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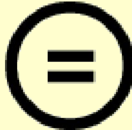
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The role of Hes1 in the regulation of metabolic disease

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The role of Hes1 in the regulation of metabolic disease

Directed by Professor Kyung-Hee Chun

The Master's Thesis
submitted to the Department of Medical Science,
The Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Master of Medical Science

Jaegyeong Lee

June 2021

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June 2021

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Abstract

The role of Hes1 in the regulation of metabolic disease

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(Directed by Professor **Kyung-Hee Chun**)

Hes1 is one of the most well-known targets of the Notch signaling pathway which is known as intracellular interaction and plays a role as a transcriptional factor. Although there are previous studies showing that Notch1, a receptor of the Notch signaling pathway, is involved in fat accumulation in the liver, little is known about the molecular mechanism. Increased Hes1 expression level was observed in liver tissues of mice fed with high-fat diet (HFD) and non-alcoholic fatty liver disease (NAFLD) patients. Based on this, in this paper, we performed whether Hes1 regulates NAFLD, a metabolic disease.

When comparing the weight and size of the liver by feeding a HFD in mice that specifically inhibited Hes1 in the liver, it was confirmed that the weight and size of liver were decreased compared to the wild-type mice. Accordingly, genes related to the inflammatory response, TNFα, F4/80, and CCL2, and genes related to fat accumulation, such as C/EBPβ,

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FABP4, CD36, SREBP, ACC1, and ACLY, were all decreased. The results were also shown in adipose tissue, and the expression of genes involved in thermogenesis, such as UCP1, was increased. In addition, when Hes1 is specifically inhibited in the liver, AST and ALT, which are indicators of liver toxicity, are decreased, but the overall metabolic rate is improved.

It was confirmed that there is a correlation between hepatokine FGF21 and Hes1, and these results suggest that Hes1 might be a new therapeutic target for NAFLD.

Key words : Hes1, obesity, NAFLD, FGF21

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I. Introduction

Over the last years, obesity has become a major public health problem which leads to chronic diseases including diabetes, cardiovascular disease and certain cancers[1]. Obesity is the most common causes of liver disease because excessive fat affects insulin resistance and inflammatory signal in liver[2]. Non-alcoholic fatty liver disease (NAFLD) is one of the most common liver disease that can progress to non-alcoholic steatohepatitis (NASH) and hepatocellular carcinoma (HCC)[3-7]. With the increasing prevalence of obesity and NAFLD in the general population, there is need for more effective therapeutic targets to block progression.

Notch signaling pathway is an evolutionarily highly conserved cell signaling system present in most animal[8]. Mammals are composed of four homologous Notch receptor (Notch 1-4), which can bind various ligands; Delta-like (DLL1, 3 and 4), Jagged (Jagged 1 and 2). These ligands induce Notch receptor and the Notch intracellular domain (NICD) is cleaved by γ-secretase, which leads to translocation of the NICD into the nucleus. In the nucleus,

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NICD interacts with the transcriptional factor RBP-J to transcript of transcriptional repressor such as Hairy and enhancer of split (Hes) family and Hes-related proteins (Hey). In the mammalian liver, Notch signaling pathway plays roles in development and regeneration[9-12]. There are researches showing that persistent activation of Notch signaling pathway is associated with liver disease, such as NAFLD, NASH, and HCC[13-15].

Hairy and enhancer of split-1 (Hes1) is one of the target genes of Notch signaling pathway, and is involved in cell proliferation and differentiation[16-18]. It has been reported that the expression level of Hes1 are significantly increased in liver of diet-induced fibrosis[19]. These findings suggested that Hes1 might be positively associated with obesity and lipid accumulation in liver. But specific molecular mechanism remains unknown.

In this study, we confirmed that the expression level of Notch signaling pathway factors in liver of high-fat diet (HFD) induced obese mice. To investigate whether Hes1 regulates metabolic disease such as NAFLD, we employed Hes1 liver-specific knockout mice (Hes1 LKO). Hes1 LKO mice were fed with HFD, and as a result, these mice had significantly lower body weight and liver sizes compared to the WT mice. The levels of AST and ALT, which are commonly used to liver function, were also decreased in Hes1 LKO mice. The liver and adipose tissues of Hes1 LKO mice show lower expression of lipogenic and inflammatory genes that those of WT mice. We also confirmed that Fibroblast growth factor 21 (FGF21), which is a hormone secreted by the liver, is positively associated with the expression level of Hes1. Collectively, these observations suggest that Hes1 plays an important role in regulating metabolic disease and might be potential therapeutic target in NAFLD.

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II. MATERIALS AND METHODS

1. Cell culture

AML12 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM):Ham's F12 medium (1:1) with 0.005 mg/ml insulin, 0.005 mg/ml transferrin, 5 ng/ml selenium, 40 ng/ml dexamethasone, 10% fetal bovine serum (Corning Cellgro), and 1% antibiotics (Invitrogen) and maintained at 37°C in humidified incubator with a 5% CO_2 atmosphere.

2. Transfection of small interfering RNA

AML12 cells were transfected with mouse Hes1 siRNA (50 nM) using Lipofectamine RNAimax (Invitrogen, Carlsbad, CA, USA) according to manufacturer's protocol. After 24 hour, media was replaced with maintenance media supplemented with 10% bovine serum.

3. Western blot

Cell lysate extractions were prepared with RIPA buffer (1% Triton X-100; 1% sodium deoxycholate; 0.1% sodium dodecyl sulfate; 150 mM NaCl; 50 mM Tris-HCl, pH 7.5; and 2 mM EDTA, pH 8.0). Cell lysates were incubated for 20 min on ice and centrifuged at 4°C for 25 min at 13,200rpm. The supernatant was transferred to a new microcentrifuge tube. The concentration of the supernatant was measured with protein assay reagent (Thermo Scientific, Waltham, MA, USA). Protein samples were loaded into wells of the SDS-PAGE gel and transferred to PVDF membranes (Merck Millipore, Billerica, MA, USA). The membranes were blocked with 5% skim milk for 1hr at room temperature. After blocking, membranes were incubated with primary antibodies (Hes1 from Invitrogen, Carlsbad, CA, USA and β-actin from Santa Cruz

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Biotechnology, Dallas, TX, USA) overnight at 4℃. The membranes were washed 3 times for 10 min with PBST and incubated with HRP-conjugated secondary antibodies (Bethyl Laboratories, Montgomery, TX, USA) for 1 hr at room temperature. The membranes were washed 3 times for 10 min with PBST. The FUSION SOLO S (Vilber, Eberhardzell, Germany) was used for image detection according to manufacturer's directions. β-actin was used loading control.

4. RNA isolation and real-time PCR

RNA was isolated using Trizol reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. Reverse transcription-polymerase chain reaction (RT-PCR) was performed using a reverse transcription system (TOYOBO, Tokyo, Japan) and primers listed in Table 1. PCR was performed using instructions given in TB Green Premix EX Taq (TaKaRa, Kyoto, Japan) with ABI instruments (Applied Biosystems Inc, Foster City, CA, USA). All results were normalized by β-actin.

5. Mouse studies

$Hes1^{fl/fl}$ mice (kindly provided by Young-Yun Kong at Seoul National University, Seoul, Korea) were bred to $Alb\text{-}Cre^{+}$ mice (kindly provide by Ho-Geun Yoon at Yonsei University, Seoul, Korea). Seven-week-old wild-type and Hes1 LKO were fed a HFD containing 60% fat for 10-12 weeks (12 hours light, 12 hours dark cycle). All animal studies were approved by the Institutional Review Board of the Yonsei University College of Medicine and were performed in specific pathogen-free facilities according to the university's guidelines for the Care and Use of Laboratory Animals (2019-0088).

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6. Mouse Primary Hepatocytes

Hepatocytes were isolated from mice at 10-12 weeks of age. Under anesthesia, the abdominal cavity was opened, and the liver was perfused via the portal vein first with a Ca^{2+}/Mg^{2+}-free Hanks' buffered salt solution (HBSS) with EDTA, second with a Ca^{2+}/Mg^{2+}-free HBSS (without EGTA), and perfusion with Ca^{2+}/Mg^{2+}-free HBSS containing 0.195 mg/ml of type I collagenase (Sigma). The hepatocytes were then gently shaken in the collagenase solution for 10 minutes. The digested liver was run through, sequentially, 10μm and 70μm nylon meshes. The suspension was centrifuge at 600 rpm (50 g) for 5 minutes at 4℃ to wash and differentially sediment hepatocytes from other cell types. The resulting cell pellet was re-suspended, and an aliquot was taken to determine cell number and viability by the trypan blue exclusion test. The cells were plated at a density of 2.0×10^5 cells/well onto collagen-coated 12-well plates (SPL Life Science, Pocheon-si, Korea) in Medium 199 (Sigma) supplemented with 10% FBS and 1% penicillin/streptomycin and allowed to adhere for 4 hours. The media were then replaced with fresh ones and 1% antibiotics (Invitrogen) and maintained at 37℃ in a humidified incubator with a 5% CO_2 atmosphere.

7. Liver digestion

Livers were extracted and finely minced. Liver tissue was additionally blended with gentle MACS Dissociator (#130-093-235, miltenyi Biotec, Berghish Glatbach, Germany) and digested with MACS Miltenyi Liver Dissociation Kit for mouse (#130-105-807, miltenyi Biotec) according to manufacturer's insturctions. Dissociated liver cells were washed with DMEM and

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passed through a 70μm cell strainer (SPL Life Science) followed by lysis of red blood cells using RBC lysis solution (Biosesang, Seongnal-si, Korea). And liver cells were then washed, and collected to analyze Flow cytometry.

8. Flow cytometry

Single-cell suspensions were made from the liver tissues. Livers were extracted and processed as described above before re-suspension in PBS buffer containing 2% FBS 20 2mM EDTA for flow cytometric analysis. LIVE/DEAD™ fixable Near-IR Dead Cell Stain Kit (L-10119, Invitrogen) was applied to cells in combination with anti-mouse CD16/CD32 Fc blocker antibody (#14-0161-81, Invitrogen) for 15 minutes on ice in the dark. Cells were washed and incubated with fluorochrome-conjugated antibody (anti-mouse Ly-6C APC, clone HK1.4, Biolegend cat. 107614; anti-mouse CD11b FITC, clone M1/70, Biolegend cat. 1001206) at manufacturer's recommended dilution for 30 minutes on ice in the dark. Celss were washed with PBS and re-suspended in FACS buffer for flow cytometric analysis on BD LSRFortessa at the flow cytometry core of the Abision Biomedical Research Center in Yonsei College of Medicine. 10,000-1,000,000 cells were analyzed per sample using BD FACS Diva Software. The data was analyzed with Flow Jo software.

9. Statistical analysis

Unpaired (two sample) t test was used to determine the p-values. P-values < 0.05 were considered to be statistically significant. Statistical analyses were using Prism (GraphPad software, La Jolla, CA, USA)

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Table 1. Primer lists and sequence for RT-PCR

primer	Sequence (5' to 3')
β-actin	Forward: GGCTGTATTCCCCTCCATCG
	Reverse: CCAGTTGGTAACAATGCCATGT
Notch1	Forward: TGAGAATGATGCCCGCACTT
	Reverse: CAGGTGCCCTGATTGTAGCA
Notch2	Forward: AGCAGGAGCAGGAGGTGATA
	Reverse: TGGGCGTTTCTTGGACTCTC
Notch3	Forward: CAGGCGAAAGCGAGAACAC
	Reverse: GGCCATGTTCTTCATTCCCA
Notch4	Forward: ATGACTCCTTGCCCTCTCTCT
	Reverse: CTCTCACCCTTTAGTCCCTCAGA
Hes1	Forward: ACACCGGACAAACCAAAGACA
	Reverse: AATGCCGGGAGCTATCTTTC
Hes3	Forward: TCAACGAGAGCCTAAGCCAGCT
	Reverse: CGCACAGTCATTTCCAGGATGTC
DLL1	Forward: CGGGCCAGGGGAGCTACACA
	Reverse: AGCTGTCCTCAAGGTCCGTGG
DLL3	Forward: TGCCCTTCCGCGATGCTTGG
	Reverse: CTCCCATGTGCCTGCGCT
DLL4	Forward: CAGCATCCCCTGGCAGTGTGC
	Reverse: GCTGGCACACTTGCTGAGTCCC
HEY1	Forward: GCCGAAGTTGCCCGTTATCTG
	Reverse: TGTGTGGGTGATGTCCGAAGG
HEY2	Forward: TCCACCTCTCTTCTGTCCGAAGG
	Reverse: GACTGGAGGCTGCGGATACC
UCP1	Forward: GGGCCCTTGTAAACAACAAA
	Reverse: GTCGGTCCTTCCTTGGTGTA
PGC1α	Forward: ATGTGTCGCCTTCTTGCTCT
	Reverse: ATCTACTGCCTGGGGACCTT
CIDEA	Forward: CATACATCCAGCTCGCCCTT
	Reverse: CGTAACCAGGCCAGTTGTGA

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Adiponectin	Forward: TACTGCAACATTCCGGGACTC
	Reverse: GAGGCCTGGTCCACATTCTT
Elovl3	Forward: CTGGGGAGTGAGTGATCTGC
	Reverse: AGGCTTGGGTCTCTAACAGC
PPARγ	Forward: AGGGCGATCTTGACAGGAAA
	Reverse: CGAAACTGGCACCCTTGAAA
C/EBPα	Forward: GACATCAGCGCCTACATCGA
	Reverse: TCGGCTGTGCTGGAAGAG
C/EBPβ	Forward: CCAGCTGAGCGACGAGTACA
	Reverse: GCTTGAACAAGTTCCGCAGG
FABP4	Forward: CATCAGCGTAAATGGGGATT
	Reverse: TCGACTTTCCATCCCACTTC
CD36	Forward: TGATACTATGCCCGCCTCTCC
	Reverse: TTTCCCACACTCCTTTCTCCTCTA
SREBP	Forward: GATCAAAGAGGAGCCAGTGC
	Reverse: TAGATGGTGGCTGCTGAGTG
ACC1	Forward: ATGCGATCTATCCGTCGGTG
	Reverse: TCCTCCAGGCACTGGAACAT
ACLY	Forward: GAAGCTGACCTTGCTGAACC
	Reverse: CTGCCTCCAATGATGAGGAT
SCD1	Forward: GTACCGCTGGCACATCAACT
	Reverse: AAGCCCAAAGCTCAGCTACTC
TNFα	Forward: CGTCAGCCGATTTGCTATCT
	Reverse: CGGACTCCGCAAAGTCTAAG
F4/80	Forward: CGTCAGCCGATTTGCTATCT
	Reverse: CGGACTCCGCAAAGTCTAAG
CCL2	Forward: TAAAAAACCTGGATCGGAACCAA
	Reverse: GCATTAGCTTCAGATTTACGGGT
CCL3	Forward: GTGACTCACCTTGTGGTCCT
	Reverse: AGGGCAGATCCCAATTGTCAG
FOXO1	Forward: GTGAACACCATGCCTCACAC
	Reverse: CACAGTCCAAGCGCTCAATA

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FOXO4	Forward: GTGCTCGCATCTCCTACTGAAG
	Reverse: CATGTCGCACTCCAGGTTCT
PCK1	Forward: AGATCATCATGCACGACCCC
	Reverse: TGTCCTTCCGGAACCAGTTG
Glut1	Forward: TCAACACGGCCTTCACTG
	Reverse: CACGATGCTCAGATAGGACATC
Glut4	Forward: GTAACTTCATTGTCGGCATGG
	Reverse: AGCTGAGATCTGGTCAAACG
AMPK	Forward: TACCTTGCCCCATCCTTTGG
	Reverse: CCTCCCAACAACGGCTTACA
FGF21	Forward: ACACAATTCCAGCTGCCTTG
	Reverse: TAGAGGCTTTGACACCCAGG
ATF4	Forward: GAGCTTCCTGAACAGCGAAGTG
	Reverse: TGGCCACCTCCAGATAGTCATC
PPARα	Forward: ATGCCAGTACTGCCGTTTTC
	Reverse: GGCCTTGACCTTGTTCATGT
Enho	Forward: AGCCCACTTCTTCCTCCTCACCTC
	Reverse: CTCCCCCAAAGCAGCAGTA

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III. Results

1. Expression levels of Hes1 is elevated in obese liver

We measured the expression level of Hes1 in obese livers. We determined that the mRNA level of Notch signaling factors including Hes1 was increased in mouse livers fed high fat diet (Figure 1A). Similarly, RT-PCR and western blot result showed up-regulated Hes1 level in HFD (Figure 1B). Also, we've determined the expression levels of Hes1 in livers of control patients, NAFLD patients and NASH patients. The mRNA level of Hes1 was increased in NAFLD patients (Figure 1C).

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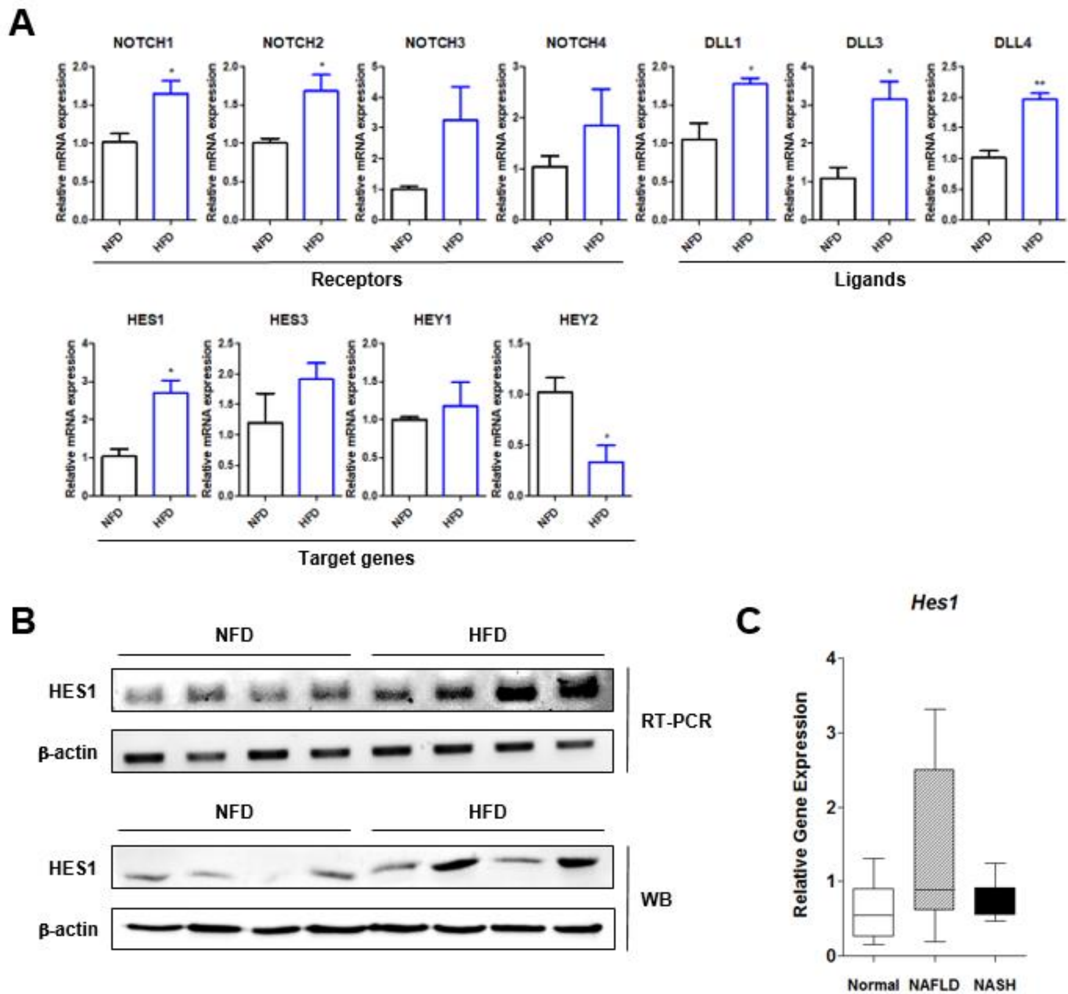

Figure 1. Expression levels of Hes1 in obese liver. (A) mRNA level of Notch signaling factors in HFD-induced obese mouse liver. (B) The expression level of Hes1 was analyzed by RT-PCR and western blot. β-actin was used as normalization control. (C) mRNA level of Hes1 in normal liver, NAFLD liver and NASH liver from patients are presented as diagrams.

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2. Liver-specific Hes1 knockout mice have reduced body weight and white adipose tissue

To identify the role of Hes1 in mouse liver, we employed conditional ablation of Hes1 genes using albumin-Cre-lox recombination, which affects hepatocytes. This model was fed with HFD to induce obesity and NAFLD. After 12 weeks, Hes1 LKO mice had significantly lower body weight than WT (Figure 2A-C). Also, Inguinal white adipose tissue (iWAT) and gonadal white adipose tissue (gWAT) of Hes1 LKO mice were also drastically lessened in Hes1 LKO mice (Figure 2D).

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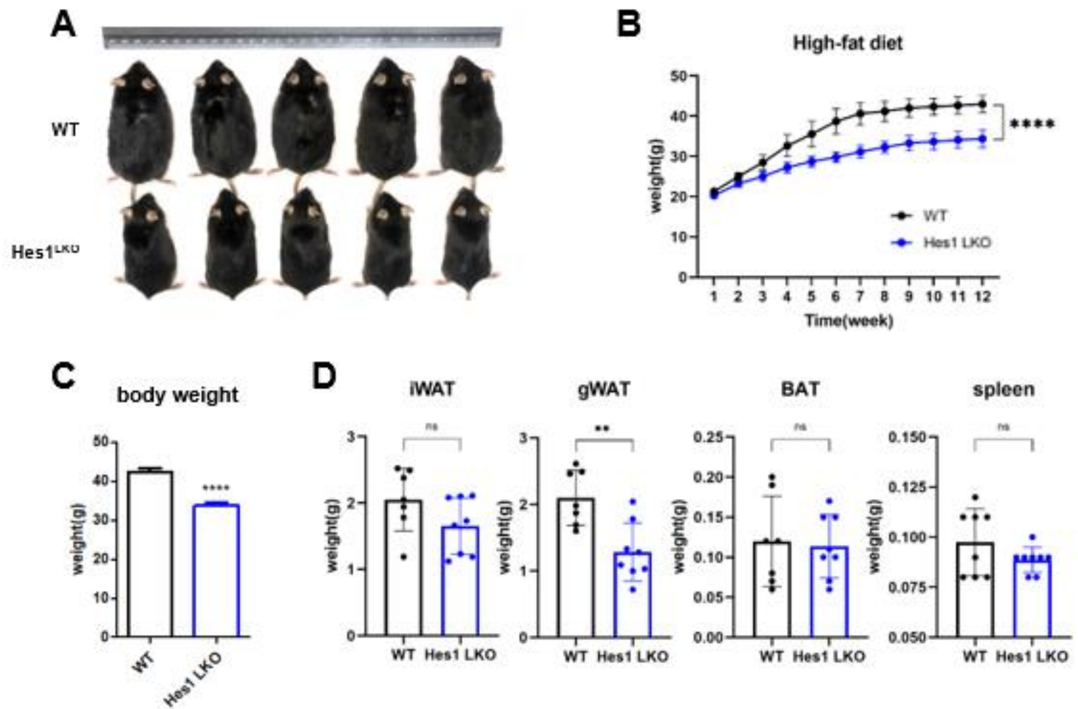

Figure 2. Hes1 ablation in obese mice is associated with metabolic rates. (A, B) Decreased body size and weight of Hes1 LKO mice fed HFD for 12 weeks. (C) Final body weight after the 12 weeks of HFD. (D) Weight of adipose tissues and spleen.

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3. Deletion of Hes1 in liver attenuates NAFLD phenotypes in HFD induced obese mice

To investigate the role of Hes1 in the liver of mouse model, we compared phenotype between the liver of wild-type mice and Hes1 LKO mice. Liver was drastically lessened in Hes1 LKO mice (Figure 3A). According to the H&E and ORO staining, larger lipid droplets were observed. IHC staining of F4/80 ensures that less inflammation had occurred in Hes1 LKO mice liver (Figure 3B). We measured the expression of genes related to lipid accumulation. Interestingly, mRNA expression of FABP4 and SREBP was reduced in liver of Hes1 LKO mice. Also, expression levels of TNFa and F4/80 were reduced in liver of Hes1 LKO mice, suggesting that the amplified inflammation did not occur in Hes1 LKO mice. (Figure 3C). We observed that increase the number of macrophages in hepatocyte of Hes1 LKO mice compared to wild-type mice (Figure 3D).

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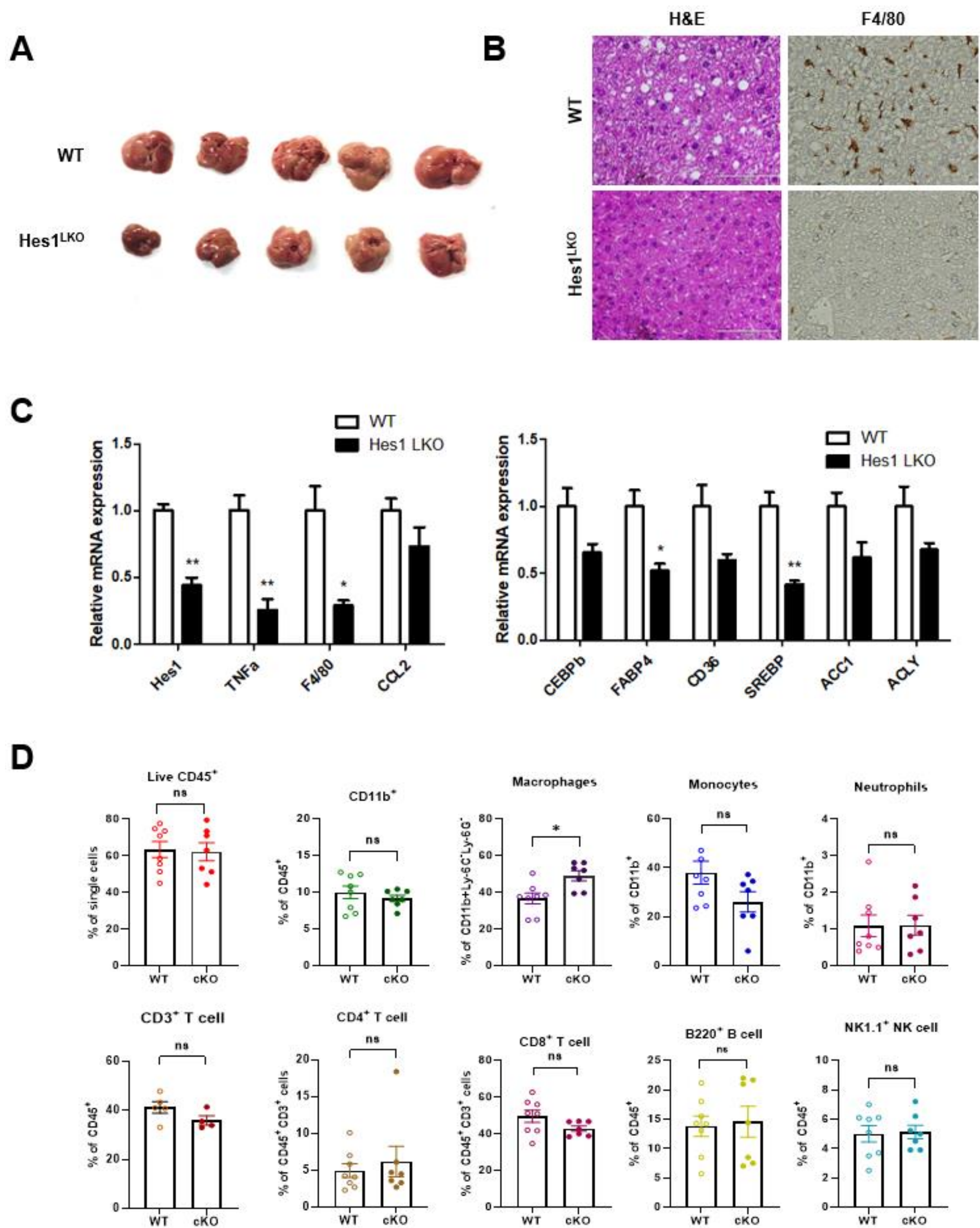

Figure 3. Hes1 deletion in obese mice decreases lipogenesis and inflammation in liver.

(A) Size of liver. (B) Liver sections of WT and Hes1 LKO mice fed with HFD for 12 weeks

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were analyzed by H&E and IHC with F4/80 staining. (C) Real-time RT-PCR analysis of liver genes including Hes1, tumor necrosis factor alpha (TNFa), F4/80, c-c motif chemokine ligand 2 (CCL2), ccaat-enhancer-binding protein alpha (C/EBPa), fatty acid binding protein (FABP4), cluster of differentiation (CD36), sterol regulatory-element binding protein (SREBP), acetyl-coA carboxylase 1 (ACC1) and ATP citrate lyase (ACLY) mRNA expression was normalized to β-actin. (D) Immune cells were isolated from hepatocyte. Data are presented as mean ± SEM (n=5 for WT and Hes1 LKO mice fed HFD) $^{*}P < 0.05$ and $^{**}P < 0.01$ for WT vs Hes1 LKO mice.

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4. Deletion of Hes1 in liver displays reduced fatty acid synthesis in adipose tissue

We further investigated whether the deletion of Hes1 in liver also affects adipose tissue. We investigated the H&E staining of iWAT and gWAT sections. From the H&E staining of adipose tissue, Hes1 LKO mice had significantly smaller adipocyte size (Figure 4A-B). Accordingly, mRNA expression levels of NAFLD markers of those livers were analyzed. Fatty acid synthesis and inflammation related gene expression levels were lower in Hes1 LKO mice (Figure 4C). Also, the mRNA level of gene related to thermogenesis was increased in BAT of Hes1 LKO mice (Figure 4D).

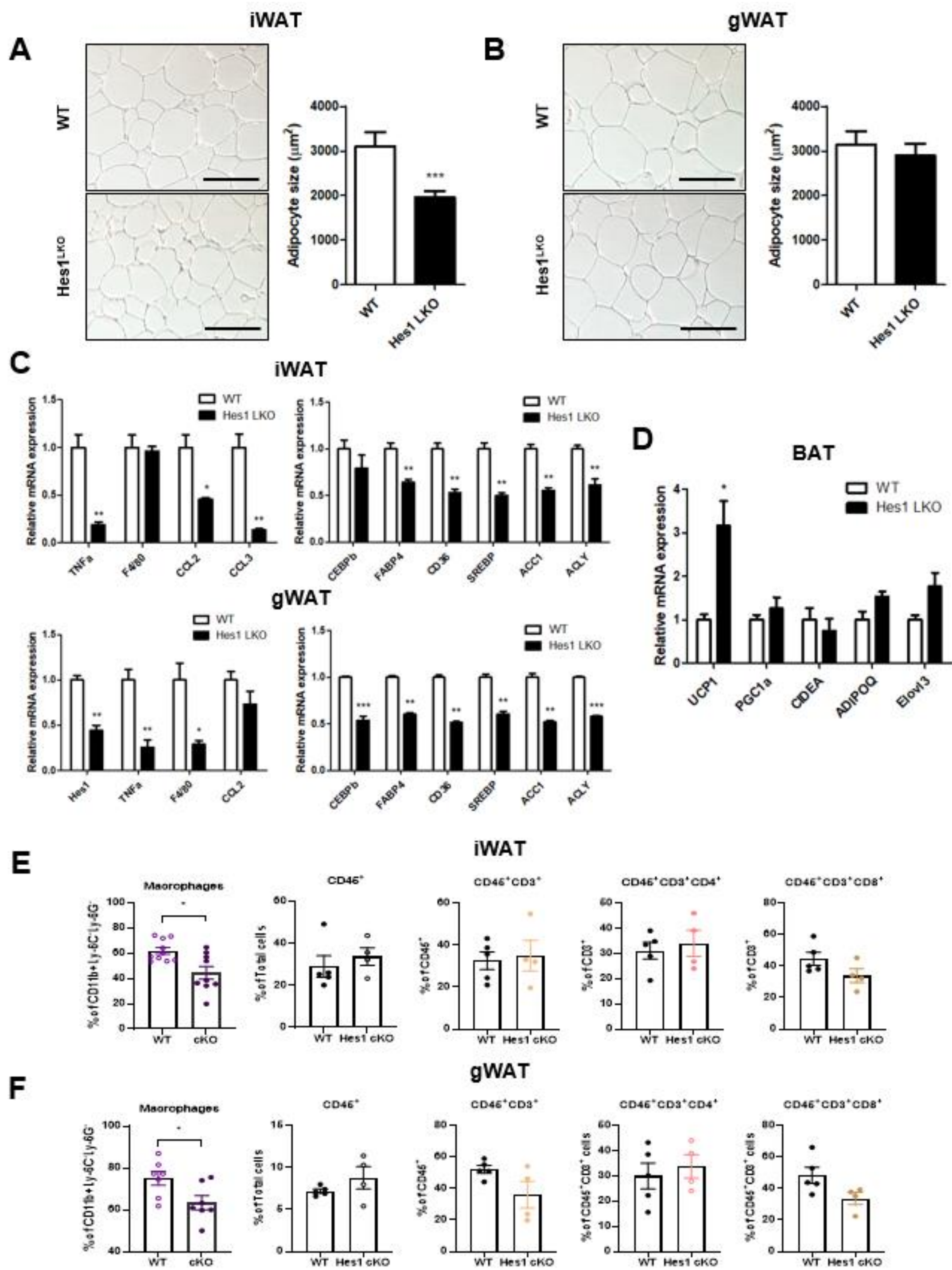
iWAT
gWAT
A
B
WT
Hes1LKO
Adipocyte size (μm²)
WT
Hes1 LKO
C
iWAT
WT
Hes1 LKO
Relative mRNA expression
TNFa
F4/80
CCL2
CCL3
CEBPb
FABP4
CD36
SREBP
ACC1
ACLY
gWAT
Hes1
D
BAT
UCP1
PGC1a
CIDEA
ADIPOQ
Elovl3
E
iWAT
Macrophages
CD45⁺
CD45⁺CD3⁺
CD45⁺CD3⁺CD4⁺
CD45⁺CD3⁺CD8⁺
cKO
Hes1 cKO
% of Total cells
F
gWAT

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Figure 4. Hes1 ablation decreases fatty acid synthesis and increases thermogenesis. (A and B) H&E staining of WT and Hes1 LKO mice iWAT and gWAT section. (C) Inflammation and fatty acid synthesis markers were analyzed from iWAT and gWAT. (D) Thermogenesis markers were analyzed from BAT. (E and F) Immune cells were isolated from iWAT and gWAT. Data are presented as mean ± SEM (n=5 for WT and Hes1 LKO mice fed with a HFD) *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ for WT vs Hes1 LKO mice.

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5. Improved metabolic characteristics in liver-specific Hes1 knockout mice

We measured metabolic characteristics of Hes1 in HFD induced obese mouse. Glucose tolerance test (GTT) and insulin tolerance test (ITT) were performed to identify the role of Hes1 in glucose metabolism. Blood glucose levels over the entire time point of GTT and ITT were lower in Hes1 LKO mice (Figure 5A). Also, blood serum of these mice were analyzed, and both AST and ALT were lower in Hes1 LKO mice (Figure 5B). To find out whether the deletion of Hes1 in liver affects the metabolic fitness, we measured mouse voiding behavior using metabolic cage system. As a result, energy expenditure of Hes1 LKO mice was decreased (Figure 5C). These data suggest that Hes1 is related to glucose metabolism and metabolic activity.

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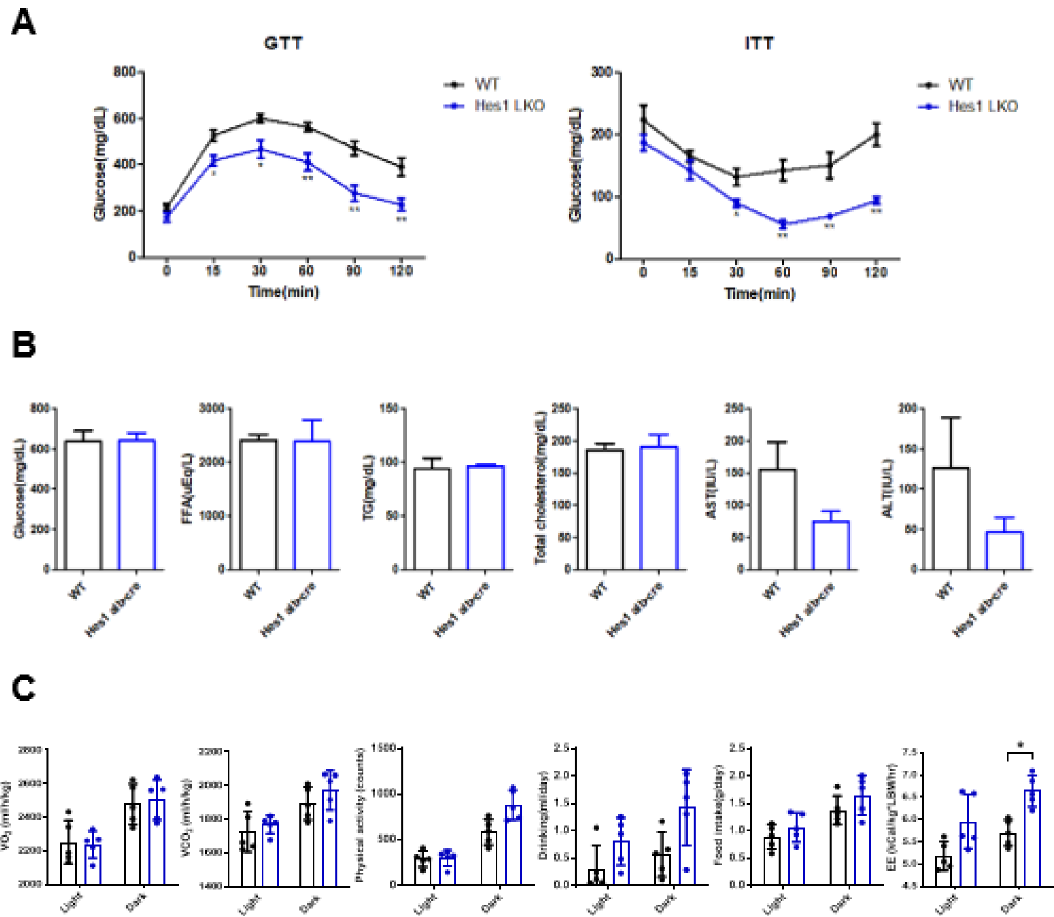

Figure 5. Metabolic activity is up-regulated in Hes1 liver-specific knockout mice. (A) Glucose tolerance test (GTT) and insulin tolerance test (ITT). Overnight fasted mice subject to an intraperitoneal injection of glucose (1g/kg) and 10 hours fasted mice subject to an intraperitoneal injection of insulin (1U/kg). (B) Measurement of AST and ALT, which are indicators of liver toxicity. (C) The metabolic activity of mice was measured in light and dark. Data are presented as mean ± SEM (n=5 for WT and Hes1 LKO mice fed with a HFD) *P < 0.05 and **P < 0.01 for WT vs Hes1 LKO mice.

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6. FGF21 is up-regulated in liver-specific Hes1 knockout mice

We further investigated how liver-specific Hes1 knockout affects entire body. We measured expression of various genes in hepatocytes by DNA microarray analysis. mRNA expression level of thermogenesis, fatty acid oxidation, energy metabolism, lipogenesis, and inflammation related gene was increased in hepatocytes of Hes1 LKO mice (Figure 6A). We also used real-time RT-PCR analysis to confirm that these mRNA expression level was relatively high in AML12 cell line and HFD induced obese mice liver tissues (Figure 6B). The presence of the Hes1 binding site was predicted as a result of analyzing the promoters of mouse FGF21 and human FGF21 to determine whether Hes1 and FGF21 interact (Figure 6C).

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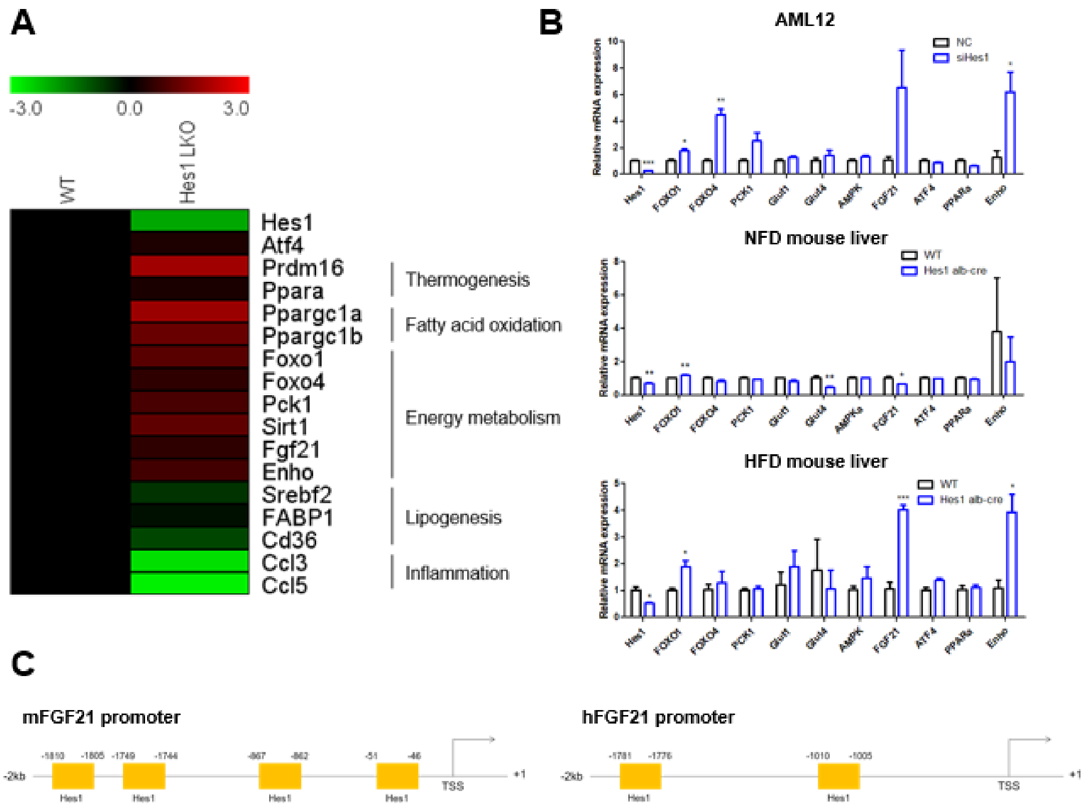

Figure 6. FGF21, a hepatokine, is up-regulated in Hes1 LKO mice. (A) Microarray analysis of hepatocytes showed increased expression of ATF4, PRDM16, PPARα, PGC1α, PGC1β, FOXO1, FOXO4, PCK1, SIRT1, FGF21, Enho, SREBF2, FABP1, CD36, CCL3, CCL5. (B) Real-time RT-PCR analysis of genes in AML12 cell and liver, including FOXO1, FOXO4, PCK1, Glut1, Glut4, AMPK, FGF21, ATF4, PPARα, Enho. (C) Hes1 binding site in the promoter of mouse FGF21 and human FGF21. Data are presented as mean ± SEM (n=5 for WT and Hes1 LKO mice fed a HFD) $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ for WT vs Hes1 LKO mice.

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IV. DISCUSSION

Obesity is one of the most common metabolic diseases[20]. According to statistics from World Health Organization, 39% of adults aged 18 years and over were overweight in 2016, and 13% were obese. Obesity is dangerous because it causes diabetes, heart disease, and even cancer[21-23]. NAFLD occurs mainly when fat builds up in the liver due to obesity[24-25]. Since NAFLD can develop into NASH and progress to HCC, it is necessary to develop an effective treatment, and this study tried to find it using Hes1.

We confirmed that Hes1 is increased in HFD induced obese liver. Hes1 expression levels were also increasing in NAFLD patients. We then used liver-specific Hes1 knockout mice (Hes1 LKO) to identify the role of Hes1 in liver. As a result, Hes1 deficiency increased resistance to HFD induced obesity and decreased the size of liver. According to H&E staining and IHC staining of F4/80, Hes1 LKO mice had lower expression levels of them. These liver tissues were lysed and their RNA was extracted, and lower mRNA expression levels of inflammation and lipid accumulation related gene were observed from real-time RT-PCR analysis. Next, we also performed real-time RT-PCR analysis in iWAT and gWAT. Inflammation and lipid accumulation related genes were down-regulated in Hes1 LKO mice and thermogenesis related genes were increased in BAT of Hes1 LKO mice. These results suggest that liver-specific Hes1 knockout is related to lipid accumulation.

Next, we found that Hes1 LKO mice has lower blood glucose levels over the entire time point of GTT and ITT. AST and ALT, the parameters of liver toxicity, were decreased in Hes1 LKO mice. To identify whether the WT and Hes1 LKO mice have differences in metabolic characteristics, we measured metabolic activity using metabolic cage system. According to

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result, Hes1 LKO mice has higher energy expenditure rate. These data indicate that liver-specific Hes1 levels are linked with energy expenditure in vivo, but we still need to further study about molecular mechanism regulating metabolism.

In this study, we suggest that FGF21 is up-regulated in hepatocytes of Hes1 LKO mice from DNA microarray analysis. FGF21 which are known as hepatokine has important roles in regulating glucose and lipid homeostasis[26-31]. FGF21 is also known as a gene therapy for obesity and insulin resistance, and studies have shown that it prevents macrophage-mediated inflammation[32-33]. Our results showed that deletion of Hes1 inhibits obesity and NAFLD by interaction with FGF21, and the increase of macrophage in the liver will suggest a new research direction that has not been previously known.

Taken together, this study demonstrates that Hes1 is regulator of lipid accumulation by affecting FGF21 and might be therapeutic target of obesity and metabolic disease.

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V. CONCLUSION

The level of Hes1 was elevated in liver of HFD induced obese mice. In addition, the Hes1 level was increased in the NAFLD patient as well. After 12 weeks of HFD, Hes1 deficiency reduced obesity in mice, and reduced liver size, lipid accumulation, and inflammation. As a result of conducting these experiments in adipose tissue, lipid accumulation and inflammation were decreased in Hes1 LKO mice, and expression of thermogenic genes was increased in BAT. When the blood glucose level was WT and Hes1 LKO mice was measured by GTT and ITT, the glucose level of Hes1 LKO mice was lowered, and AST and ALT, which are used as indicators of liver toxicity, were also lowered. The energy expenditure was high in Hes1 LKO mice from the result of measuring the metabolic rates using a metabolic cage. DNA microarray analysis results showed that the expression of genes related to thermogenesis, fatty acid oxidation, and energy metabolism was elevated in the hepatocyte of Hes1 LKO, and the high level of FGF21, a hepatokine, was confirmed by real-time RT-PCR. There are four binding sites in the Hes1 promoter, and we suggest that Hes1 interacts with FGF21 to regulate metabolic activity and might be a therapeutic target on the NAFLD.

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ABSTRACT (IN KOREAN)

대사성 질환에서 전사 인자 Hes1의 기능 연구

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이재경

Hes1은 세포 간 상호작용 시스템으로 알려진 Notch 신호 전달 시스템의 가장 잘 알려진 타겟 유전자 중 하나로 전사 인자의 역할을 수행한다. Notch 신호 전달 시스템의 수용체인 Notch1이 간에서의 지방 축적에 관여한다는 기존 연구 결과가 있지만, 분자 연구 기전에 대해서는 알려진 바가 거의 없다. 고지방 식이를 한 마우스의 간 조직과 비알코올성 지방간질환 (NAFLD) 환자의 간 조직에서 Hes1의 발현이 증가해 있는 것을 관찰하였고, 이를 바탕으로 본 논문에서는 Hes1이 대사성 질환인 비알코올성 지방간질환 (NAFLD)을 조절할 수 있는지에 대한 연구를 진행하였다.

간에서 특이적으로 Hes1 을 저해한 (Hes1 LKO) 마우스에 고지방 식이를 진행하여 체중과 간의 크기를 비교하였을 때, WT 마우스에 비해 모두 감소해

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있는 것을 확인하였다. 이에 따라 염증 반응과 관련된 유전자들인 TNFα, F4/80, CCL2 와 지방 축적에 관련된 유전자들인 C/EBPβ, FABP4, CD36, SREBP, ACC1, ACLY 의 발현이 모두 감소해 있었다. 이는 지방 조직에서도 같은 경향을 보였으며, UCP1 과 같이 열 발생에 관여하는 유전자의 발현은 증가해 있었다. 또, 간에서 특이적으로 Hes1 을 저해할 경우, 간 독성을 나타내는 지표인 AST 와 ALT 는 감소하지만 전반적인 대사율은 향상된 것을 확인할 수 있다.

추가적인 실험을 통해 간에서 분비되는 호르몬인 FGF21 와 Hes1 사이에 상관관계가 존재함을 확인하였고, 이러한 결과들은 Hes1 이 비알코올성 지방간질환 (NAFLD)에 대한 새로운 치료 타겟이 될 수 있음을 시사한다.

핵심되는 말 : Hes1, 비만, 비알코올성 지방간질환, FGF21