Title page

The Gene Knockout of Angiotensin II Type 1a Receptor Improves High-fat Diet-Induced Obesity in rat *via* Promoting Adipose Lipolysis

AT1aR knockout improves obesity

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Abstract

Aims: The renin-angiotensin system (RAS) is over-activated and the serum angiotensin II (Ang II) level increased in obese patients, while their correlations were incompletely understood. This study aims to explore the role of Ang II in diet-induced obesity by focusing on adipose lipolysis.

Methods: Rat model of AT1aR gene knockout were established to investigate the special role of Ang II. Wild-type (WT) and AT1aR gene knockout ($AT1aR^{-/-}$) SD rats were fed with normal diet or high-fat diet for 12 weeks. Adipose morphology and adipose lipolysis were examined.

Results: AT1aR deficiency activated lipolysis-related enzymes and increased the levels of NEFAs and glycerol released from adipose tissue in high-fat diet rats, while did not affect triglycerides synthesis. Besides, AT1aR knockout promoted energy expenditure and fatty acids oxidation in adipose tissue. cAMP levels and PKA phosphorylation in the adipose tissue were significantly increased in $AT1aR^{-/-}$ rats fed with high-fat. Activated PKA could promote adipose lipolysis and thus improved adipose histomorphology and insulin sensitivity in high-fat diet rats.

Conclusions: AT1aR deficiency alleviated adipocyte hypertrophy in high-fat diet rats by promoting adipose lipolysis via cAMP/PKA pathway, and thereby delayed the onset of obesity and related metabolic diseases.

Key Words: Angiotensin II type 1a receptor, obesity, adipose lipolysis, cAMP/PKA

1. Introduction

Obesity is a growing health problem that induces major metabolic disorders, such as diabetes, cardiovascular disease, and hypertension. Obesity has been officially listed as a disease by the World Health Organization in 2000. It has been predicted that more than 57.8% adults and about 3.3 billion

people worldwide will facing overweight or obesity problems by 2030[1]. With an increasing prevalence of obesity, it is urgent to elucidate the pathophysiological mechanism for obesity and develop effective therapy strategies for treatment of obesity and its associated disorders.

Adipose tissue plays major role in the development of obesity. Excess energy is stored in the adipose tissue in the form of triacylglycerol (TAG), while fatty acid mobilization via lipolysis releases fatty acids to increase hepatic fatty acid oxidation for energy supply[2]. Physiologically, adipose tissue maintains a dynamic balance between lipid synthesis and decomposition. However, adipose tissue stores excess energy by expansion and remodeling when the storage capacity of adipocytes is exceeded in response to overfeeding, resulting in fat accumulation and adipocyte hypertrophy[3, 4]. Therefore, inhibiting adipocyte hypertrophy can be an effective strategy for obesity treatment.

The renin-angiotensin system (RAS) is a dynamic physiologic system and its classic function is to regulate blood pressure and fluid and electrolyte balance. Angiotensin-converting enzyme inhibitor (ACEI) and angiotensin II receptor blockers (ARB) has been clinically used as antihypertensive drugs and play a crucial role in the treatment of cardiovascular diseases such as hypertension and heart failure. However, it has been found that these drugs have a positive effect in metabolic diseases such as obesity, insulin resistance and diabetes[5, 6], indicating the relations between RAS and metabolic diseases. Animal studies have shown that mice with renin gene knockout are resistant to diet-induced obesity[7]. Besides, excessive activation of RAS is a common feature in obese patients[8, 9], and the serum levels of angiotensinogen (AGT) and angiotensin II (Ang II) in the obese are higher than those in normal population[10-12]. Adipose tissue is the most abundant source of AGT outside liver. It is showed that AGT expression increased in the adipose tissue of obese animal models and adipocyte-specific enhancement of AGT lead to insulin resistance[13], indicating the special role of adipose tissue

RAS in regulation of metabolic homeostasis. Adipose RAS has attracted more and more attention due to its close relationship with obesity and adipose dysfunction over the years[14-16]. However, the underline mechanism by which RAS activation in adipose tissue results in obesity and other metabolic diseases remain unclear.

Angiotensin II (Ang II), the predominant peptide of the RAS, exerts effects mainly by binding with angiotensin type 1 receptor (AT1R) in adipose tissue[14]. Hence, we established an obese rat model with AT1aR gene knockout to explore the role of adipose RAS in the develop process of obesity. The results showed that the gene knockout of AT1aR ameliorated adipocyte hypertrophy by promoting adipose lipolysis, and thereby improving obesity and related metabolic disorders. Our finding suggests that targeting RAS signaling in adipose tissue may become a promising therapy avenue for obesity and associated metabolic abnormalities.

2. Materials and Methods

2.1 Experimental animals

Wild type male Sprague-Dawley (SD) rats purchased from the Experimental Animal Center of Shanxi Medical University and AT1aR$^{-/-}$ male SD rats (Nanjing University-Nanjing Institute of Biology) were all fed in the SPF laboratory animal environmental facilities with 12 h light/dark cycles under standard room temperature (22 ± 2°C) and free access to water and food. AT1aR gene knockout rats have been verified as homozygous by polymerase chain reaction (PCR) (S1 Fig). All animal experiments were in accordance with the guidelines for the management of animals for medical experiments issued by the Ministry of Health of the People's Republic of China (No. 55) and animal ethics standards of Shanxi Medical University, and approved by the ethics committee.

4-week male wild type (WT) rats and AT1aR$^{-/-}$ rats were randomly divided into normal diet group

(ND) and high-fat-diet group (HFD). The HFD group rats were fed with 60% high-fat feed (D12492, Whitby Technology Co., Ltd. Beijing,China) and the ND group rats were fed with normal feed for 12 weeks. Body weight was recorded weekly. At the end of feeding, rats were fasted overnight and anesthesia, blood sample was collected from the abdominal aorta for the assay of blood glucose, nonesterified free fatty acids (NEFAs), triglyceride (TG), total cholesterol (T-CHO), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) contents with commercial kits (Jiancheng Bioengineering Institute, Nanjing, China). Serum glycerol content was measured with glycerol (liquid sample) enzymatic determination kit (Applygen Gene Technology Co., Ltd, Beijing). Epididymal adipose tissue were isolated immediately or stored at minus 80 degrees Celsius until analysis. Epididymal fat index were calculated as the ratio of epididymal fat weight to the body weight.

2.2 Glucose tolerance test and insulin tolerance test

For glucose tolerance test, rats were fasted overnight and administrated with glucose (2 g/kg) by gavage. For insulin tolerance test, rats were fasted for 6h and intraperitoneally injected insulin (1 IU/kg). Blood glucose contents at 0 min, 15 min, 30 min, 60 min, 90 min, and 120 min were recorded and blood glucose area under curve (AUC) was calculated.

2.3 Blood pressure measurement

At the end of 12-week feeding, rats were anesthetized and blood pressure were measured by carotid artery cannulation and monitored with the BL-420 system.

2.4 Morphological examination of adipose tissue

Isolated epididymal adipose tissue was fixed with 4% paraformaldehyde, dehydrated with gradient alcohol and then embedded in paraffin. Embedded wax block was sliced and epididymal sections were stained with hematoxylin for 20 min and with eosin for 15 min, respectively. After sealing slide with

neutral gum, the morphology changes of adipocytes were visualized using optical microscope (Olympus, Japan) and the cross-sectional area of adipocytes were calculated with Image J software.

2.5 Quantitative real time RT-PCR

Total mRNA of epididymal adipose tissue was extracted with Trizol (Takara Bio Inc., Japan). The concentration and purity of extracted total mRNA were measured using NANODROP ONE (Thermo Scientific, USA). cDNA was synthesized with the PrimeScript^{TM}RT reagent kit (Takara Bio Inc., Japan) following the manufacturers' instructions. Relative quantitative PCR was conducted with a TB Green Primer Ex Taq II (Takara Bio Inc., Japan) using LightCycler® 96 Real-Time PCR System (Roche, USA). The primer sequences were obtained from Takara and listed in supplementary Table 1. Gene expressions were normalized with β-actin. Statistically relative quantification was analyzed with equation $2^{-\Delta\Delta CT}$. Ct is the threshold cycle to detect fluorescence.

Table 1

Primer sequences used for quantitative real time RT-PCR

Name	Forward Primer (5'-3')	Reverse Primer (5'-3')
FAS	ACCTCATCACTAGAAGCCACCAG	GTGGTACTTGGCCTTGGGTTTA
ACC	TACAACGCAGGCATCAGAAG	TGTGCTGCAGGAAGATTGAC
CPT1a	CTGCCAGTTCCATTAAGCCACA	CAGCTATGCAGCCTTTGACTACCA
PPARα	GGCAATGCACTGAACATCGAG	GCCGAATAGTTCGCCGAAAG
PPARδ	TGGCCCTGTTCCTAGAATTGATG	GCAAACTCTGCCTGTGAGCTG
Dio2	CTGTGGTTGGATGTAGTCACACGA	CTTTGCACCAGGACCCAAATG
β-actin	ACGGTCAGGTCATCACTATCG	GGCATAGAGGTCTTTACGGATG

2.6 Measurement of free fatty acids and glycerol in adipose tissue

At the end of 12-week feeding, rats were anesthetized and sterilized with alcohol. Epididymal adipose tissue was isolated and 200 mg adipose tissue was weight and cut into pieces, and then

127 incubated in serum-free DMEM for 24 hours. DMEM was collected to measure NEFAs and glycerol
128 contents in the supernatant with commercial Kits following the manufacturer's instructions.

129 **2.7 Measurement of cAMP concentration**

130 Epididymal adipose tissue supernatants were obtained by homogenate and centrifugation. Cyclic
131 AMP in the supernatants were measured using cAMP assay kit (4339, Cell Signaling Technology)
132 according to the manufacturer's protocol.

133 **2.8 Western blot analysis**

134 Epididymal adipose tissue was homogenized with a tissue grinder at 4℃ temperature and the
135 lysate were centrifugation at 12000 rpm for 20 minutes. The protein concentrations were measured
136 with BCA kit (KeyGEN BioTECH Corp., Ltd, Nanjin, China). The protein samples were denatured,
137 separated by SDS-PAGE and then transferred to the PVDF membrane. Membranes were blocked with
138 5% skim milk or bovine serum albumin (BSA) for 3 hours, and then incubated overnight at 4℃ with
139 specific primary antibodies as follows: ATGL (2138S, Cell Signaling Technology), P-HSL $^{(ser660)}$
140 (4126S, Cell Signaling Technology), HSL (4107S, Cell Signaling Technology), P-GSK-3β $^{(ser9)}$ (5558S,
141 Cell Signaling Technology), GSK-3β (9315S, Cell Signaling Technology), β-actin (AP0060, Bioworld)
142 and PKA (5842S, Cell Signaling Technology). Membranes were washed and incubated with secondary
143 antibodies (BA1054, BOSTER Biological Technology) at room temperature for 3 hours. After
144 washing, membranes were exposed by Super ECL Prime (SEVEN BIOTECH) with ChemiDoc™
145 Imaging System (BIO-RAD, USA). The images were analyzed quantitatively by densitometry with
146 Image J software.

147 **2.9. Statistical analysis**

148 Results were shown as mean ± SEM. Data were analyzed by using Student's t test for comparison

the difference of two groups and the comparison among multiple groups was analyzed by one-way ANOVA. GraphPad Prism 6 was used for statistical analysis. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1 AT1aR knockout improved insulin sensitivity and metabolic disorders in high-fat diet rats

Glucose tolerance test and insulin tolerance test are important indicators that generally to be used to measure glucose tolerance and insulin sensitivity, respectively[17]. In response to glucose load or insulin injection, there was no significant differences between AT1aR$^{-/-}$ rats and WT rats with normal diet, while the area under the curve (AUC) in HFD-fed AT1aR$^{-/-}$ rats was significantly lower than that in the HFD-fed WT rats (Fig 1A, B), indicating that AT1aR knockout improved insulin sensitivity in high-fat-diet rats. Consistent with this, AT1aR knockout reduced the fasting blood glucose compared with WT rats fed with high-fat-diet (Fig 1C). Moreover, rats blood pressure was monitored through carotid artery intubation and the result showed that AT1aR gene knockout could reduce hypertension caused by high-fat feeding (Fig 1D).

Next, lipid metabolism-related indicators in serum were detected with commercial kits. The results showed that serum levels of TG, T-CHO, NEFAs, glycerol, LDL and HDL in high-fat diet WT rats were significantly higher than those of WT rats fed with normal diet, whereas these alterations were significantly reversed by AT1aR gene knockout, demonstrating that AT1aR knockout improved metabolic disorders in obese rats (Fig 1E-J). Besides, serum LDL level of AT1aR$^{-/-}$ rats were also lower than that of the WT rats fed with normal diet (Fig 1I).

Fig 1. AT1aR knockout improved insulin resistance and metabolic disorders in high-fat diet rats. A: Oral glucose tolerance test (OGTT) curve and area under the curve. B: Insulin tolerance test

(ITT) curve and area under the curve. C: Fasting blood glucose (FBG). D: Blood pressure. E: Serum triglyceride (TG) levels. F: Serum total cholesterol (T-CHO). G: Serum free fatty acid. H: Serum glycerol. I: Serum low-density lipoprotein (LDL). J: Serum high-density lipoprotein (HDL). *p < 0.05 vs WT-ND rats; #p < 0.05 vs WT-HFD rats. Data were presented as Mean ± S.E.M. n = 6

3.2 AT1aR knockout alleviated high-fat diet-induced adipocyte hypertrophy

Body weight of rats were recorded weekly and the results showed that body weight gain of WT rats were increased by high-fat diet feeding, while body weight gain of high-fat diet AT1aR$^{-/-}$ rats were much lower than that of high-fat diet WT rats (Fig 2A, B). Epididymal adipose tissues were isolated and their physical morphology were photographed. As showed in Fig 2C, physical morphology of epididymal adipose tissue in AT1aR$^{-/-}$ rats was smaller than that of WT rats. Besides, the epididymal fat index of high-fat diet AT1aR$^{-/-}$ rats was also decreased compared to high-fat diet WT rats (Fig 2D). Moreover, the histomorphology of adipose were visualized by HE staining and adipocyte area of WT rats were significantly enlarged by high-fat diet feeding, whereas this alteration were significantly reversed in high-fat diet AT1aR$^{-/-}$ rats (Fig 2E). These results indicated that AT1aR gene knockout improved adipose histomorphology and inhibited adipocyte hypertrophy.

Fig 2. AT1aR knockout improved epididymal histomorphology induced by high fat diet. A, B: Body weight of rats was recorded weekly and body weight gain was calculated by weight increase in 12 weeks. C: Physical morphology of epididymal fat; D: Epididymal fat index (showed as epididymal fat weight/body weight). E: Adipocyte morphology was visualized by HE staining and adipocyte area were calculate by Image J. *p < 0.05 vs WT-ND rats; #p < 0.05 vs WT-HFD rats. Data were presented as Mean ± S.E.M. n = 6

3.3 AT1aR gene knockout promoted adipose lipolysis

The main function of white adipose tissue is to store energy in the form of triglycerides and the fat mass is systematically regulated by lipid synthesis and decomposition. In order to know the underlining mechanism by which AT1aR gene knockout inhibited adipocyte hypertrophy, we detected the expressions of major enzymes for triglyceride synthesis and lipolysis in the adipose tissue. Fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) are rate-limiting enzymes involved in biosynthesis of fatty acids, an important step of lipogenesis[18]. Gene expressions of FAS and ACC were measured with QPCR and the results showed that there were no significant differences between WT and AT1aR$^{-/-}$ rats neither fed with normal diet nor with high-fat diet (Fig 3A, B). The hydrolysis of triglycerides is initiated by adipose triglyceride lipase (ATGL)[19], and hormone sensitive lipase (HSL) is the main hydrolase for triacylglycerol (TAG) and diacylglycerol (DAG)[20]. As showed in Fig 3C, protein expression of ATGL and phosphorylation of HSL in AT1aR$^{-/-}$ rats were much higher than those of WT rats both in the normal and high-fat diet rats. Furthermore, the adipose tissue was cultured in the DMEM for 24 hours and the levels of NEFAs and glycerol in the culture medium were detected. Compared with WT rats fed with high-fat diet, the levels of NEFAs and glycerol released from adipose tissue of high-fat diet AT1aR$^{-/-}$ rats increased significantly (Fig 3D, E). These results proved that AT1aR knockout improved adipose histomorphology in high-fat diet rats by promoting adipose lipolysis, while did not affect lipid synthesis.

Fig 3. AT1aR knockout promoted adipose lipolysis. A, B: Gene expressions of key enzymes for lipid synthesis in adipose tissue, FAS (A) and ACC (B). C: Protein expressions of adipose lipolysis related enzymes, ATGL and HSL. D, E: Levels of NEFAs (D) and glycerol (E) in culture medium released by adipose tissue within 24h. *$p < 0.05$ vs WT-ND rats; +$p < 0.05$ vs AT1aR-/--ND rats. Data were presented as Mean ± S.E.M. n = 6

3.4 AT1aR knockout accelerated adipose energy expenditure and fatty acids oxidation

Subsequently, adipose lipid utilization was detected. Peroxisome proliferator activated receptor δ (PPARδ) is a transcription factor that promote oxidative metabolism in adipose[21] and deiodinase 2 (Dio2) could activate thyroid hormone to promote energy expenditure[22]. The results showed that by comparison of WT rats fed with high-fat diet, the gene expressions of PPARδ and Dio2 in adipose tissue were significantly increased in high-fat diet AT1aR$^{-/-}$ rats (Fig 4A, B), indicating increased energy consumption adipose tissue of AT1aR$^{-/-}$ rats. Besides, the levels of fatty acid oxidation in adipose tissue were also measured. Peroxisome proliferator-activated receptors α (PPARα) acts as a transcription factor to regulate a series of genes involved in fatty acid oxidation like carnitine palmitoyltransferase 1 (CPT1) [23, 24]. The results showed that the gene expressions of PPARα and CPT1 were significantly increased in AT1aR$^{-/-}$ rats compared with WT rats fed with high fat diet. However, there was not difference between the WT and AT1aR$^{-/-}$ rats fed a normal diet (Fig 4C, D). These results indicated enhanced fatty acid oxidation in high-fat diet AT1aR$^{-/-}$ rats.

Fig4. AT1aR knockout accelerated adipose energy expenditure and fatty acids oxidation. A, B: Gene expressions of PPARδ (A) and Dio2 (B) in adipose tissue. C, D: Gene expressions of PPARα and CPT1 in adipose tissue. +$p < 0.05$ vs AT1aR-/--ND rats. Data were presented as Mean ± S.E.M. n = 6

3.5 AT1aR knockout activated cAMP/PKA pathway

It has been reported that the binding of Ang Ⅱ to AT1R activates Gi protein against the effect of cAMP/PKA pathway[25]. As showed in Fig 5A and 5B, PKA and cAMP levels were significantly increased in AT1aR$^{-/-}$ rats fed with high-fat. Protein kinase A (PKA) that activated by cyclic adenosine monophosphate (cAMP) mainly phosphorylates HSL to mediate adipose lipolysis [26]. Apart from

HSL, GSK-3β is also an important phosphorylation substrate of PKA[27]. The phosphorylation level of GSK-3β in AT1aR$^{-/-}$ rats was higher than WT rats both in the normal and high-fat diet (Fig 5A), further confirmed the enhancement of PKA activity in AT1aR$^{-/-}$ rats. These results suggested that gene knockout of AT1aR could activate cAMP/PKA pathway.

Fig 5. AT1aR knockout activated cAMP/PKA pathway. A: Protein expressions of PKA, P-GSK-3β (ser9) and GSK-3β in adipose tissue. B: cAMP levels in adipose tissue. C: The proposed pathway for Ang II inhibiting adipose lipolysis. By binding to AT1R, Ang II activates inhibitory Gi which in turn reduces cAMP production. As a result, PKA activation is limited and HSL phosphorylation is decreased, leading to inhibitory triglyceride hydrolysis. +$p < 0.05$ vs AT1aR-/--ND rats. Data were presented as Mean ± S.E.M. n = 6

4. Discussion

Obesity is the common pathological basis of many metabolic diseases and its mechanism exploration has attracted more and more attention. RAS is generally considered acting in the regulation of blood pressure and organism water-salt balance. However, clinical studies have demonstrated that type 2 diabetes can be delayed by treatment with ACEI and ARB compared with other antihypertensive drugs[28]. Besides, captopril, one of ACEI drugs, was showed to not only lower blood pressure but also reduce the weight of high-fat diet rats[29]. These studies indicated that RAS plays an important role in obesity and subsequent metabolic diseases, whereas the regulatory mechanism remains unclear. In the present study, we found that the gene knockout of AT1aR improved high-fat diet induced obesity by promoting lipolysis through cAMP/PKA pathway, providing new sight for the prevention and treatment of obesity and related metabolic disorders.

As the primary peptide of the RAS, Ang II exerts its biological activity mainly through AT1R in

adipose tissue. Different from human beings, AT1R is coded by two gene subtypes, AT1aR and AT1bR, in rodent mammals[30]. AT1aR has the most homology with human, mainly involved in the vasoconstriction and blood pressure regulation. While AT1bR is related to the thirst response of mammals and existed in certain areas of the central nervous system and adrenal tissues [31]. So, in the present study, we constructed AT1aR$^{-/-}$ rats to explore the relation between RAS and obesity.

Obesity is closely correlated with insulin resistance and diabetes. Besides storage energy, adipose tissue also acts as an endocrine organ. In obesity, adipocytes secrete inflammatory adipokines which damage insulin signaling pathway, resulting in disorders of glucose and lipid metabolism[32]. Here, we showed that AT1aR gene knockout improved glucose intolerance and insulin sensibility in high-fat diet induced obese rats. Consistent with our results, Kengo Azushima et al. has demonstrated that upregulation of AT1R-related protein (ATRAP), a protein limiting AT1R's effects by promoting its internalization, could improve insulin resistance[17].

Lipid stocks in the adipose tissue fluctuate by lipid synthesis and lipolysis. Our results showed that AT1aR gene knockout improved adipocyte hypertrophy in obese rats mainly by promoting lipolysis. However, enhanced lipolysis increases circulatory NEFAs which leading to ectopic deposition of lipids. Interestingly, serum levels of NEFAs were decreased in high-fat diet AT1aR$^{-/-}$ rats despite significant enhancement of lipolysis in adipose tissue. In this regard, energy expenditure and NEFAs oxidation in adipose tissue were measured. The results demonstrated that genes expressions of proteins involved in adipose tissue oxidative metabolism were elevated. Given that increasing lipolysis in adipose tissue result in elevated circulating NEFAs, our results showed that AT1aR gene knockout promoted adipose energy expenditure and fatty acids utilization, and thus lowering lipolysis-derived circulating NEFAs levels.

By binding to AT1R, a G protein-coupled receptor, Ang Ⅱ activates inhibitory Gi protein which in turn decreases cAMP production[33, 34]. The cAMP/PKA pathway plays an important role in energy balance and metabolic regulation. As an important second messenger, increased cellular cAMP activates PKA which in turn phosphorylates HSL[35]. AT1aR gene knockout increased PKA protein expression and cellular levels of cAMP in the adipose tissue. Activated PKA can be confirmed by increased phosphorylation of GSK-3β, a main phosphorylation substrate of PKA[27].

In conclusion, our results showed that RAS predominant peptide Ang II promoted adipocyte hypertrophy by inhibition of cAMP/PKA and subsequent adipose lipolysis via binding to AT1aR. However, AT1aR gene knockout activated cAMP/PKA signaling and elevated adipose lipolysis by phosphorylation of HSL, and thus improving obesity and insulin resistance (Fig 5C). Our findings emphasized the special role of RAS signaling in adipose tissue in the progress of obesity and associated metabolic abnormalities, and provided potential drug target for their therapy.

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Author contributions

Aiyun Li: Conceptualization, Methodology, Writing - Review & Editing. **Wenjuan Shi:** Conceptualization, Methodology, Investigation, Writing - Original Draft. **Jin Wang:** Resources, Project administration, Validation. **Xuejiao Wang:** Investigation, Software. **Yan Zhang:** Validation, Software. **Zhandong Lei:** Data Curation, Software. **Xiangying Jiao:** Conceptualization, Writing - Review & Editing, Supervision.

References

1. Kelly T, Yang W, Chen CS, Reynolds K, He J. Global burden of obesity in 2005 and projections to 2030. Int J Obes (Lond). 2008;32(9):1431-7. Epub 2008/07/09. doi: 10.1038/ijo.2008.102. PubMed PMID: 18607383.

2. Ahmadian M, Duncan RE, Jaworski K, Sarkadi-Nagy E, Sul HS. Triacylglycerol metabolism in adipose tissue. Future Lipidol. 2007;2(2):229-37. Epub 2007/04/01. doi: 10.2217/17460875.2.2.229. PubMed PMID: 19194515; PubMed Central PMCID: PMCPMC2633634.

3. Sun K, Kusminski CM, Scherer PE. Adipose tissue remodeling and obesity. J Clin Invest. 2011;121(6):2094-101. Epub 2011/06/03. doi: 10.1172/JCI45887. PubMed PMID: 21633177; PubMed Central PMCID: PMCPMC3104761.

4. Choe SS, Huh JY, Hwang IJ, Kim JI, Kim JB. Adipose Tissue Remodeling: Its Role in Energy Metabolism and Metabolic Disorders. Front Endocrinol (Lausanne). 2016;7:30. Epub 2016/05/06. doi: 10.3389/fendo.2016.00030. PubMed PMID: 27148161; PubMed Central PMCID: PMCPMC4829583.

5. Henriksen EJ, Jacob S, Kinnick TR, Teachey MK, Krekler M. Selective angiotensin II receptor antagonism reduces insulin resistance in obese Zucker rats. Hypertension. 2001;38(4):884-90. Epub 2001/10/20. doi: 10.1161/hy1101.092970. PubMed PMID: 11641303.

6. Shiuchi T, Iwai M, Li HS, Wu L, Min LJ, Li JM, et al. Angiotensin II type-1 receptor blocker valsartan enhances insulin sensitivity in skeletal muscles of diabetic mice. Hypertension. 2004;43(5):1003-10. Epub 2004/03/24. doi: 10.1161/01.HYP.0000125142.41703.64. PubMed PMID: 15037562.

7. Takahashi N, Li F, Hua K, Deng J, Wang CH, Bowers RR, et al. Increased energy expenditure,

325 dietary fat wasting, and resistance to diet-induced obesity in mice lacking renin. Cell Metab.
326 2007;6(6):506-12. Epub 2007/12/07. doi: 10.1016/j.cmet.2007.10.011. PubMed PMID: 18054319;
327 PubMed Central PMCID: PMCPMC2174204.
328 8. Rahmouni K, Mark AL, Haynes WG, Sigmund CD. Adipose depot-specific modulation of
329 angiotensinogen gene expression in diet-induced obesity. Am J Physiol Endocrinol Metab.
330 2004;286(6):E891-5. Epub 2004/01/30. doi: 10.1152/ajpendo.00551.2003. PubMed PMID: 14749209.
331 9. Prasad A, Quyyumi AA. Renin-angiotensin system and angiotensin receptor blockers in the
332 metabolic syndrome. Circulation. 2004;110(11):1507-12. Epub 2004/09/15. doi:
333 10.1161/01.CIR.0000141736.76561.78. PubMed PMID: 15364819.
334 10. Engeli S, Schling P, Gorzelniak K, Boschmann M, Janke J, Ailhaud G, et al. The adipose-tissue
335 renin–angiotensin–aldosterone system: role in the metabolic syndrome? The International Journal of
336 Biochemistry & Cell Biology. 2003;35(6):807-25. doi: 10.1016/s1357-2725(02)00311-4.
337 11. Kalupahana NS, Massiera F, Quignard-Boulange A, Ailhaud G, Voy BH, Wasserman DH, et al.
338 Overproduction of angiotensinogen from adipose tissue induces adipose inflammation, glucose
339 intolerance, and insulin resistance. Obesity (Silver Spring). 2012;20(1):48-56. Epub 2011/10/08. doi:
340 10.1038/oby.2011.299. PubMed PMID: 21979391; PubMed Central PMCID: PMCPMC4465436.
341 12. Jones BH, Standridge MK, Taylor JW, Moustaid N. Angiotensinogen gene expression in adipose
342 tissue: analysis of obese models and hormonal and nutritional control. Am J Physiol. 1997;273(1 Pt
343 2):R236-42. Epub 1997/07/01. doi: 10.1152/ajpregu.1997.273.1.R236. PubMed PMID: 9249555.
344 13. Pahlavani M, Kalupahana NS, Ramalingam L, Moustaid-Moussa N. Regulation and Functions of
345 the Renin-Angiotensin System in White and Brown Adipose Tissue. Compr Physiol. 2017;7(4):1137-50.
346 Epub 2017/09/16. doi: 10.1002/cphy.c160031. PubMed PMID: 28915321; PubMed Central PMCID:

PMCPMC7561006.

14. Jing F, Mogi M, Horiuchi M. Role of renin–angiotensin–aldosterone system in adipose tissue dysfunction. Molecular and Cellular Endocrinology. 2013;378(1-2):23-8. doi: 10.1016/j.mce.2012.03.005.

15. Wang CH, Li F, Takahashi N. The renin angiotensin system and the metabolic syndrome. Open Hypertens J. 2010;3:1-13. Epub 2010/12/07. doi: 10.2174/1876526203010001. PubMed PMID: 21132096; PubMed Central PMCID: PMCPMC2995894.

16. de Kloet AD, Pioquinto DJ, Nguyen D, Wang L, Smith JA, Hiller H, et al. Obesity induces neuroinflammation mediated by altered expression of the renin-angiotensin system in mouse forebrain nuclei. Physiol Behav. 2014;136:31-8. Epub 2014/02/11. doi: 10.1016/j.physbeh.2014.01.016. PubMed PMID: 24508821; PubMed Central PMCID: PMCPMC4125551.

17. Azushima K, Ohki K, Wakui H, Uneda K, Haku S, Kobayashi R, et al. Adipocyte - Specific Enhancement of Angiotensin II Type 1 Receptor - Associated Protein Ameliorates Diet - Induced Visceral Obesity and Insulin Resistance. Journal of the American Heart Association. 2017;6(3). doi: 10.1161/jaha.116.004488.

18. Wakil SJ, Stoops JK, Joshi VC. Fatty acid synthesis and its regulation. Annu Rev Biochem. 1983;52:537-79. Epub 1983/01/01. doi: 10.1146/annurev.bi.52.070183.002541. PubMed PMID: 6137188.

19. Schreiber R, Xie H, Schweiger M. Of mice and men: The physiological role of adipose triglyceride lipase (ATGL). Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids. 2019;1864(6):880-99. doi: 10.1016/j.bbalip.2018.10.008.

20. Duncan RE, Ahmadian M, Jaworski K, Sarkadi-Nagy E, Sul HS. Regulation of Lipolysis in Adipocytes. Annual Review of Nutrition. 2007;27(1):79-101. doi: 10.1146/annurev.nutr.27.061406.093734.

21. Roberts LD, Murray AJ, Menassa D, Ashmore T, Nicholls AW, Griffin JL. The contrasting roles of PPARdelta and PPARgamma in regulating the metabolic switch between oxidation and storage of fats in white adipose tissue. Genome Biol. 2011;12(8):R75. Epub 2011/08/17. doi: 10.1186/gb-2011-12-8-r75. PubMed PMID: 21843327; PubMed Central PMCID: PMCPMC3245615.

22. Castillo M, Hall JA, Correa-Medina M, Ueta C, Kang HW, Cohen DE, et al. Disruption of thyroid hormone activation in type 2 deiodinase knockout mice causes obesity with glucose intolerance and liver steatosis only at thermoneutrality. Diabetes. 2011;60(4):1082-9. Epub 2011/02/22. doi: 10.2337/db10-0758. PubMed PMID: 21335378; PubMed Central PMCID: PMCPMC3064082.

23. Sun B, Jia Y, Hong J, Sun Q, Gao S, Hu Y, et al. Sodium Butyrate Ameliorates High-Fat-Diet-Induced Non-alcoholic Fatty Liver Disease through Peroxisome Proliferator-Activated Receptor alpha-Mediated Activation of beta Oxidation and Suppression of Inflammation. J Agric Food Chem. 2018;66(29):7633-42. Epub 2018/07/03. doi: 10.1021/acs.jafc.8b01189. PubMed PMID: 29961332.

24. Yoon S, Kim J, Lee H, Lee H, Lim J, Yang H, et al. The effects of herbal composition Gambigyeongsinhwan (4) on hepatic steatosis and inflammation in Otsuka Long-Evans Tokushima fatty rats and HepG2 cells. J Ethnopharmacol. 2017;195:204-13. Epub 2016/11/16. doi: 10.1016/j.jep.2016.11.020. PubMed PMID: 27845265.

25. Crajoinas RO, Polidoro JZ, Carneiro de Morais CP, Castelo-Branco RC, Girardi AC. Angiotensin II counteracts the effects of cAMP/PKA on NHE3 activity and phosphorylation in proximal tubule cells. Am J Physiol Cell Physiol. 2016;311(5):C768-C76. Epub 2016/11/03. doi: 10.1152/ajpcell.00191.2016.

391 PubMed PMID: 27510906.

392 26. London E, Bloyd M, Stratakis CA. PKA functions in metabolism and resistance to obesity: lessons
393 from mouse and human studies. J Endocrinol. 2020;246(3):R51-R64. Epub 2020/06/03. doi:
394 10.1530/JOE-20-0035. PubMed PMID: 32485681; PubMed Central PMCID: PMCPMC7385994.

395 27. Fang X, Yu SX, Lu Y, Bast RC, Jr., Woodgett JR, Mills GB. Phosphorylation and inactivation of
396 glycogen synthase kinase 3 by protein kinase A. Proc Natl Acad Sci U S A. 2000;97(22):11960-5. Epub
397 2000/10/18. doi: 10.1073/pnas.220413597. PubMed PMID: 11035810; PubMed Central PMCID:
398 PMCPMC17277.

399 28. Abuissa H, Jones PG, Marso SP, O'Keefe JH, Jr. Angiotensin-converting enzyme inhibitors or
400 angiotensin receptor blockers for prevention of type 2 diabetes: a meta-analysis of randomized
401 clinical trials. J Am Coll Cardiol. 2005;46(5):821-6. Epub 2005/09/06. doi: 10.1016/j.jacc.2005.05.051.
402 PubMed PMID: 16139131.

403 29. de Kloet AD, Krause EG, Kim DH, Sakai RR, Seeley RJ, Woods SC. The effect of angiotensin-
404 converting enzyme inhibition using captopril on energy balance and glucose homeostasis.
405 Endocrinology. 2009;150(9):4114-23. Epub 2009/06/06. doi: 10.1210/en.2009-0065. PubMed PMID:
406 19497971; PubMed Central PMCID: PMCPMC2736089.

407 30. Sasamura H, Hein L, Krieger JE, Pratt RE, Kobilka BK, Dzau VJ. Cloning, characterization, and
408 expression of two angiotensin receptor (AT-1) isoforms from the mouse genome. Biochemical and
409 Biophysical Research Communications. 1992;185(1):253-9. doi: 10.1016/s0006-291x(05)80983-0.

410 31. Burson JM, Aguilera G, Gross KW, Sigmund CD. Differential expression of angiotensin receptor
411 1A and 1B in mouse. Am J Physiol. 1994;267(2 Pt 1):E260-7. Epub 1994/08/01. doi:
412 10.1152/ajpendo.1994.267.2.E260. PubMed PMID: 8074205.

413 32. Bays HE, Gonzalez-Campoy JM, Bray GA, Kitabchi AE, Bergman DA, Schorr AB, et al. Pathogenic
414 potential of adipose tissue and metabolic consequences of adipocyte hypertrophy and increased
415 visceral adiposity. Expert Rev Cardiovasc Ther. 2008;6(3):343-68. Epub 2008/03/11. doi:
416 10.1586/14779072.6.3.343. PubMed PMID: 18327995.
417 33. Liu FY, Cogan MG. Angiotensin II stimulates early proximal bicarbonate absorption in the rat by
418 decreasing cyclic adenosine monophosphate. J Clin Invest. 1989;84(1):83-91. Epub 1989/07/01. doi:
419 10.1172/JCI114174. PubMed PMID: 2544631; PubMed Central PMCID: PMCPMC303956.
420 34. Anand-Srivastava MB. Modulation of Gi Proteins in Hypertension: Role of Angiotensin II and
421 Oxidative Stress. Curr Cardiol Rev. 2010;6(4):298-308. Epub 2011/11/02. doi:
422 10.2174/157340310793566046. PubMed PMID: 22043206; PubMed Central PMCID:
423 PMCPMC3083811.
424 35. Schweiger M, Schreiber R, Haemmerle G, Lass A, Fledelius C, Jacobsen P, et al. Adipose
425 triglyceride lipase and hormone-sensitive lipase are the major enzymes in adipose tissue
426 triacylglycerol catabolism. J Biol Chem. 2006;281(52):40236-41. Epub 2006/11/01. doi:
427 10.1074/jbc.M608048200. PubMed PMID: 17074755.

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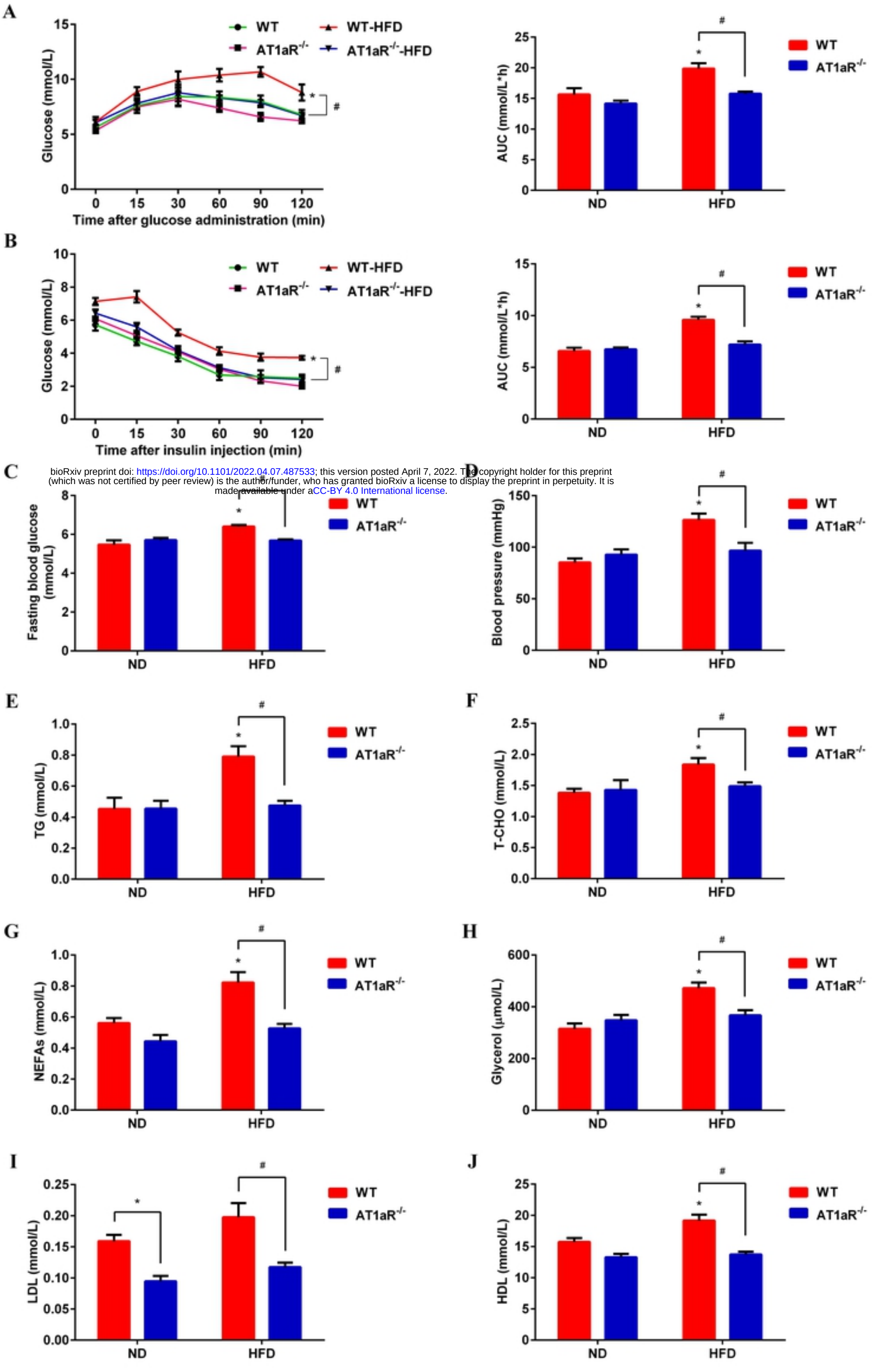
430 **Supporting information**

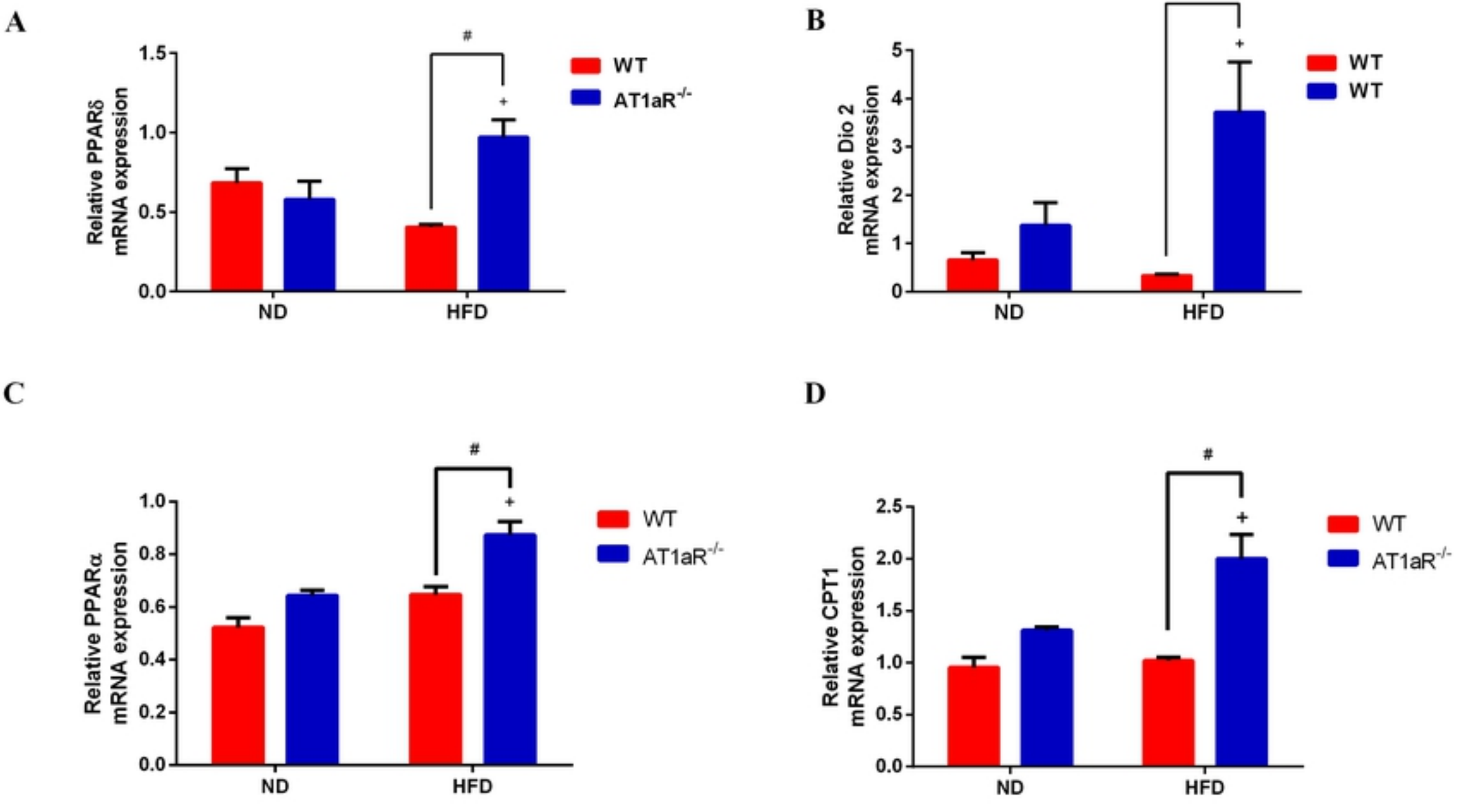
431 **S1 Fig.** PCR results for AT1aR-/- rats and WT rats. M: marker; N: negative control; P: positive control



Figure

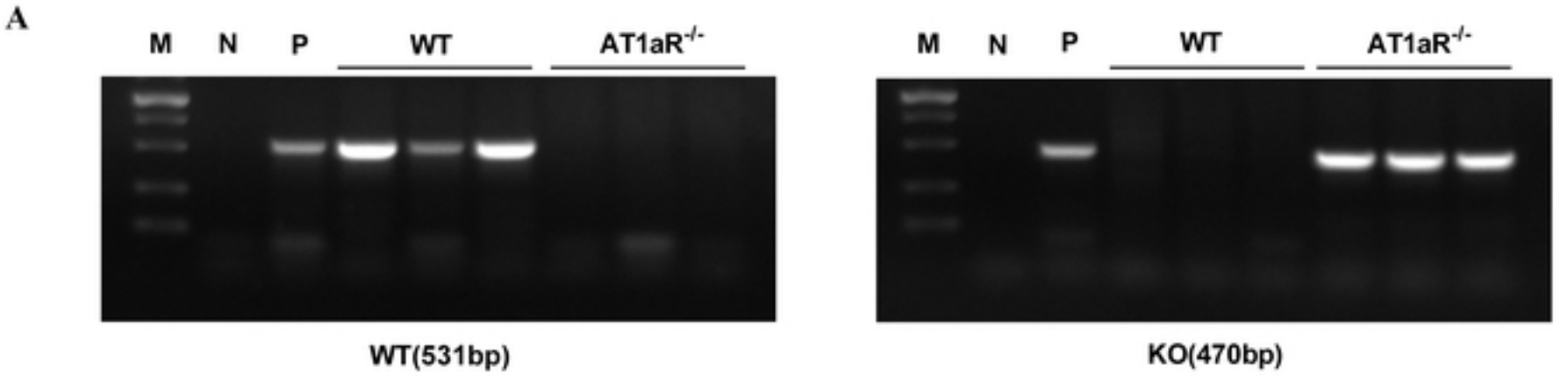
Figure

Figure


A
Relative PPARδ mRNA expression
1.5
1.0
0.5
0.0
ND
HFD
#
+
WT
AT1aR-/-
B
Relative Dio 2 mRNA expression
5
4
3
2
1
0
ND
HFD
#
+
WT
WT
C
Relative PPARα mRNA expression
1.0
0.8
0.6
0.4
0.2
0.0
ND
HFD
#
+
WT
AT1aR-/-
D
Relative CPT1 mRNA expression
2.5
2.0
1.5
1.0
0.5
0.0
ND
HFD
#
+
WT
AT1aR-/-

Figure

Figure



Figure