Original Article
Yonsei Med J 2022 Jun;63(6):530-538
https://doi.org/10.3349/ymj.2022.63.6.530
Yonsei Medical Journal
YMJ
pISSN: 0513-5796 · eISSN: 1976-2437

Fimasartan Ameliorates Deteriorations in Glucose Metabolism in a High Glucose State by Regulating Skeletal Muscle and Liver Cells

Yoo Na Jang[1,2*], Yong Jik Lee[1,3,4*], Yoon Mi Han[1], Hyun Min Kim[1,5], Hong Seog Seo[1,5], Ji Hoon Jeong[3,6], Seung Yeon Park[3,6], and Tae Woo Jung[3]

[1]Cardiovascular Center, Korea University Guro Hospital, Seoul;
[2]Department of Medicine, Graduate School, College of Medicine, Chung-Ang University, Seoul;
[3]Department of Pharmacology, College of Medicine, Chung-Ang University, Seoul;
[4]Laboratory of Genomics and Translational Medicine, Department of Internal Medicine, Gachon University College of Medicine, Incheon;
[5]Department of Medical Science, Korea University College of Medicine, Seoul;
[6]Department of Global Innovative Drugs, Graduate School of Chung-Ang University, Seoul, Korea.

Purpose: Since diabetes and hypertension frequently occur together, it is thought that these conditions may have a common pathogenesis. This study was designed to evaluate the anti-diabetic function of the anti-hypertensive drug fimasartan on C2C12 mouse skeletal muscle and HepG2 human liver cells in a high glucose state.

Materials and Methods: The anti-diabetic effects and mechanism of fimasartan were identified using Western blot, glucose uptake tests, oxygen consumption rate (OCR) analysis, adenosine 5'-triphosphate (ATP) enzyme-linked immunosorbent assay (ELISA), and immunofluorescence staining for diabetic biomarkers in C2C12 cells. Protein biomarkers for glycogenolysis and glycogenesis were evaluated by Western blotting and ELISA in HepG2 cells.

Results: The protein levels of phosphorylated 5' adenosine monophosphate-activated protein kinase (p-AMPK), p-AKT, insulin receptor substrate-1 (IRS-1), and glucose transporter type 4 (Glut4) were elevated in C2C12 cells treated with fimasartan. These increases were reversed by peroxisome proliferator-activated receptor delta (PPARδ) antagonist. ATP, OCR, and glucose uptake were increased in cells treated with 200 μM fimasartan. Protein levels of glycogen phosphorylase, glucose synthase, phosphorylated glycogen synthase, and glycogen synthase kinase-3 (GSK-3) were decreased in HepG2 cells treated with fimasartan. However, these effects were reversed following the addition of the PPARδ antagonist GSK0660.

Conclusion: In conclusion, fimasartan ameliorates deteriorations in glucose metabolism as a result of a high glucose state by regulating PPARδ in skeletal muscle and liver cells.

Key Words: Fimasartan, angiotensin II type 1 receptor blocker, glucose metabolism, PPARδ

INTRODUCTION

Obesity, diabetes, cardiovascular disease, and metabolic syndrome have all seen significant increases in the last few decades, and this trend is expected to continue into the future. These increases are likely the result of changes in modern lifestyle, such as an overabundance of nutrition and a lack of exercise.[1] Among these conditions, diabetes may be the most dangerous as it causes not only vascular disease, but also various other complications, including neuropathy and arteriosclerosis, all of which pose serious social and economic burden for both patients and society. There are two types of diabetes mellitus: type 1, or insulin-dependent diabetes, which is caused by

Received: October 5, 2021 **Revised:** February 11, 2022
Accepted: February 15, 2022
Co-corresponding authors: Tae Woo Jung, PhD, Department of Pharmacology, College of Medicine, Chung-Ang University, 84 Heukseok-ro, Dongjak-gu, Seoul 06974, Korea.
Tel: 82-2-820-5342, Fax: 82-2-813-5387, E-mail: twjung@cau.ac.kr and
Hong Seog Seo, MD, PhD, Cardiovascular Center, Korea University Guro Hospital, 148 Gurodong-ro, Guro-gu, Seoul 08308, Korea.
Tel: 82-2-2626-3018, Fax: 82-2-2626-2024, E-mail: mdhsseo@korea.ac.kr

*Yoo Na Jang and Yong Jik Lee contributed equally to this work.
•The authors have no potential conflicts of interest to disclose.

the destruction of pancreatic beta cells by the immune system, and type 2, or non-insulin-dependent diabetes, which is induced by insufficient glucose uptake and degradation due to insulin resistance in endodermal tissue cells.[2,3] Currently, most diabetes patients are type 2, and insulin resistance, which is a major factor in the development of diabetes, is known to play a key role in this metabolic syndrome.

Several papers have reported a correlation between diabetes and other metabolic diseases. The incidence of hypertension is proportional to that of diabetes, and hypertension itself is one of the major risk factors for aggravating insulin sensitivity.[4-6] Because the survival rate of hypertensive patients with diabetes is lower than that of hypertensive patients without diabetes, the development of appropriate therapeutic and preventive medicines based on their shared pathogenesis is necessary.

Fimasartan (2-n-butyl-5-dimethylaminothiocarbonylmethyl-6-methyl-3-[1]pyrimidine-(3H)-one potassium salt trihydrate) is a new angiotensin II type 1 receptor (AT1R) blocker approved by the Korean Food and Drug Administration in 2010 for the treatment of hypertension.[7] AT1R blockers lower blood pressure by inhibiting the binding of angiotensin II to AT1R and are therefore widely used in the treatment of hypertension and related vascular diseases.[8] One recent study reported that when angiotensin II-AT1R binding is blocked, angiotensin II binds to AT2R instead, inducing various anti-hypertension, anti-stroke, anti-arteriosclerosis, and anti-diabetes effects.[9] Additionally, another study reported that the use of an AT1R blocker lowered the prevalence rate of diabetes and heightened regulation effects on lipid and glucose metabolism, compared to other antihypertensive drugs.[10] In addition, AT1R blockers have been found to reduce the progression of diabetes and to be effective in preventing cardiovascular complications in patients with type 2 diabetes and hypertension.[11] Although many studies have suggested a relationship between AT1R blockers and diabetes, the underlying reaction mechanism remains unclear.

Skeletal muscle is a major organ involved in glucose uptake, utilization, and storage.[12] Furthermore, skeletal muscle is the main site of insulin resistance in obesity and type 2 diabetes.[13] The liver also plays a major role in the homeostasis of blood glucose by maintaining a balance between glucose uptake and storage via glycogenesis, glycogenolysis, and gluconeogenesis.[14] Although skeletal muscle and the liver are known to be involved in the regulation of glucose metabolism in a variety of ways, a comprehensive evaluation is lacking on the the effects of peroxisome proliferator-activated receptor delta (PPARδ) and 5' adenosine monophosphate-activated protein kinase (AMPK) on insulin signal transduction in both muscle and liver tissues.

Accordingly, this study was designed to clarify how fimasartan regulates glucose metabolism and insulin signal transduction in type 2 diabetes using in vitro models, focusing particularly on the roles of PPARδ and AMPK.

MATERIALS AND METHODS

Cell culture

C2C12 (mouse myoblast), HepG2 (human liver cancer cell), and Raw264.7 (mouse macrophage) cell lines (Korean Cell Line Bank, Seoul, Korea) were cultured in low glucose Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic solution (AA) (Welgene, Gyeongsan, Korea). The medium was replaced every 48–72 h. When the C2C12 cells reached confluence, they were placed in differentiation medium containing 1% FBS and 1% AA and differentiated to myotubes for 72 h. Afterwards, the medium was changed to high-glucose DMEM containing 1% FBS and 1% AA. The cells were treated with 200 μM fimasartan (Boryung Pharmaceutical Co., Ltd., Seoul, Korea), 50 μM GSK0660, and 1 μM compound C (Sigma-Aldrich, St. Louis, MO, USA) for 24 h.

When HepG2 and Raw264.7 cells reached 80% confluence, the medium was changed to high-glucose DMEM containing 1% FBS and 1% AA, and the cells were then treated with 200 μM fimasartan and 50 μM GSK0660 for 24 h.

When 3T3-L1 preadipocytes (Korean Cell Line Bank, Seoul, Korea) reached confluence, differentiation medium was applied to cells, together with fimasartan (30 μmole). The differentiation medium included 10% FBS and a differentiation cocktail solution consisting of 1.5 μg/mL insulin, 1 μM dexamethasone, 500 μM 3-isobutyl-1-methylxanthine, and 1 μM rosiglitazone. After differentiation for 2 days, the medium was replaced with insulin medium containing 1.5 μg/mL insulin and 10% FBS. After incubation in insulin media for 2–4 days, the medium was changed to maintenance medium that contained only 10% FBS.

Membrane protein extraction

Membrane proteins from the C2C12 cells were extracted using the Mem-PER Plus Membrane Protein Extraction Kit (Pierce Biotechnology, Rockford, IL, USA) according to the manufacturer's protocol.

After collection, the precipitated cells were resuspended in a cell wash solution and then transferred to a new tube and centrifuged. The cell supernatant was then discarded, and the cell pellet was resuspended in permeabilization buffer before being briefly agitated. The permeabilized cells were centrifuged, and the supernatant containing cytosolic proteins was transferred to new tubes. Solubilization buffer was added to the cell pellets and resuspended. After centrifugation, the supernatant containing the solubilized membrane and membrane-associated proteins was transferred to a new tube.

Western blot analysis

Cells were lysed in PRO-PREP™ (iNtRON, Seongnam, Korea) and centrifuged. The supernatant was then collected, and proteins were separated by SDS-PAGE (10% w/v) and transferred

to nitrocellulose membranes. After blocking with 5% skimmed milk solution or 1% BSA in Tris-buffered saline containing 0.05% Tween 20 (TBS-T), the membranes were incubated at room temperature for 2 h with the following primary antibodies: AMPK, p-AMPK (at Thr172), AKT, p-AKT, insulin receptor substrate-1 (IRS-1), glycogen phosphorylase, liver form (PYGL), glycogen synthase (GS), p-GS (Cell Signaling, Danvers, MA, USA), glucose transporter type 4 (Glut4), and β-Actin (Santa Cruz, CA, USA). After the membranes were incubated with appropriate horseradish peroxidase-conjugated secondary antibodies (Santa Cruz) at room temperature for 1 h, the membranes were treated with the Clarity™ Western ECL Substrate kit (Bio-Rad, Hercules, CA, USA), and images were obtained manually using developer fixer reagents (Kodak, Rochester, NY, USA) and X-ray film. The results were analyzed using Image J software (National Institutes of Health, Bethesda, MD, USA).

Reverse transcription polymerase chain reaction

Total RNA was extracted using TRIzol reagent according to the manufacturer's instructions. Complementary DNA synthesis was performed according to the protocol provided by the PrimeScript™ RT Master Mix (Takara Bio Inc, Shiga, Japan). The temperature protocol for RT was as follows: 37°C for 15 min, followed by 85°C for 5 sec. The primer sequences used were as follows: forward 5'-CATCTTCTCAAAATTCGAGTGACAA-3' and reverse 5'-GGGAGTAGACAAGGTACAACCC-3' for mouse TNF-α; forward 5'-TAAAACGCAG CTCAGTAACAGTCCG-3' and reverse 5'-TGGAATCCTGTGGACTCCATGAAAC-3' for mouse β-actin. For quantitative PCR, TOPreal™ qPCR 2X PreMIX (Enzynomics Co Ltd, Daejeon, Korea) and CFX96TM Real-Time System (Bio-Rad) were used. All reactions were repeated three times under the following conditions: initial denaturation at 95°C for 10 min, followed by 35 cycles at denaturation step for 10 sec at 95°C, annealing for 15 sec at 60°C, and elongation for 15 sec at 72°C. Relative gene expression values were determined using the $2^{-\Delta\Delta Cq}$ method.

Cell mito stress test

The oxygen consumption rate (OCR) in C2C12 cells was estimated using the Cell Mito Stress kit and Seahorse XFp system (Agilent, Santa Clara, CA, USA) according to the manufacturer's protocol. Cells (1×10^4/well) were plated, allowed to settle, and treated with fimasartan before being incubated overnight. A sensor cartridge and utility plate (cartridge+utility) containing the calibrant was incubated overnight in a CO_2-free incubator at 37°C. Oligomycin, carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP), and antimycin A/rotenone were separately injected into each drug port in the sensor cartridge+utility plate and incubated in a CO_2-free incubator for 10 min, after which OCR was measured.

ELISA for ATP, AMPK, glycogen synthase kinase-3, and pyruvate carboxylase

C2C12 and HepG2 cells (2×10^5/well) were seeded in 1 mL of medium per well in a 12-well plate and incubated for 48 h. The supernatants from these cells were then assayed for ATP, AMPK and glycogen synthase kinase-3 (GSK-3) using commercial ELISA kits (MyBioSource, San Diego, CA, USA) according to the manufacturer's instructions. Absorbance was measured using a microplate reader (Molecular Devices LLC., Sunnyvale, CA, USA) at 450 nm.

Glucose uptake test

C2C12 myotubes were washed twice with PBS and starved in 100 μL of PBS containing 2% (w/v) bovine serum albumin for 40 min. The myotubes were treated with insulin (100 μM) for 20 min, and 2-deoxyglucose was added to the myotubes and incubated for 20 min. After three washes with PBS, myotubes were extracted using an extraction buffer. These extracts were centrifuged at 500 rpm for 1–2 min, and the supernatants were transferred to fresh tubes. The enzyme mixture, glutathione reductase, substrate, and recycling mixture were added to diluted extracts with assay buffer, and the reaction mixture was incubated for 10 min. Optical density was then measured at 412 nm.

Immunofluorescence

C2C12 myotubes were treated with fimasartan (200 μM) and GSK0660 (50 μM) in the presence of 25 mM glucose (high glucose state) for 24 h. Immunofluorescence analysis was performed using primary antibodies against Glut4. Green fluorescence indicates Glut4 expression. The nuclei in all groups were stained blue with 4',6-diamidino-2-phenylindole (DAPI), and all images were acquired using a fluorescence microscope (EVOS FL Auto Imaging System, Thermo Fisher Scientific Inc, Bothell, WA, USA) at 200× magnification.

Statistical analyses

Data analyses were performed using GraphPad Prism 7.0 (GraphPad Software Inc., San Diego, CA, USA), and statistical significance was assessed using Student's t-test or one-way analysis of variance. Statistical significance was defined as a two-tailed p value<0.05.

RESULTS

Effects of fimasartan on C2C12 cells

Fimasartan in the range of 50–200 μmole increased the cell viability of C2C12 cells. Cell viability was the highest at 200 μmole and decreased at over 250 μmole (Fig. 1A). Compared to the high glucose control group, cells treated with fimasartan showed elevated levels of p-AMPK, IRS-1, p-AKT, and ATP. However, these increases were reversed following the addi-

https://doi.org/10.3349/ymj.2022.63.6.530

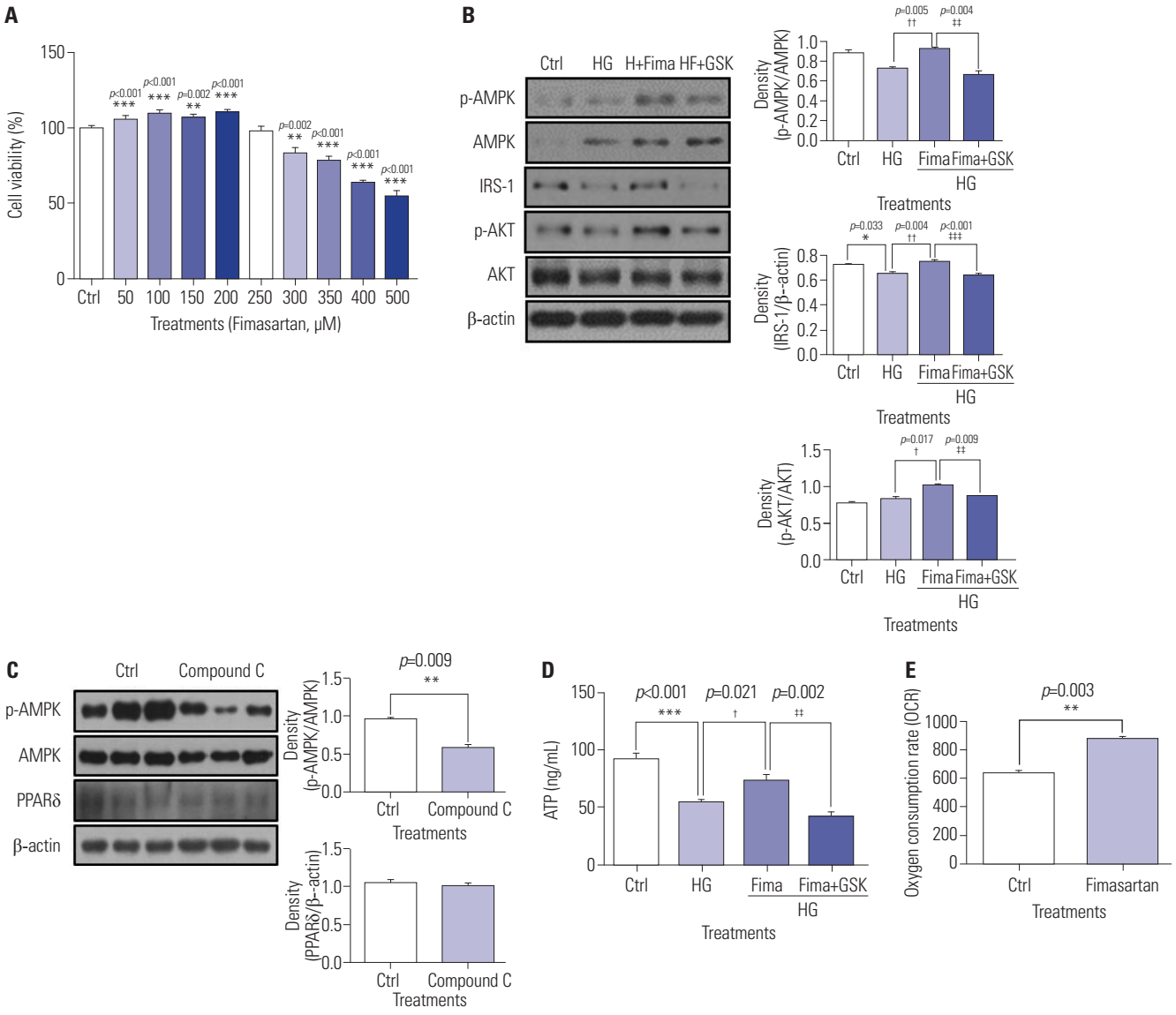

Fig. 1. Effects of fimasartan on C2C12 cells. (A) The viability of C2C12 cells was increased with fimasartan treatment at 50–200 μmole. $^{**}p<0.01$, $^{***}p<0.001$ vs. Ctrl. (B and D) Protein levels of p-AMPK, IRS-1, and phosphorylated AKT (p-AKT) and ATP concentrations were elevated with fimasartan, compared to the high glucose group. The increases were reversed by PPARδ antagonist (GSK0660) treatment. (B) $^{*}p<0.05$ vs. Ctrl, $^{\dagger}p<0.05$, $^{\dagger\dagger}p<0.01$ vs. HG, $^{\ddagger}p<0.01$, $^{\ddagger\ddagger}p<0.001$ vs. Fima. (D) $^{***}p<0.001$ vs. Ctrl, $^{\dagger}p<0.05$ vs. HG, $^{\ddagger}p<0.01$ vs. Fima. (C) The AMPK antagonist compound C decreased the levels of p-AMPK, but did not affect PPARδ expression. $^{**}p<0.01$ vs. Ctrl. (E) Fimasartan elevated OCRs. $^{**}p<0.01$ vs. Ctrl. Values represent means± SEM (n=3 or 8). p-AMPK, phosphorylated 5' adenosine monophosphate-activated protein kinase; IRS-1, insulin receptor substrate 1; ATP, adenosine 5'-triphosphate; PPARδ, peroxisome proliferator-activated receptor delta; GSK, glycogen synthase kinase.

tion of the PPARδ antagonist GSK0660 (Fig. 1B and D). PPARδ protein expression in C2C12 myotubes grown in the presence of high glucose was not changed in response to treatment with the AMPK antagonist compound C (Fig. 1C). Fimasartan treatment also increased OCRs in C2C12 myocytes (Fig. 1E).

Fimasartan increases glucose uptake and Glut4 expression in C2C12 cells

Fimasartan increased membranous Glut4 expression, but its effect was offset by GSK0660 (Fig. 2A and B). The uptake of 2-deoxy-glucose, which decreased under high glucose conditions, was increased in cells treated with fimasartan or insulin (Fig. 2C).

Fimasartan inhibits glycogenolysis, but alleviates glycogenesis in HepG2 cells

We determined the effects of fimasartan on glucose metabolism in liver cells treated with high glucose by evaluating the expression of various glycogen production and glucose homeostasis biomarkers, including PGYL, GS, and GSK-3, in HepG2 cells. Protein levels of PYGL, p-GS, and GSK-3 were decreased in fimasartan-treated cells compared to the high glucose control group, and these changes were attenuated in the presence of GSK0660. GS protein levels were higher in the fimasartan-

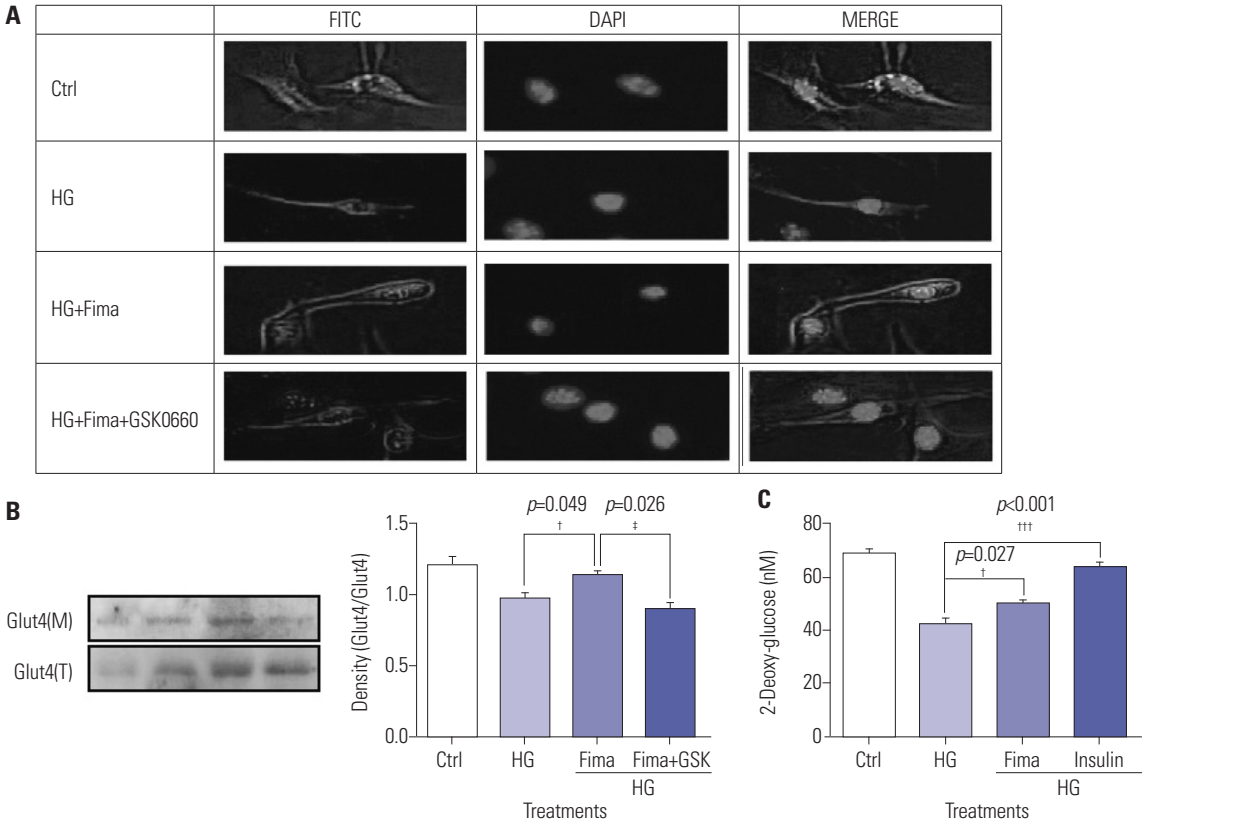

Fig. 2. The effects of fimasartan on glucose uptake and Glut4 expression in C2C12 cells. Glut4 was stained using a green fluorescent dye, and nuclei were stained blue using DAPI in all groups. Glut4 expression decreased in the high glucose treatment group, but this decrease was reversed in response to fimasartan. (A) The effect of fimasartan was attenuated by the addition of PPARδ antagonist GSK0660. (B) Western blot against Glut4 supported the immunofluorescence assays. $^{\dagger}p<0.05$ vs. HG, $^{\ddagger}p<0.05$ vs. Fima. (C) Decreases in 2-deoxy-glucose uptake induced by high glucose were reversed in response to fimasartan. $^{\dagger}p<0.05$, $^{\dagger\dagger\dagger}p<0.001$ vs. HG. Values represent means±SEM (n=3). Glut4; glucose transporter type 4; PPARδ, peroxisome proliferator-activated receptor delta; GSK, glycogen synthase kinase.

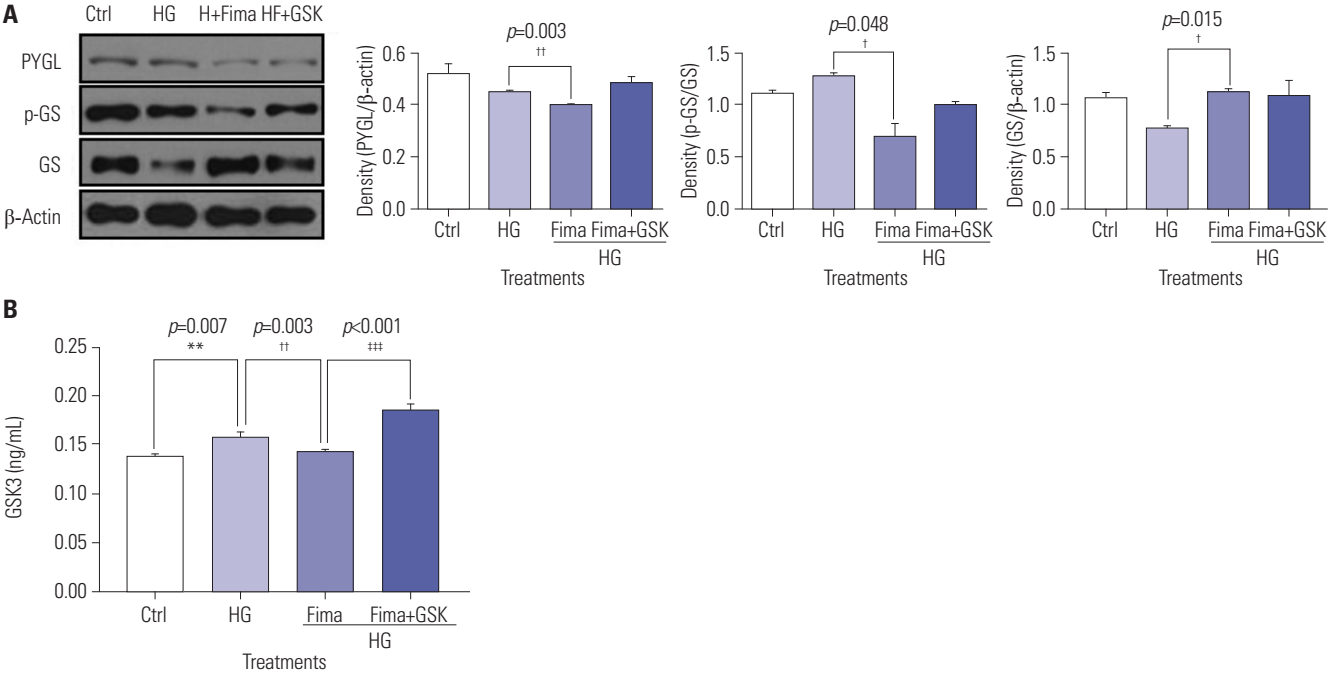

Fig. 3. The effects of fimasartan on glycogenolysis and glycogenesis in HepG2 Cells. (A) PYGL and p-GS expression decreased in response to fimasartan treatment, while GS expression increased. $^{\dagger}p<0.05$, $^{\dagger\dagger}p<0.01$ vs. HG. (B) GSK-3 concentrations decreased in the fimasartan-treated group, although this decrease was reversed following the addition of GSK0660. $^{**}p<0.01$ vs. Ctrl, $^{\dagger\dagger}p<0.01$ vs. HG, $^{\ddagger\ddagger}p<0.001$ vs. Fima. Values represent means± SEM (n=3).

https://doi.org/10.3349/ymj.2022.63.6.530

treated group than in the high glucose control group (Fig. 3).

DISCUSSION

Angiotensin 1–7 has been reported to exert anti-diabetic effects mediated by phosphorylation of AKT via the modulation of G protein coupled- and Mas receptors.[15] The angiotensin 1–7, Mas receptor, angiotensin II, and angiotensin II receptor pathways protect β-cells against oxidative stress and reduce insulin resistance induced by the accumulation of ectopic fat.[16] Research has shown that the AT1R blocker candesartan improves insulin resistance by increasing adiponectin levels[17] and that another AT1R blocker, telmisartan, ameliorates glucose intolerance in obese mice.[18] While several studies have demonstrated anti-diabetic effects for AT1R blockers, the underlying mechanism of these effects remains unknown. Meanwhile, recent reports suggest that the new AT1R blocker fimasartan elicits positive effects in several diseases, including nonalcoholic fatty liver disease and inflammation.[19-21]

Tumor necrosis factor-α (TNF-α), which is primarily secreted by activated macrophages, induces insulin resistance.[22] In this study, fimasartan reduced the increased expression of TNF-α mRNA elicited by high glucose in Raw264.7 macrophages; however, the effect of fimasartan was reversed by the PPARδ antagonist GSK0660 (Supplementary Fig. 1, only online). This suggests that that the anti-diabetic effects of fimasartan can be partially attributed to the downregulation of TNF-α dependent on PPARδ.

Although a recent clinical study reported an effect of increased insulin secretion for fimasartan in type 2 diabetic patients,[23] relatively few studies have described the effects or mechanism of fimasartan-mediated improvements in glucose metabolism.

Skeletal muscle is the major organ regulating the uptake, decomposition, and storage of glucose and accounts for approximately 75% of the total body glucose absorption under insulin stimulation. The activation of insulin signaling molecules, such as IRS-1 and AKT, stimulates glucose uptake in skeletal muscle.[24] It is also well known that skeletal muscle is the major tissue inducing insulin resistance in obesity and type 2 diabetes, and several studies have identified both AMPK and PPARδ as major therapeutic targets for metabolic disease.[25,26]

AMPK acts as an intracellular energy state sensor and is activated by an energy shortage in which the concentration of AMP is increased in comparison to ATP. AMPK is composed of a catalytic α-subunit and two regulatory β- and α-subunits. When cells are identified as energy deficient, AMPK undergoes phosphorylation of the 172 threonine residue in the α-subunit, which activates its signaling cascade.[27] AMPK is an important regulator of diabetes-related transcription factors, and AMPK activity regulates hepatic and intramuscular glucose transport.[28]

PPARs regulate gene transcription associated with fatty acid and glucose metabolism, and they interact with the renin-angiotensin system. Moreover, PPARs play important roles in insulin signal transduction and fat burning, and improve metabolic diseases, such as diabetes, obesity, hyperlipidemia, and atherosclerosis.[29] It has been reported that treatment with AT1 receptor blockers improves insulin sensitivity by activating PPARγ, although these interactions have been largely inferred based on the molecular structure of the drug.[30] Thus, studies on the relationship between AT1 receptor blockers and PPARs have primarily focused on PPARγ, which is expressed mainly in adipose tissues for fat storage; however, it is not expressed in skeletal muscle, which is the major tissue associated with insulin resistance.[31] PPARδ is relatively highly expressed in skeletal muscle and is known to play an important role in glucose metabolism and insulin action.[32] In addition, the activation of PPARδ has been found to induce an increase in fatty acid transport and glucose uptake in cells and to elevate the expression of genes related to lipid and glucose metabolism in skeletal muscle.[33,34] However, few studies have demonstrated how AMPK and PPARδ interact with AT1 receptor blockers in glucose metabolism.

Decreased mitochondrial function, including reduced ATP production rate and increased ROS generation, in skeletal muscle tissue results in insulin resistance, diabetes, and obesity.[35-37] Mitochondrial DNA mutations impair ATP-generation in the mitochondria, leading to a deficit in ATP, which is required for the transport of glucose and insulin.[38] OCR is directly proportional to the cellular concentration of ATP, and if the intracellular mitochondria are damaged and there are any problems in the oxidative phosphorylation process, the mitochondria do not consume oxygen and ultimately stop producing ATP. Therefore, the amount of oxygen consumed in the mitochondrial electron transport system is an important indicator for evaluating mitochondrial function, and it can be an important tool in the diagnosis and treatment of metabolic syndrome.[39] In our study, fimasartan was found to elevate the protein expression levels of IRS-1, P-AKT, P-AMPK, and membranous Glut4 in skeletal muscle cells treated with high glucose. Moreover, the effects of fimasartan on glucose utilization were shown to be PPARδ dependent, and the final effect of fimasartan on glucose metabolism appeared to be related with increases in OCR and ATP levels.

The liver is an important organ in both glucose and lipid metabolism. The liver is responsible for the storage and release of glucose: the liver will begin to store carbohydrates in the form of glycogen when there is a nutritional oversupply, which can then be released in the form of glucose when the body enters low blood glucose conditions.[40] Therefore, the liver plays an essential role in maintaining glucose homeostasis in the body. Glycogen is a multi-branched polysaccharide consisting of glucose, which functions as a readily available energy source and is primarily stored in the liver and muscle. Thus, the regulation of glycogen content is important for the homeostasis of glu-

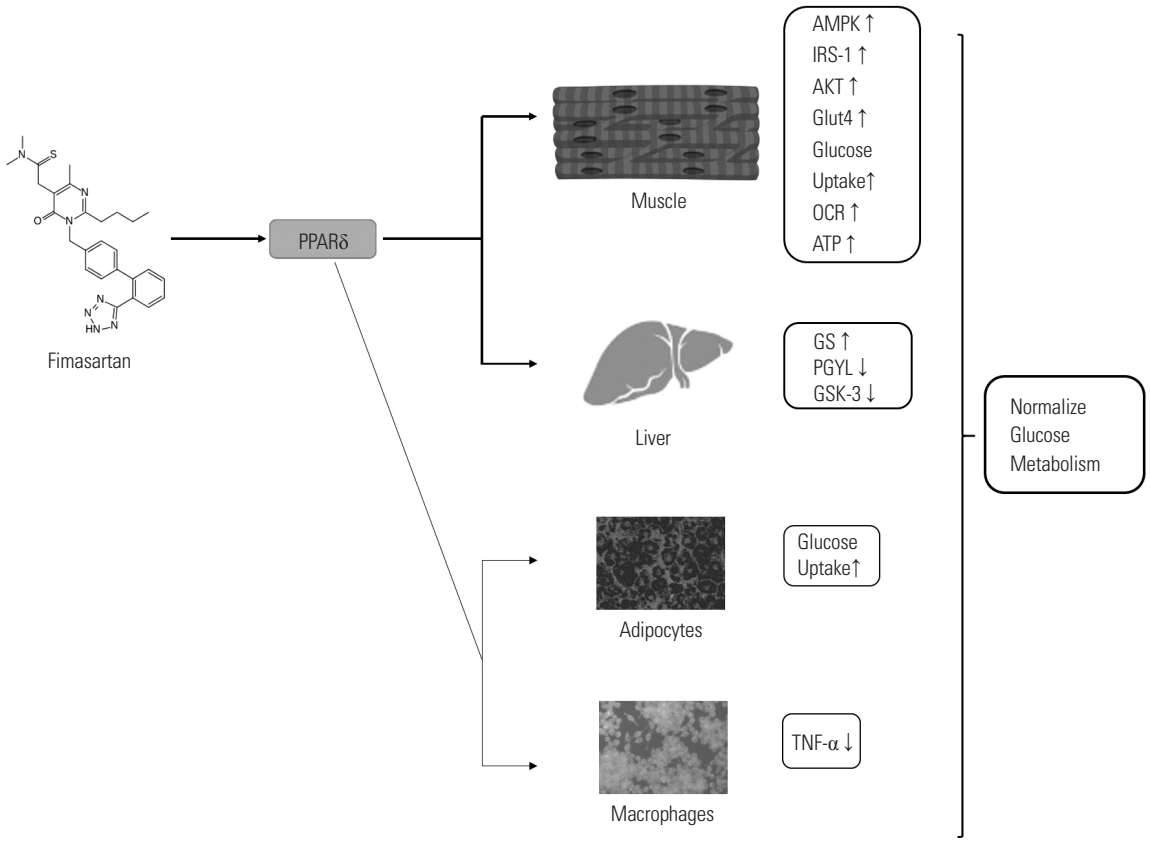

Fig. 4. Hypothetical diagram showing the effects and mechanism of fimasartan on the regulation of biomarkers related to glucose metabolism in several cells. Fimasartan can ameliorate glucose metabolism disturbed by a high glucose state primarily through the stimulation of insulin signal transduction and ATP-producing biomarkers in skeletal muscle cells and through elevated glycogen synthase and reduced glycogen phosphorylase in hepatocytes. Furthermore, fimasartan appears to elicit elevated glucose uptake in adipose cells and decreased inflammation in macrophages. Meaning of symbols: horizontal arrow means stimulation, up arrow means increase, and down arrow means decrease. AMPK, 5' adenosine monophosphate-activated protein kinase; OCR, oxygen consumption rate; ATP, adenosine 5'-triphosphate; IRS-1, insulin receptor substrate 1; Glut4; glucose transporter type 4; PPARδ, peroxisome proliferator-activated receptor delta; GSK, glycogen synthase kinase.

cose metabolism, with glycogen synthase and glycogen phosphorylase acting as the key regulators in glycogen metabolism. Glycogen synthase and glycogen phosphorylase regulate the synthesis and degradation of polysaccharides, respectively, and the level of cellular glycogen can be controlled by suppressing their catalytic activity.[41]

In hepatic glycogen metabolism, GSK-3 activity, which is a negative regulator of glycogen synthase, is inhibited in response to hyperglycemic conditions.[42] Here, we found that PPARδ was stimulated by fimasartan, which increased the expression of biomarkers related to glucose utilization in skeletal muscle cells and decreased concentrations of GSK-3. This downregulation of GSK-3 may inhibit glycogen phosphorylase in hepatocytes, thereby altering glycogen metabolism in these cells. This suggests that fimasartan may contribute to the amelioration of glycogen metabolism via modulation of PPARδ in hyperglycemic hepatocytes.

Consequently, fimasartan may ameliorate glucose metabolism dysfunction in response to increased glucose availability through modulation of PPARδ in skeletal and liver cells (Fig. 4). Interestingly, fimasartan did not affect pyruvate kinase, which is a key enzyme in gluconeogenesis (Supplementary Fig. 2B, only online). This suggests that fimasartan is involved in the regulation of glucose levels through the control of glycogen metabolism in hepatocytes.

Additionally, adipocytes uptake glucose and converse to lactate, thus participating in the regulation of blood glucose levels.[43] In this study, fimasartan significantly increased glucose uptake rates in differentiated 3T3-L1 cells (Supplementary Fig. 2A, only online). It is supposed that fimasartan can ameliorate elevated blood glucose levels through activation of glucose uptake in both skeletal muscle cells and adipocytes.

Research has indicated that telmisartan has anti-diabetic function and that the PPARδ-AMPK pathway is essential in the treatment of various diseases, including diabetes.[44,45] However, our study, for the first time, systematically investigated the anti-diabetes related functions of a new angiotensin II receptor blocker, fimasartan, by examining the effects thereof on insulin signal transduction in skeletal muscle cells and glycogen metabolism in hepatocytes. Notwithstanding, the precise mechanism of fimasartan awaits elucidation through further experiments aiming to document the effects and mechanisms of fi-

https://doi.org/10.3349/ymj.2022.63.6.530

masartan on insulin secretion in islet cells and diabetic animal models.

Fimasartan was originally developed to treat hypertension. Also, treating concentration (200 μmole) of fimasartan used in this study was limited to only cell-based studies. In this study, 200 μmole fimasartan was optimal in cell viability tests, ELISA for PPARδ and AMPK, and glucose uptake test; however, at the same concentration, telmisartan was not effective in view of cell physiology (Supplementary Fig. 3, only online). Accordingly, we deemed that fimasartan may better ameliorate biomarkers related to diabetes disturbed by high glucose at a relatively high concentration, compared to telmisartan. Thus the fimasartan concentration used in our cell-based diabetic study may be inappropriate in clinical applications.

ACKNOWLEDGEMENTS

This work was supported by a grant (O1801781) from Korea University Medical Center and Korea University Guro Hospital and by the Chung-Ang University Research Scholarship Grants in 2021.

AUTHOR CONTRIBUTIONS

Conceptualization: Yong Jik Lee and Hong Seog Seo. Data curation: Yong Jik Lee and Yoo Na Jang. Formal analysis: Hyun Min Kim and Seung Yeon Park. Funding acquisition: Hong Seog Seo and Tae Woo Jung. Investigation: Yong Jik Lee, Yoo Na Jang, and Yoon Mi Han. Methodology: Yong Jik Lee. Project administration: all authors. Resources: all authors. Software: Ji Hoon Jeong. Supervision: Yong Jik Lee, Hong Seog Seo, and Tae Woo Jung. Validation: Yong Jik Lee and Yoo Na Jang. Visualization: Yong Jik Lee and Yoo Na Jang. Writing—original draft: Yong Jik Lee and Yoo Na Jang. Writing—review & editing: Yong Jik Lee and Yoo Na Jang. Approval of final manuscript: all authors.

ORCID iDs

Yoo Na Jang https://orcid.org/0000-0002-0014-9783
Yong Jik Lee https://orcid.org/0000-0001-8814-0099
Yoon Mi Han https://orcid.org/0000-0002-1554-4823
Hyun Min Kim https://orcid.org/0000-0001-8807-0764
Hong Seog Seo https://orcid.org/0000-0002-3228-7988
Ji Hoon Jeong https://orcid.org/0000-0001-8257-403X
Seung Yeon Park https://orcid.org/0000-0002-3850-0567
Tae Woo Jung https://orcid.org/0000-0002-1167-1892

REFERENCES

1. Chellan N, Muller CJ, de Beer D, Joubert E, Page BJ, Louw J. An in vitro assessment of the effect of Athrixia phylicoides DC. aqueous extract on glucose metabolism. Phytomedicine 2012;19:730-6.
2. Tisch R, McDevitt H. Insulin-dependent diabetes mellitus. Cell 1996;85:291-7.
3. Hales CN, Barker DJ. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetologia 1992;35:595-601.
4. Horr S, Nissen S. Managing hypertension in type 2 diabetes mellitus. Best Pract Res Clin Endocrinol Metab 2016;30:445-54.
5. UK Prospective Diabetes Study Group. Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. BMJ 1998;317:703-13.
6. Tatsumi Y, Ohkubo T. Hypertension with diabetes mellitus: significance from an epidemiological perspective for Japanese. Hypertens Res 2017;40:795-806.
7. Kim JH, Lee JH, Paik SH, Kim JH, Chi YH. Fimasartan, a novel angiotensin II receptor antagonist. Arch Pharm Res 2012;35:1123-6.
8. Karnik SS, Unal H, Kemp JR, Tirupula KC, Eguchi S, Vanderheyden PM, et al. International Union of Basic and Clinical Pharmacology. XCIX. Angiotensin receptors: interpreters of pathophysiological angiotensinergic stimuli. Pharmacol Rev 2015;67:754-819.
9. Chrysant SG, Chrysant GS. The pleiotropic effects of angiotensin receptor blockers. J Clin Hypertens (Greenwich) 2006;8:261-8.
10. Savoia C, Touyz RM, Volpe M, Schiffrin EL. Angiotensin type 2 receptor in resistance arteries of type 2 diabetic hypertensive patients. Hypertension 2007;49:341-6.
11. Sica DA. Cardiovascular morbidity and mortality in patients with diabetes in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. Curr Hypertens Rep 2002;4:321-3.
12. Smith AG, Muscat GE. Skeletal muscle and nuclear hormone receptors: implications for cardiovascular and metabolic disease. Int J Biochem Cell Biol 2005;37:2047-63.
13. Kelley DE, Goodpaster BH, Storlien L. Muscle triglyceride and insulin resistance. Annu Rev Nutr 2002;22:325-46.
14. Nordlie RC, Foster JD, Lange AJ. Regulation of glucose production by the liver. Annu Rev Nutr 1999;19:379-406.
15. Prasannarong M, Santos FR, Henriksen EJ. ANG-(1-7) reduces ANG II-induced insulin resistance by enhancing Akt phosphorylation via a Mas receptor-dependent mechanism in rat skeletal muscle. Biochem Biophys Res Commun 2012;426:369-73.
16. Favre GA, Esnault VL, Van Obberghen E. Modulation of glucose metabolism by the renin-angiotensin-aldosterone system. Am J Physiol Endocrinol Metab 2015;308:E435-49.
17. Furuhashi M, Ura N, Higashiura K, Murakami H, Tanaka M, Moniwa N, et al. Blockade of the renin-angiotensin system increases adiponectin concentrations in patients with essential hypertension. Hypertension 2003;42:76-81.
18. Shiota A, Shimabukuro M, Fukuda D, Soeki T, Sato H, Uematsu E, et al. Telmisartan ameliorates insulin sensitivity by activating the AMPK/SIRT1 pathway in skeletal muscle of obese db/db mice. Cardiovasc Diabetol 2012;11:139.
19. Lee YJ, Jang YN, Han YM, Kim HM, Jeong JM, Seo HS. Fimasartan ameliorates nonalcoholic fatty liver disease through PPARδ regulation in hyperlipidemic and hypertensive conditions. PPAR Res 2017;2017:8048720.
20. Kim S, Kim SJ, Yoon HE, Chung S, Choi BS, Park CW, et al. Fimasartan, a novel angiotensin-receptor blocker, protects against renal inflammation and fibrosis in mice with unilateral ureteral obstruction: the possible role of Nrf2. Int J Med Sci 2015;12:891-904.
21. Yang XL, Kim CK, Kim TJ, Sun J, Rim D, Kim YJ, et al. Anti-inflammatory effects of fimasartan via Akt, ERK, and NFκB pathways on astrocytes stimulated by hemolysate. Inflamm Res 2016;65:115-23.
22. De Taeye BM, Novitskaya T, McGuinness OP, Gleaves L, Medda M, Covington JW, et al. Macrophage TNF-α contributes to insulin resistance and hepatic steatosis in diet-induced obesity. Am J Physiol Endocrinol Metab 2007;293:E713-25.
23. Yang YS, Lim MH, Lee SO, Roh E, Ahn CH, Kwak SH, et al. Fimasartan increases glucose-stimulated insulin secretion in patients with type 2 diabetes and hypertension compared with amlodipine. Dia-

betes Obes Metab 2018;20:1670-7.
24. Long YC, Cheng Z, Copps KD, White MF. Insulin receptor substrates Irs1 and Irs2 coordinate skeletal muscle growth and metabolism via the Akt and AMPK pathways. Mol Cell Biol 2011;31:430-41.
25. Zhang BB, Zhou G, Li C. AMPK: an emerging drug target for diabetes and the metabolic syndrome. Cell Metab 2009;9:407-16.
26. Reilly SM, Lee CH. PPAR delta as a therapeutic target in metabolic disease. FEBS Lett 2008;582:26-31.
27. Woods A, Vertommen D, Neumann D, Turk R, Bayliss J, Schlattner U, et al. Identification of phosphorylation sites in AMP-activated protein kinase (AMPK) for upstream AMPK kinases and study of their roles by site-directed mutagenesis. J Biol Chem 2003;278:28434-42.
28. Rutter GA, Da Silva Xavier G, Leclerc I. Roles of 5'-AMP-activated protein kinase (AMPK) in mammalian glucose homoeostasis. Biochem J 2003;375:1-16.
29. Cho MC, Lee K, Paik SG, Yoon DY. Peroxisome proliferators-activated receptor (PPAR) modulators and metabolic disorders. PPAR Res 2008;2008:679137.
30. Monsalve FA, Pyarasani RD, Delgado-Lopez F, Moore-Carrasco R. Peroxisome proliferator-activated receptor targets for the treatment of metabolic diseases. Mediators Inflamm 2013;2013:549627.
31. Schupp M, Janke J, Clasen R, Unger T, Kintscher U. Angiotensin type 1 receptor blockers induce peroxisome proliferator-activated receptor-gamma activity. Circulation 2004;109:2054-7.
32. Tontonoz P, Hu E, Graves RA, Budavari AI, Spiegelman BM. mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. Genes Dev 1994;8:1224-34.
33. Krämer DK, Al-Khalili L, Guigas B, Leng Y, Garcia-Roves PM, Krook A. Role of AMP kinase and PPARdelta in the regulation of lipid and glucose metabolism in human skeletal muscle. J Biol Chem 2007;282:19313-20.
34. Luquet S, Lopez-Soriano J, Holst D, Fredenrich A, Melki J, Rassoulzadegan M, et al. Peroxisome proliferator-activated receptor delta controls muscle development and oxidative capability. FASEB J 2003;17:2299-301.
35. Lowell BB, Shulman GI. Mitochondrial dysfunction and type 2 diabetes. Science 2005;307:384-7.
36. Rong JX, Qiu Y, Hansen MK, Zhu L, Zhang V, Xie M, et al. Adipose mitochondrial biogenesis is suppressed in db/db and high-fat diet-fed mice and improved by rosiglitazone. Diabetes 2007;56:1751-60.
37. Short KR, Bigelow ML, Kahl J, Singh R, Coenen-Schimke J, Raghavakaimal S, et al. Decline in skeletal muscle mitochondrial function with aging in humans. Proc Natl Acad Sci U S A 2005;102:5618-23.
38. McInnes J. Mitochondrial-associated metabolic disorders: foundations, pathologies and recent progress. Nutr Metab (Lond) 2013;10:63.
39. Gan Z, Burkart-Hartman EM, Han DH, Finck B, Leone TC, Smith EY, et al. The nuclear receptor PPARβ/δ programs muscle glucose metabolism in cooperation with AMPK and MEF2. Genes Dev 2011;25:2619-30.
40. Hartman ML, Shirihai OS, Holbrook M, Xu G, Kocherla M, Shah A, et al. Relation of mitochondrial oxygen consumption in peripheral blood mononuclear cells to vascular function in type 2 diabetes mellitus. Vasc Med 2014;19:67-74.
41. Akatsuka A, Singh TJ, Huang KP. Comparison of the liver glycogen synthase from normal and streptozotocin-induced diabetic rats. Arch Biochem Biophys 1983;220:426-34.
42. Han HS, Kang G, Kim JS, Choi BH, Koo SH. Regulation of glucose metabolism from a liver-centric perspective. Exp Mol Med 2016;48:e218.
43. Mårin P, Rebuffé-Scrive M, Smith U, Björntorp P. Glucose uptake in human adipose tissue. Metabolism 1987;36:1154-60.
44. Feng X, Luo Z, Ma L, Ma S, Yang D, Zhao Z, et al. Angiotensin II receptor blocker telmisartan enhances running endurance of skeletal muscle through activation of the PPAR-δ/AMPK pathway. J Cell Mol Med 2011;15:1572-81.
45. Amoghimath S, Suresha RN. Effect of telmisartan on blood glucose levels and blood lipid levels in streptozotocin induced diabetic rats. Biomed Pharmacol J 2019;12:1303-8.

https://doi.org/10.3349/ymj.2022.63.6.530