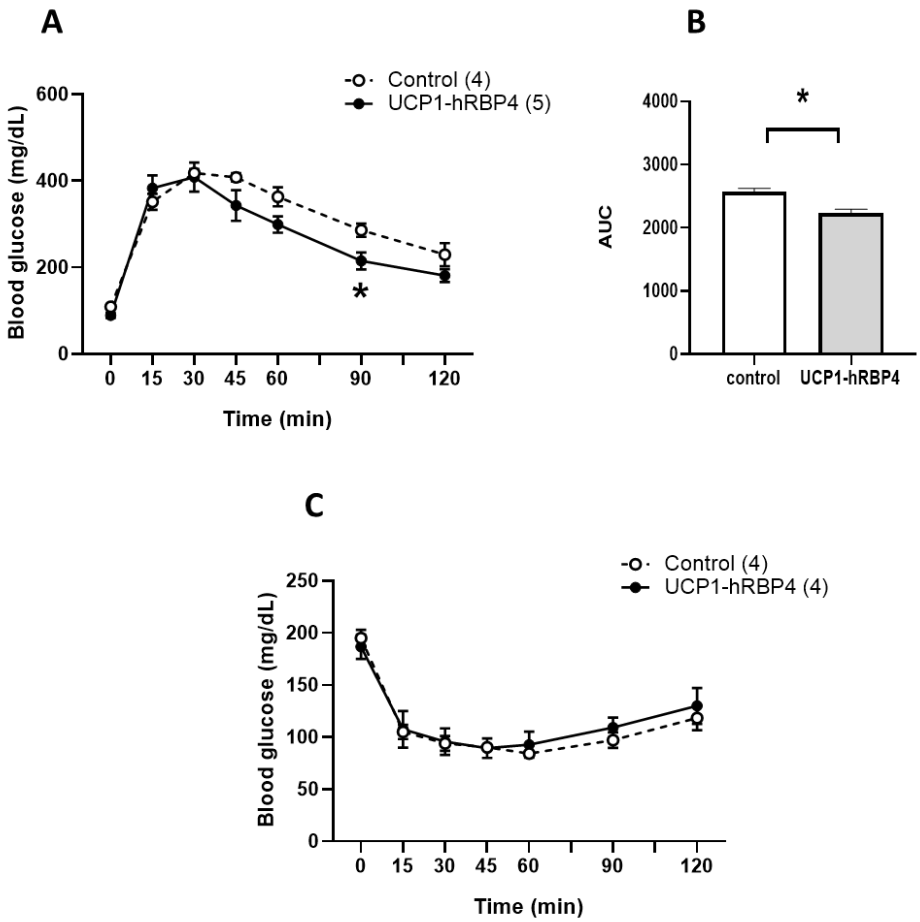

Figure 5. Glucose tolerance test and the calculated area under the curve, and insulin tolerance test.

(A) Blood glucose level curves and (B) the area under the curve graphs for both genotypes tested for glucose tolerance (intraperitoneal injection of 2g of 20% D-glucose/kg body weight, 16 h after food removal) (n=5-7). *, $p<0.05$ vs. control. (C) Insulin tolerance test (intra-peritoneal injection of 1U insulin /kg body weight, 5 h after food removal (n=4/group). Data are presented as mean ± SEM.

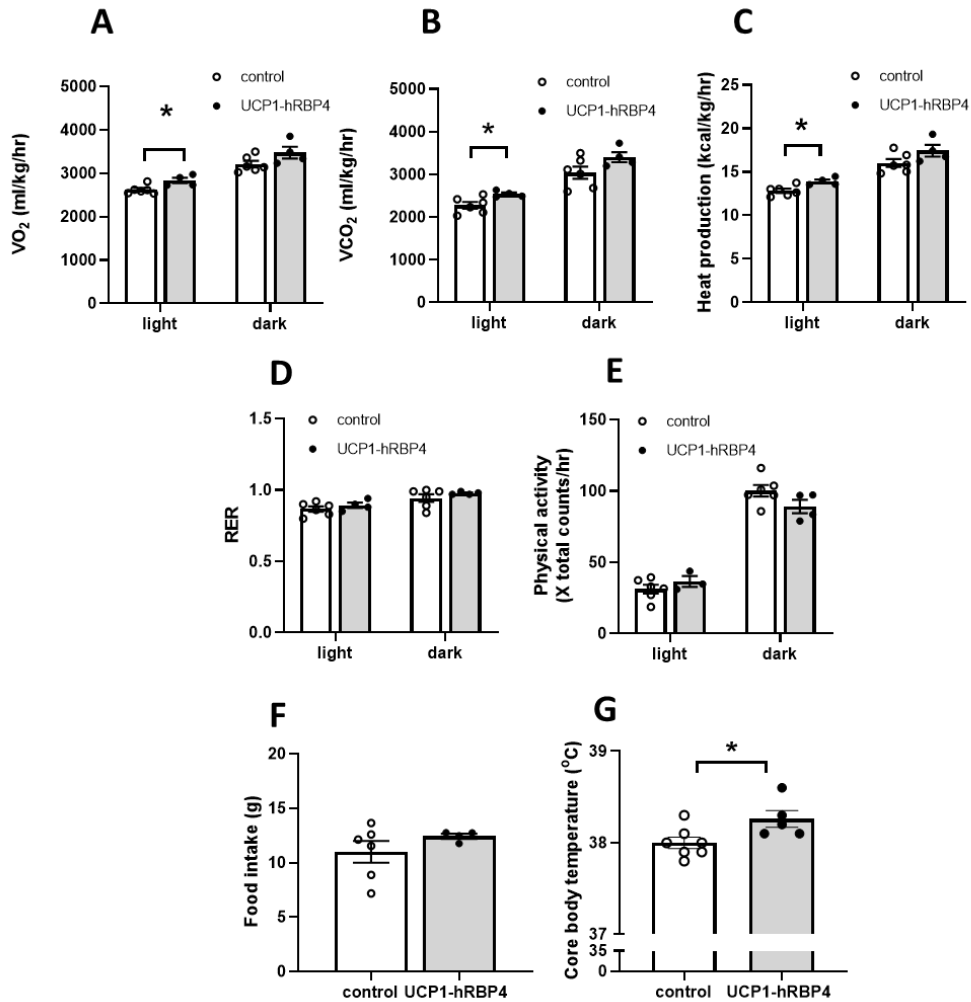

Figure 6. Metabolic parameters and core body temperature.

Metabolic parameters obtained by Comprehensive Lab Animal Monitoring System (CLAMS). (A) Oxygen consumption, (B) carbon dioxide production, (C) heat production, (D-F) respiration exchange ratio, physical activity, and food intake were calculated by the packaged program. (G) Rectal core body temperature measured before daytime. (n=5-7). *, $p<0.05$ vs. control. Data are presented as mean ± SEM.

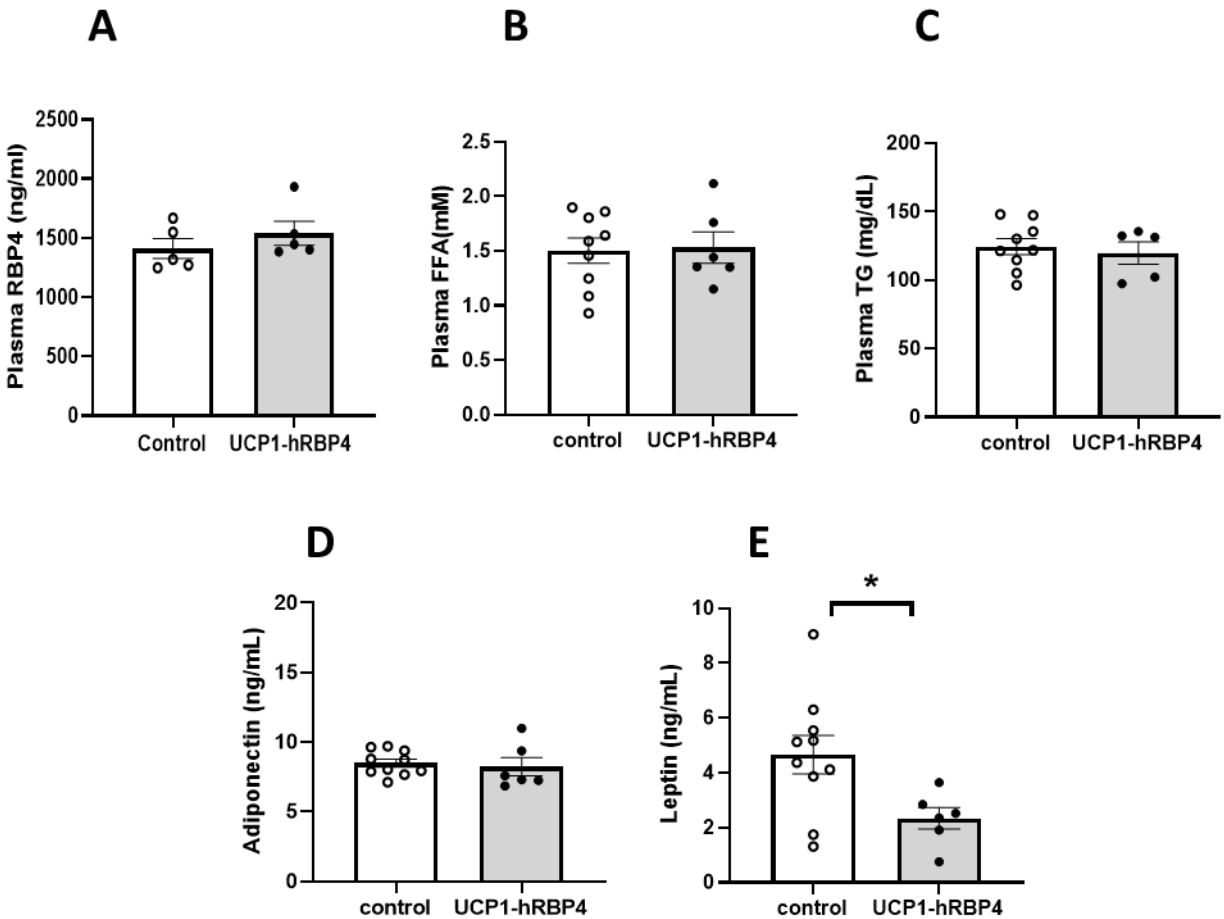

Figure 7. Plasma parameters of UCP1-hRBP4 mice.

Plasma levels of (A) RBP4, (B) free fatty acid, (C) triglyceride, (D) adiponectin, and (E) leptin. Plasma was collected upon sacrifice after 16 h fasting. (n=5-6) (n=6-10). *, $p<0.05$ vs. control. Data are presented as mean ± SEM.

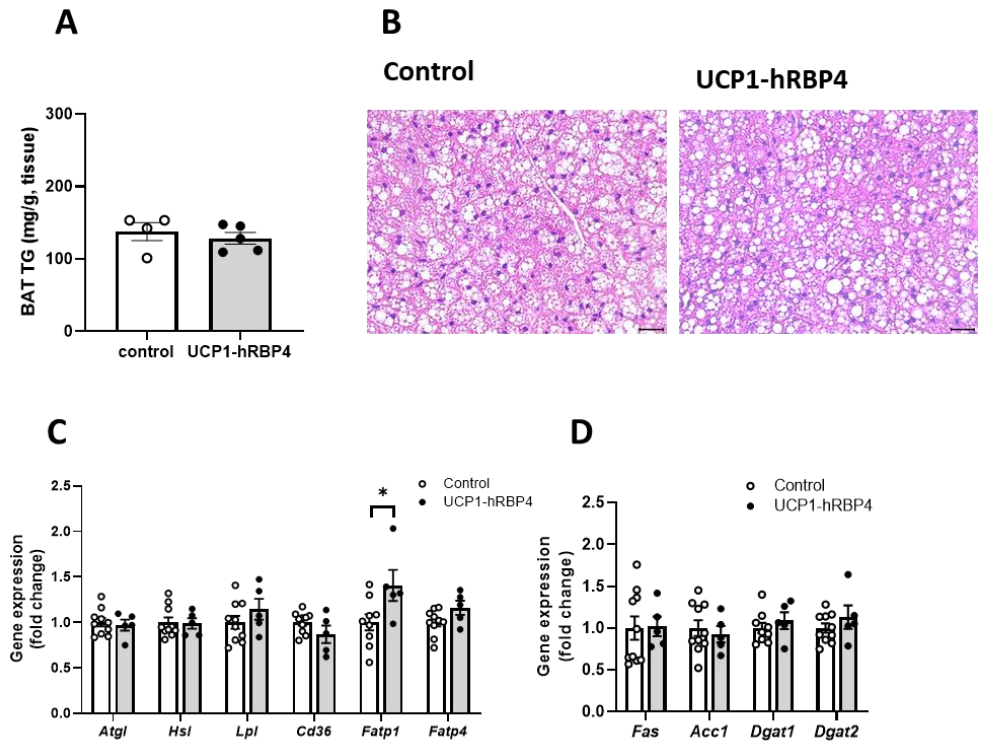

Figure 8. Lipogenesis and lipolysis in BAT through triglyceride level and gene expression measurement.

(A) Triglyceride levels in BAT of control and UCP1-hRBP4 mice, and (B) their representative H&E staining. (n=5-7). Real-time PCR results in BAT for mRNA expression associated with (C) lipolysis, fatty acid uptake, and (D) lipogenesis. (n=6-10). Data are presented as mean ± SEM.

RBP4 promotes higher fatty acid oxidation in BAT and increases thermogenic gene expression

Because metabolic rate and basal body temperatures were increased in the UCP1-hRBP4 mice (Figure 6), I further scrutinized the functions of RBP4 in BAT. Since mitochondria utilize lipids as an energy source to produce heat, the lipids must undergo several degradation processes such as fatty acid oxidation. Fatty acid oxidation is an aerobic mitochondrial processing of fatty acids into acetyl-CoA units [19]. Since free fatty acids are potent activators of the UCP1-induced uncoupling in BAT, I have measured the fatty acid oxidation rate (Figure 9A) and analyzed the mRNA levels of the related genes including carnitine palmitoyl transferases, acyl-CoA synthetase-1, medium-chain acyl-CoA dehydrogenase (*Cpt1b, Cpt2, Acsl1, Mcad*) (Figure 9B). This has revealed that the fatty acid oxidation rate is significantly increased by 1.5 times in the BAT of the UCP1-hRBP4 mice as compared to the control group, but no statistical difference was observed in the expression of related genes.

Further gene expression analysis of thermogenic mRNA levels (Figure 9C) revealed that no statistical difference was seen in the *Ucp1* expression between the UCP1-hRBP4 and control mice, as well as the expression of inducing dffa like effector a, and Adrenoceptor Beta 3 (*Cidea*, *Adrb3)*. However, the representative thermogenic gene expression levels including elongation of very long chain fatty

acids, type II iodothyronine deiodinase, and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*Elovl3, Dio2, Pgc1a*) have substantially increased to as much as 4 times as compared to those of the control group. However, genes associated with mitochondrial respiration and TCA cycle such as nuclear respiratory factor 1, mitochondrial transcription factor A and cytochrome c oxidases (*Nrf1, Tfam, Cox7a1, Cox8b*) were not significantly different in both groups (Figure 9D).

Systemic *in vivo* lipolysis is increased in the UCP1-hRBP4 mice.

To account for the increase in fatty acid oxidation levels in BAT, I have investigated the changes in lipid metabolism. Activation of the ß-adrenoreceptors in brown adipocytes leads to an increase in lipolysis [5]. Moreover, along with the studies which show that ablations of ATGL and HSL in mice result in down-regulation of genes involved in fatty acid oxidation and lipid metabolism in BAT [29,30], the possibility that RBP4 could enhance fatty acid oxidation through the changes in the regulation of lipolysis was recognized.

After ß-adrenergic stimulation with Cl316,243, changes in vivo lipolysis were analyzed in the UCP1-hRBP4 mice and their littermate controls. Plasma glycerol levels were significantly higher at the basal level as well as after 4 hours of adrenergic stimulation in the UCP1-hRBP4 mice as compared to the controls (Figure 10A). Plasma free fatty acid levels, or the systemic lipolysis in the transgenic were also significantly higher at 2-hour and 4-hour time points (Figure 10B).

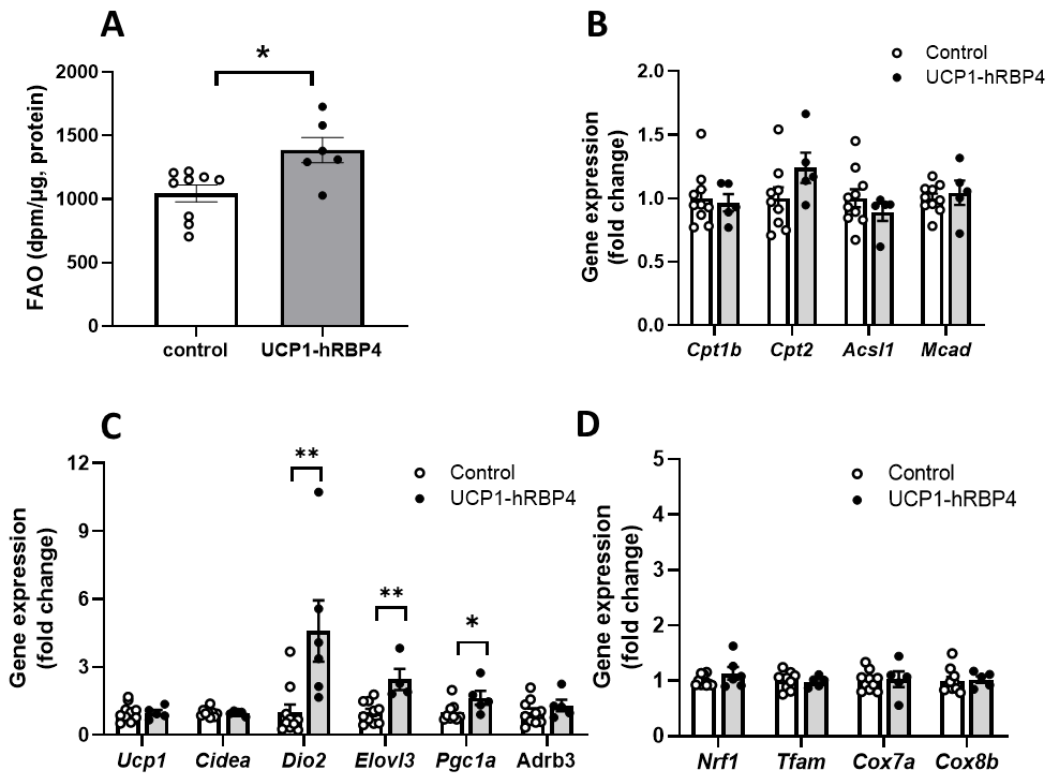

Figure 9. Fatty acid oxidation measurement in BAT, and mRNA levels of fatty acid oxidation, thermogenesis, and mitochondrial biogenesis.

(A) Fatty acid oxidation rate measured in BAT of the UCP1-hRBP4 mice and their littermate controls. (B) mRNA levels of fatty acid oxidation genes (*Cpt1b, Cpt2, Acsl1, Mcad*), (C) thermogenic genes (*Ucp1, Cidea, Dio2 Elovl3,, Pgc1a, Adrb3*) and (D) mitochondrial biogenesis genes (*Nrf1, Tfam, Cox7a, Cox8b* in BAT. (n=6-10). *, $p<0.05$, **, $p<0.01$ vs. control. Data are presented as mean ± SEM.

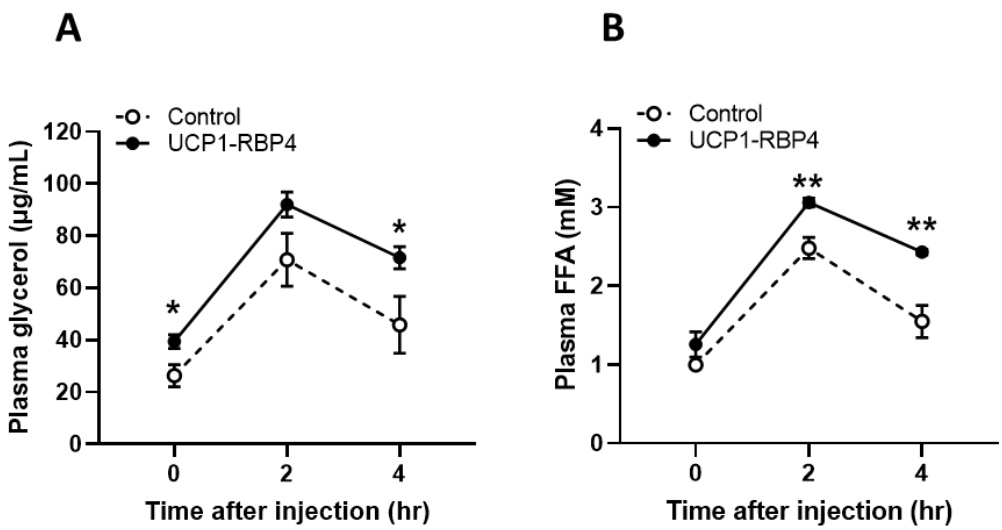

Figure 10. Plasma glycerol and free fatty acid measurements of *in vivo* lipolysis assay in the UCP1-hRBP4 mice.

(A) Plasma glycerol and (B) plasma free fatty acid levels were measured at 0,2,4-hour time points after intra-peritoneal injection of CL316,243 (1mg/kg body weight, 16h after food removal). (n=4-5). *, $p<0.05$, **, $p<0.01$ vs. control. Data are presented as mean ± SEM.

RBP4 overexpression in the brown fat causes browning in subcutaneous fat as a secondary effect

Another type of thermogenic fat other than the brown adipose tissue is the beige fat. Beige adipocytes are thermogenic adipocytes that reside in the WAT depots and when stimulated by external or internal cues, are capable of expressing similar levels of UCP1 as brown adipocytes [5]. In the RBP4 deficient mice, upon thermogenic stimulation, browning of the subcutaneous fat was perturbed compared to the wild-type mice, which showed the importance of retinol transport in the formation of beige fats [22]. Therefore, in the RBP4 overexpressing mice, I have also investigated for the possible changes in the subcutaneous white fats.

Since the hallmark of browning of WAT is the development of UCP1-positive multilocular adipocytes, immunohistochemistry with anti-UCP1 antibody was conducted on subcutaneous fat tissue sections (Figure 11A). UCP1-positive adipocytes were more abundant in the subcutaneous fat of UCP1-hRBP4 mice, as compared to those of the control mice. Despite the difference observed in UCP1-staining, mRNA levels of thermogenic genes were similar in both genotypes, but genes associated with ß-adrenergic signaling (*Adrb3, Hsl*) and fatty acid transport (*Fatp1*) were significantly increased in the UCP1-hRBP4 mice compared to those of the controls. (Figure 11B, C)

DEG analysis and the possible pathways associated with RBP4's effect on BAT

To study the underlying mechanisms of the effect of RBP4 in BAT, an unbiased mRNA sequencing was performed on the BAT of both genotypes. In UCP1-hRBP4 mice, 225 genes were upregulated and 148 genes were down-regulated as compared to the control group, and the heat map shows the hierarchical clustering (Figure 12A). The top 10 terms of KEGG analysis for the DEGs revealed that metabolic pathways were most altered in expression, followed by other equally important pathways such as cAMP signaling and calcium signaling pathways (Figure 12B).

KEGG analysis confirmed that *Elovl3* is upregulated in the biosynthesis of very long chain fatty acids (VLCFAs), and revealed that *Acot2* is also upregulated in this process (Figure 12C). Along with the previous qPCR data where *Dio2* expression was increased by 4 folds in BAT (Figure 9C), KEGG analysis confirmed the upregulation of the *Dio2* gene (Figure 12D).

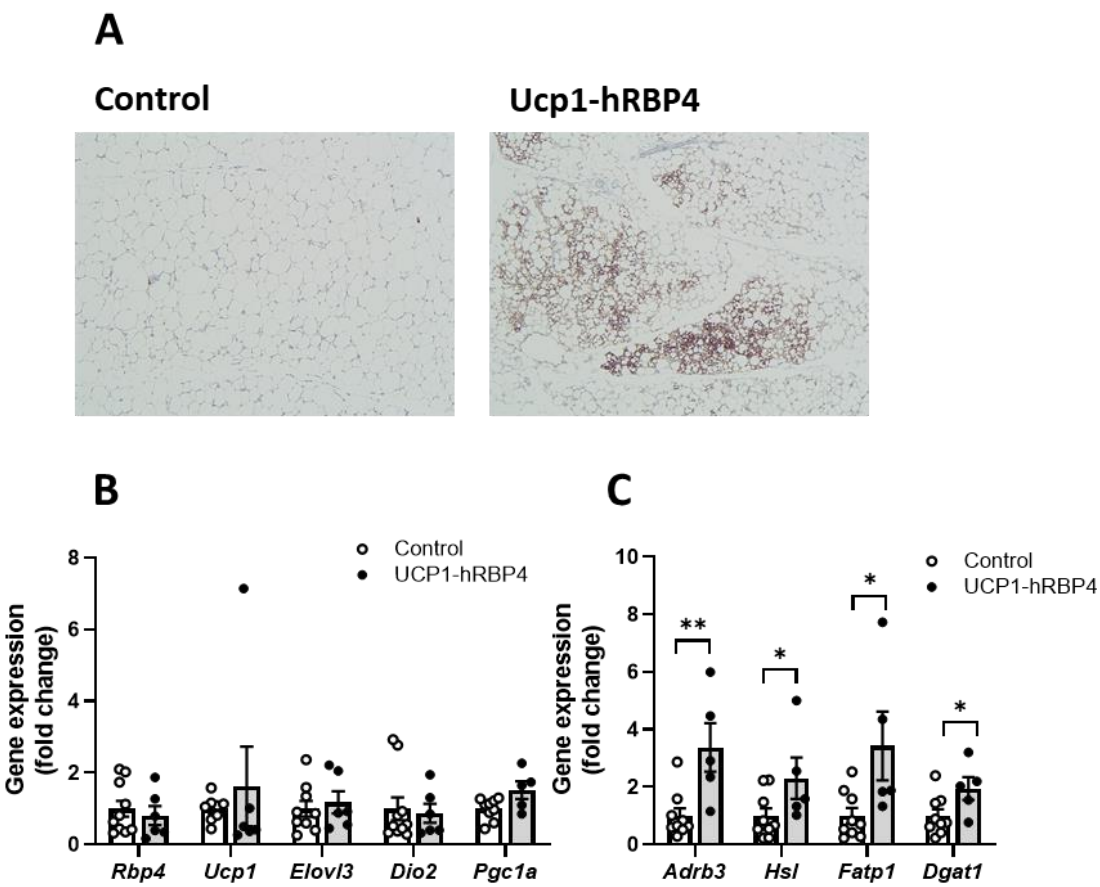

Figure 11. Immunohistochemistry of UCP1 and mRNA levels of thermogenic genes in the subcutaneous fat.

(A) Representative immunohistochemistry image of subcutaneous fat tissue sections stained with anti-UCP1 antibody. (n=5-7) (B) mRNA expression of endogenous RBP4 and thermogenic genes (*Ucp1, Elovl3, DIo2, Pgc1a*), (C) as well as genes related to noradrenergic activation, lipolysis, fatty acid uptake, and lipogenesis (*Adrb3, Hsl, Fatp1, Dgat1*) (n=6-10). *, p<0.05, **, p<0.01 vs. control. Data are presented as mean ± SEM.

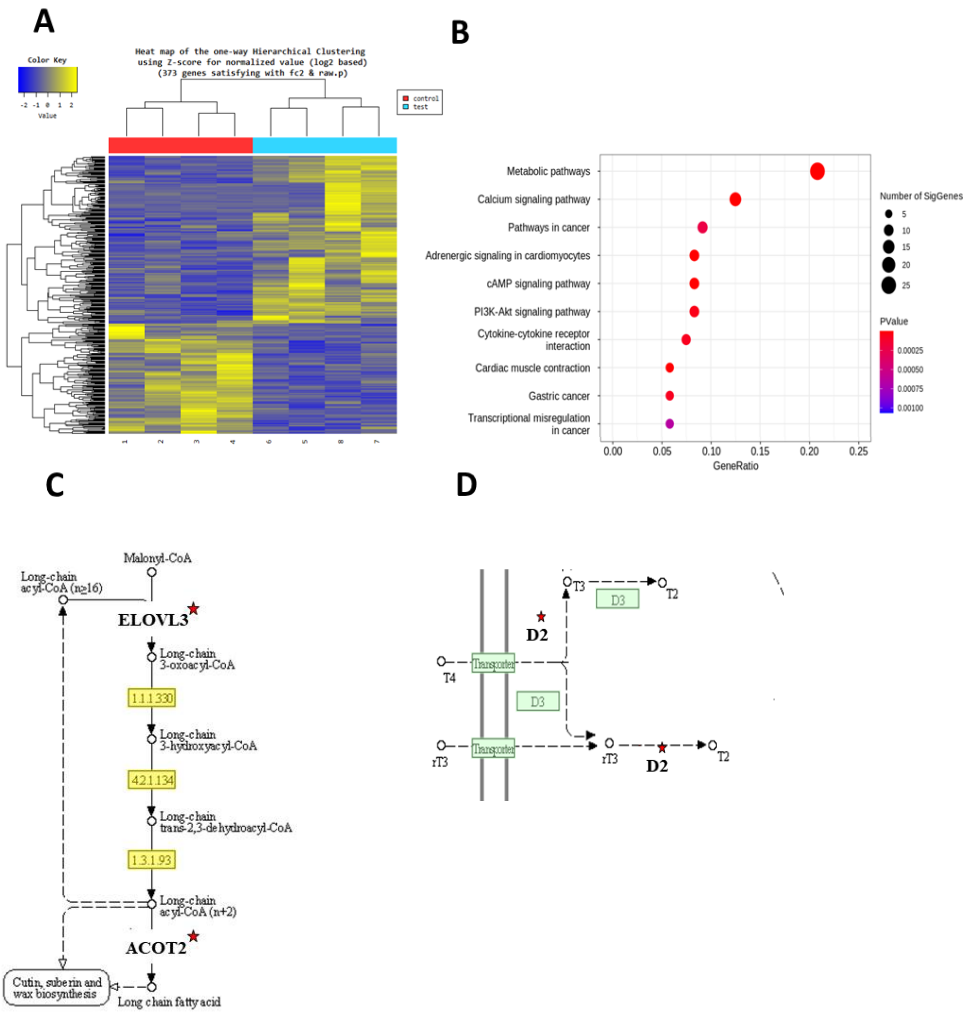

Figure 12. Analysis of uniquely regulated gene programs in the BAT of UCP1-hRBP4 mice.

(A) Heat map of the one-way hierarchical clustering of 373 genes. (B) Top 10 terms of KEGG analysis for the DEGs in the BAT of UCP1-hRBP4 mice. Differentially expressed genes of the (C) fatty acid elongation and (D) thyroid hormone signaling pathways.

Discussion

The current studies on RBP4 are rather controversial in defining its relation to insulin resistance, obesity, and metabolic diseases. Nevertheless, there is a fair consensus on the fact that RBP4 expressed in WAT, especially in visceral fat, is positively correlated to obesity. It was first discovered that in the absence of GLUT4, insulin resistance induced by obesity was interconnected to elevated levels of RBP4 in WAT and in serum [13, 20]. Similarly, in mice that specifically overexpress human RBP4 in adipose tissues, hepatic steatosis was induced, but the adverse consequences occurred even before elevations of circulating RBP4 levels were detectable [14]. In BAT, however, RBP4 is proposed to take a contrasting role. When BAT is activated upon noradrenergic stimulus, not only UCP1 but also RBP4 expression levels are substantially increased [15]. Moreover, since the previous studies have comprehensively established that retinoid signaling is important in the whole-body energy metabolism and UCP1 induction, I have investigated the role of RBP4 in BAT.

To examine the function of RBP4 in brown adipose tissue, the UCP1-hRBP4 mice models that specifically overexpress human RBP4 in the BAT were generated. These mice displayed several important characteristics that indicate a positive correlation between RBP4 and anti-obesity effects. Lower body weight and decreased fat mass in UCP1-hRBP4 mice suggest a change in the whole-body energy

balance (Figure 4). The plasma leptin levels were significantly lower in the transgenic mice (Figure 7E), and this correlates with the decrease in fat mass since adipose tissues are known to secrete leptin [18]. Furthermore, the overexpression of RBP4 in brown adipose tissue led to an improvement in glucose tolerance (Figure 5). Other prominent features of the transgenic mice of this study are that metabolic respiration rates were enhanced, and the higher body temperature, despite the similar levels in food intake and physical activity directed a change in the energy expenditure paradigm (Figure 6). In line with the finding in which RBP4 overexpression in adipose tissue preceded an elevation in circulating RBP4 in chow-fed mice [14], I have also shown that the plasma profile was not changed despite the overexpression of RBP4 in BAT (Figure 7). According to the previous studies, neither of the mice with adiponectin-driven overexpression of human RBP4 nor UCP1-driven overexpression of human RBP4 show statistical difference in the circulating levels of RBP4 on a chow diet, but since hepatocyte-specific RBP4 knockout or liver-specific RBP4 overexpression directly translates to changes in the serum levels of RBP4, it can be assumed that the liver is the major tissue contributing to the circulating levels of RBP4 [21]. Thus, it can be deduced that RBP4 overexpressed in BAT would rather be more active in an autocrine or paracrine manner.

One of the most important traits of the UCP1-hRBP4 mice was that fatty acid oxidation in BAT was significantly increased compared to the controls (Figure 9A). The increase of ß-oxidation rate indicates an elevation of BAT activity and a

promotion of energy expenditure. However, despite the increase in ß-oxidation rates, there were no changes in the genes associated with the oxidation enzymes (Figure 9B). To comprehend the increase in the fatty acid oxidation in BAT, I have examined the mechanisms behind fatty acid oxidation, and have come up with a possible explanation. Usually, carnitine palmitoyl transferase 1 (CPT1) is known as the rate-limiting enzyme in the regulation of mitochondrial fatty acid oxidation, and it is inhibited by malonyl-CoA in high-energy conditions [31]. Additionally, AMP-activated protein kinase (AMPK) activation is also known to promote fatty acid oxidation by decreasing malonyl-CoA levels and thereby enhancing the activity of CPT1 [32]. However, since RBP4 in BAT seems to be associated with the activation of the ß-adrenergic pathway (Figure 2), I have investigated its correlation to fatty acid oxidation. In BAT, when ß-adrenergic signaling is activated, the increase in cAMP levels stimulates activation of lipases such as HSL and ATGL that results in increased lipolysis of triglycerides [5]. In WAT, previous studies have shown that RBP4 induces inflammation in adipocytes that elevates the levels of TNFα, and the expression levels of HSL and ATGL [14,20]. Along with the studies which show that ablation of HSL and ATGL results in decreased fatty acid oxidation and thermogenesis [29,30], I proposed that RBP4 could affect the noradrenergic pathway to increase the hydrolysis of intracellular lipid droplets, resulting in elevated levels of free fatty acids supplied as substrates to increase ß-oxidation. However, since the expression levels of ATGL and HSL, which are central lipases in intracellular lipolysis, were unchanged (Figure 8C), another possibility is that the activity of the

lipases could be upregulated via phosphorylation by PKA, and that RBP4 could act as an activator to this process (Figure 1). The in vivo lipolysis assay revealed that the UCP1-hRBP4 display higher systemic lipolysis rates as compared to that of the controls. However, it is difficult to establish the causal relationship between systemic lipolysis and overexpression of RBP4 in BAT because the source of increased lipolysis is unclear. Thus, further study is required to confirm this hypothesis, and the methods would be to perform a western blot analysis to observe levels of phospho-ATGL and phospho-HSL in the BAT of UCP1-hRBP4 mice. Moreover, lipolysis assays could be conducted *ex vivo* as well as *in vitro* to validate the increase in lipolysis.

Moreover, gene expression associated with fatty acid elongation and thyroid hormone signaling pathways were significantly altered as a result of RBP4 overexpression in the BAT (Figure 12 B, C). Firstly, fatty acid elongase 3 (*Elovl3*) and Acyl-CoA thioesterase (*Acot2*) were upregulated in BAT of the transgenic mice as shown by the DEG analysis (Figure 12 C), of which the increase in *Elovl3* expression was also confirmed by the mRNA analysis (Figure 8C). In BAT, sufficient lipid turnover is critical, and maximal thermogenesis relies on the maintenance of long chain fatty acid (LCFA) pool. Literature supports that upon cold challenge, expression of genes associated with procurement and combustion of LCFAs are increased [24]. Additionally, in the study of *Elovl3*-ablated mice, the mice were unable to hyperactivate BAT during cold, and depended on muscle shivering to maintain body temperature [25]. Thus, in application to the UCP1-hRBP4 mice, the

upregulation of *Elovl3* signifies that if lipolysis was increased as previously suggested, the increased demand for fatty acid supply could have stimulated the expression of *Elovl3* to replenish the intracellular very long chain fatty acids (VLCFAs). Moreover, the upregulation of *Acot2* could also explain the increase in β-oxidation since *Acot2* is an enzyme that hydrolyzes long-chain acyl-CoA to free fatty acids, supplementing mitochondria for active fatty acid oxidation [26].

Thyroid hormone signaling is also critical in regulating energy expenditure and the activity of BAT. *Dio2* is a gene that encodes for D2 protein, and its expression is increased by 10- to 50- times during thermogenic stimulation [27]. D2 activates the intracellular T4 to T3, and the activated T3 enhances the cAMP response. Additionally, it is also associated with the expression of UCP1, because the thyroid hormone-responsive element is located 2000bp upstream of the UCP1 transcription point. In the UCP1-hRBP4 mice, it was shown that *Dio2* expression is significantly increased by over 4-folds (Figure 9C), and the KEGG analysis also confirmed the upregulation of *Dio2* (Figure 12D). The importance of DIO2 in thermogenesis was elaborated in a study where D2 knockout mice exhibited decreased expression levels of UCP1 and PGC1α, and showed impaired oxidative functions [28]. In my study, I have observed an increase in PGC1α expression along with DIO2 (Figure 9C), which proposes that RBP4 could be an important factor in the thyroid hormone signaling in regards to thermogenesis, but further investigation is required to understand how RBP4 affects the process.

Finding the mechanism behind the WAT browning has been the key question of many researchers for the past decades [8]. Since the beige fat possesses the capability to express similar levels of UCP1 as the brown adipocytes, it poses great potential in energy expenditure and possibly treatment of obesity [5]. The importance of RBP4 with regards to WAT browning, was shown in a study of RBP4-deficient mice, in which the lack of RBP4 disrupted the formation of beige fats even under thermogenic activation [22]. In my model of RBP4 overexpression in the BAT, I have observed the presence of UCP1-positive multilocular adipocytes (Figure 11A), as well as an increase in the expression of *Adrb3, Hsl,* and *Fatp1* genes (Figure 11C). Although I have yet been unable to identify the exact drivers for this change, the possible explanations are that an unknown factor produced by the RBP4-driven BAT activation could have induced the secondary changes (Figure 1), or that RBP4 secreted by BAT could have directly acted upon the subcutaneous fat. In a study reported by Wang B et al., it was found that retinoic acid promotes browning in WAT by activating the vascular endothelial growth factor (VEGF) signaling via binding to the RAR and RXR nuclear receptors. Retinoic acid also activates the VEFG-VEGFR2 that stimulates the proliferation of platelet-derived growth factor receptor α positive (PDGFRα+) adipose precursor cells. It was suggested that the increase in angiogenesis enhanced the energy expenditure of the active adipose tissue and resulted in the browning of the BAT [31]. In relation to the browning observed in the UCP1-hRBP4 mice, it is possible that the RBP4 overexpression in BAT could have acted in an autocrine or paracrine manner to affect the overall retinoid metabolism.

However, since this research is still at a preliminary stage, further examinations such as fatty acid oxidation measurement, lipid turnover rates, and measurement of retinoic acid in the subcutaneous fat of the UCP1-hRBP4 mice are necessary to comprehend the role of RBP4 in the BAT and its secondary effects.

Acknowledgments

I would like the acknowledge Dr. Seung Ah Lee for her profound insights and guidance, as well as Eun Kyung Joo and Jong Yeon Park for their help in conducting the glucose tolerance test and *in vivo* lipolysis assay.

국문초록

갈색지방 조직에서의 Retinol-Binding Protein 4 의 역할

비만의 근본적인 요인은 영양 섭취와 소비의 장기적 불균형이고, 여분의 에너지는 체지방의 형태로 축적이 된다. 지방조직은 에너지를 저장하는 역할로 많이 알려져 있었으나, 갈색지방은 열발생을 통해 에너지를 사용하는 것이 밝혀졌다. Retinoid 의 대사, 더 구체적으로 retinol-binding protein 4(RBP4)는, 에너지 대사에 중요한 영향을 끼친다. RBP4 는 비타민 A 로 알려진 retinol 을 혈액속에서 운반하는 단백질이다. RBP4 는 간과 지방 조직에서 가장 많이 발현이 되며, 지방에서 합성된 RBP4 는 TNFα 의 증가를 통해 지방분해를 촉진시키고, 이는 인슐린 저항성과 간경화와 같은 대사 질환과 연관이 있다고 알려져 있다. 하지만 갈색지방애서의 RBP4 의 연구들에 따르면, 조직이 활성화됐을 때 UCP1 과 더불어 RBP4 발현이 증가한다고 보고된 바 있고, 추위 노출로 인한 백색지방의 갈색화에 RBP4 가 중요하다고 밝혀졌다. 하지만 아직까지 갈색지방에서의 RBP4 의 역할은 정확하게 규명되지 않았다. 그래서 해당 연구는 갈색지방에서의 RBP4 의 대사적인 역할을 알아보기 위해 갈색지방에서 RBP4 를 특이적으로 과발현시킨 생쥐 모델을

사용하였다. RBP4 과발현 쥐는 대조군에 비해 체성분과, 당내성이 개선된 것을 볼 수 있었고, 기초 대사량과 중심체온이 유의미하게 높은 것으로 나타났다. 또한 갈색지방에서의 지방산화가 유의미하게 증가하였고 이러한 결과 봤을 때 갈색지방에서의 RBP4 는 지방산화와 갈색지방의 활성화에 주요한 역할을 할 수 있다고 관찰하였지만 자세한 기작을 밝히기 위한 추가적인 연구가 필요하다.

주요어: Retinol-Binding Protein 4, 갈색지방세포, 갈색화, 지방산산화, 비만

학번: 2019-24521

Chapter 5. Bibliography

1. Caballero B. The global epidemic of obesity: an overview. Epidemiol Rev. 2007; 29:1–5.

2. Blüher, M. Obesity: global epidemiology and pathogenesis. Nat Rev Endocrinol.2019; 15, 288–298

3. Dietrich, M, Horvath, T. Limitations in anti-obesity drug development: the critical role of hunger-promoting neurons. Nat Rev Drug Discov.2012; 11, 675–691.

4. Trayhurn P. Origins and early development of the concept that brown adipose tissue thermogenesis is linked to energy balance and obesity. Biochimie. 2017; 134:62070.

5. Ikeda K et al. The Common and Distinct Features of Brown and Beige Adipocytes. Trends in Endocrinology & Metabolism. 2018; 29 (3).

6. Rothwell NJ, Stock MJ. Luxuskonsumption, diet-induced thermogenesis and brown fat: the case in favour. Clin Sci (Lond). 1983 Jan;64(1):19-23.

7. Betz, M, Enerbäck, S. Targeting thermogenesis in brown fat and muscle to treat obesity and metabolic disease. Nat Rev Endocrinol. 2018; 14, 77–87.

8. Townsend K, Tseng YH. Brown adipose tissue: Recent insights into development, metabolic function and therapeutic potential. Adipocyte. 2012;1(1):13-24. doi:10.4161/adip.18951

9. Bonet L et al. Lipid metabolism in mammalian tissues and its control by retinoic acid. Molecular and Cell Biology of Lipids. 2012; 1821(1).

10. Herz C & Kiefer F. The Transcriptional Role of Vitamin A and the Retinoid Axis in Brown Fat Function. Front Endocrinol. 2020; 11(608)

11. Guo H et al. Lipocalin2, a Regulator of Retinoid Homeostasis and Retinoid-mediated Thermogenic Activation in Adipose Tissue. Journal of Biological Chemistry. 291(21)p11216-11229.

12. Villarroya, F., Cereijo, R., Villarroya, J. et al. Brown adipose tissue as a secretory organ. Nat Rev Endocrinol. 2017; 13, 26–35

13. Yang, Q, Graham T, Mody, N. et al. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature. 2005; 436, 356–362.

14. Lee SA et al. Adipocyte-specific overexpression of retinol-binding protein 4 causes hepatic steatosis in mice. Hepatology. 2016; 64(5) p1534-1546.

15. Rosell M et al. Proliferator-Activated Receptors-α and -γ, and cAMP-Mediated Pathways, Control Retinol-Binding Protein-4 Gene Expression in Brown Adipose Tissue. Endocrinology. 153(3)p1162-1173

16. Leiva M et al. Uncovering the Role of p38 Family Members in Adipose Tissue Physiology. Front.Endocrionol. 2020.

17. Bowe J et al. METABOLIC PHENOTYPING GUIDELINES: Assessing glucose homeostasis in rodent models, Journal of Endocrinology. 2014; 222(3)

18. Zhao S et al. Leptin: Less Is more. Diabetes. 2020; 69(5) 823-829.

19. Houten S et al. The Biochemistry and Physiology of Mitochondrial Fatty Acid β-Oxidation and Its Genetic Disorders. 2016; Annual Review of Physiology. 78:23-44.

20. Kilicarslan M et al. RBP4 increases lipolysis in human adipocytes and is associated with increased lipolysis and hepatic insulin resistance in obese women. Faseb J. 2020; 34(5):6099-6110.

21. Steinhoff J et al. Biological Functions of RBP4 and Its Relevance for Human Diseases. Front Physiol. 2021.

22. Fenzl A, Kulterer O, Kiefer F et al. Intact vitamin A transport is critical for cold-mediated adipose tissue browning and thermogenesis. Molecular Metabolism, 2020.

23. Westerberg R, Jacobsson A, et al. ELOVL3 Is an Important Component for Early Onset of Lipid Recruitment in Brown Adipose Tissue. Journal of Biological Chemistry. 2005; 281 (8).

24. Yu X et al. Cold elicits the simultaneous induction of fatty acid synthesis and β-oxidation in murine brown adipose tissue: prediction from differential gene expression and confirmation in vivo. The FASEB Journal. 2002; 16(2).

25. Mottillo E et al. Coupling of lipolysis and de novo lipogenesis in brown, beige, and white adipose tissues during chronic β3-adrenergic receptor activation. Journal of Lipid Research. 2014; 55(11).

26. Moffat, Cynthia et al. Acyl-CoA thioesterase-2 facilitates mitochondrial fatty acid oxidation in the liver. Journal of lipid research. 2014; vol. 55,12: 2458-70.

27. de Jesus L et al. The type 2 iodothyronine deiodinase is essential for adaptive thermogenesis in brown adipose tissue. The Journal of Clinical Investigation. 2001;108(9):1379-1385.

28. Hall J, Ribich S et al. Absence of Thyroid Hormone Activation during Development Underlies a Permanent Defect in Adaptive Thermogenesis. Endocrinology. 2010; 151(9): 4573–4582.

29. Pinent M et al. Differential transcriptional modulation of biological processes in adipocyte triglyceride lipase and hormone-sensitive lipase-deficient mice. Genomics.

2008; 92(1):26-32.

30. Haemmerle G et al. Defective Lipolysis and Altered Energy Metabolism in Mice Lacking Adipose Triglyceride Lipase. Science. 2006; 312,734.

31. Qu Q et al. Fatty acid oxidation and carnitine palmitoyltransferase I: emerging therapeutic targets in cancer. Cell Death & Disease. 2016;7(5):e2226.

32. Wang B et al. Retinoic acid induces white adipose tissue browning by increasing adipose vascularity and inducing beige adipogenesis of PDGFRα+ adipose progenitors. Cell Discovery. 2017; 17036

33. Cypess A et al. Identification and Importance of Brown Adipose Tissue in Adult Humans. N Engl J Med. 2009; 360:1509-1517

감사의 글

지난 2년간 많은 도움을 주신 분들께 감사의 마음을 전하고자 합니다.

우선 석사과정 동안 세심한 지도로 많은 가르침을 주신 박경수 교수님께 진심으로 감사드립니다. 또한 처음 연구실에 들어왔을 때 여러 조언해주신 정성수 박사님께도 감사드립니다. 그리고 항상 격려해주시고, 같이 고민해주시고 실험을 가르쳐 주신 이승아 박사님께 감사드립니다. 교수님과 박사님들 덕분에 연구자로서 생각하고 계획하는 방법을 배우고 성장할 수 있었습니다. 그리고 저의 논문심사를 맡아 주신 김태유 교수님과 박영주 교수님께도 감사드립니다.

연구실에서 많은 도움을 받았던 지선언니, 금연언니, 예슬언니, 종연 선생님, 수빈 선생님, 찬미 선생님과 내분비내과 선생님들께 진심으로 감사의 말씀 전합니다.

마지막으로 먼 타국에서도 항상 저를 응원해주시고 믿어 주신 부모님과, 사랑하는 할아버지, 할머니, 동생 명선이와 가족들, 그리고 힘들 때 마다 버팀목이 되어준 친구들 모두 감사하고 사랑합니다.

2021년 8월

하은선