Fibroblast Growth Factor 21 Improves Insulin Resistance and Ameliorates Renal Injury in *db/db* Mice

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Directed by Professor Shin-Wook Kang

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<ABSTRACT>

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(Directed by Professor Shin-Wook Kang)

Despite the emerging importance of fibroblast growth factor 21 (FGF21) as a metabolic hormone regulating energy balance, its direct effects on renal function remain unexplored. FGF21 was injected intraperitoneally daily for 12 weeks into *db/db* mice. Compared to control vehicle injection, FGF21 treatment significantly improved lipid profiles and insulin resistance, and resulted in significantly higher serum adiponectin level. In contrast, serum insulin and 8-isoprostane levels were significantly decreased. Interestingly, FGF21 and its receptor components in the kidneys were found to be significantly upregulated in *db/db* mice, which suggests an FGF21-resistant state. FGF21 treatment significantly downregulated FGF21 receptor components and activated extracellular signal-regulated kinase (ERK) phosphorylation. FGF21 administration also markedly decreased urinary albumin excretion and mesangial expansion, and suppressed profibrotic molecule synthesis. Furthermore, FGF21 improved renal lipid metabolism and oxidative stress injury. In cultured renal cells, FGF21 was mainly expressed in mesangial cells and knockdown of FGF21 expression by stealth small interfering RNA further aggravated high-glucose-induced pro-fibrotic cytokine synthesis in mesangial cells. These results suggest that FGF21 improves insulin resistance and protects against renal injury through both improvement of systemic metabolic alterations and anti-fibrotic effects in type 2 diabetic nephropathy. Targeting FGF21 could therefore provide a potential candidate approach for a therapeutic strategy in type 2 diabetic nephropathy.

Key words: diabetic nephropathy, fibroblast growth factor 21, insulin resistance

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I. INTRODUCTION

Fibroblast growth factor 21 (FGF21) is a member of the FGF family and has been identified as an important regulator of energy metabolism[1]. While other classical fibroblast growth factors (FGFs) require heparin for efficient binding between FGF and fibroblast growth factor receptors (FGFRs), along with FGF19 and FGF23, FGF21 has a unique feature of lacking heparin-binding domains, and this enables these factors to readily diffuse into circulation and act in an endocrine manner[2]. FGF21 is mainly expressed in the liver but also in other tissues such as thymus, adipose tissue, islet β-cells, and skeletal muscle[3-4] and exerts its effects through four classic FGF receptor (FGFR) isotypes 1-4[5].

The role of FGF21 in metabolic regulation was first demonstrated as facilitating glucose uptake by adipocytes through upregulating transcription of the glucose transporter GLUT1[6]. The same study reported that overexpression of FGF21 in the liver of transgenic mice improved insulin sensitivity, glucose clearance, and resistance to weight gain despite a significant increase in caloric intake, whereas others indicated that adenoviral knockdown of FGF21 in mice leads to fatty liver and a marked increase in triglyceride levels[6-7]. In addition, 7-day administration of recombinant FGF21 to various diabetic murine models

showed similar effects such as reduction in plasma levels of glucose, insulin, glucagon, and triglyceride[6]. Likewise, a more prolonged injection of FGF21 in genetically obese mice increased energy expenditure, fat utilization, and lipid excretion as well as reduced hepatosteatosis[8], and similar results were also observed in diet-induced obese mice[9]. Furthermore, despite its therapeutic potential, FGF21 administration did not induce serious adverse events such as hypoglycemia or edema, which is commonly observed during diabetic therapies, in experiments using various animal models[4]. Taken together, in terms of both efficacy and safety, FGF21 could be an excellent candidate for a therapeutic strategy in obesity-related diabetic conditions.

Kidney is a well-known target of metabolic disorders. Obesity-related morbidities have been regarded as strong independent risk factors for chronic kidney disease (CKD) and end-stage renal disease (ESRD)[10]. Also notably, previous studies have demonstrated that various types of fibroblast growth factor receptors (FGFRs) are also expressed and localized in adult as well as developing murine kidneys[11-12]. Taken together, these findings suggest that the kidneys may be one of the target organs for FGF21 treatment and be protected against obesity-related diabetic injury by the treatment of FGF21. However, to date, there has been no convincing demonstration of this association.

In this study, I administered recombinant FGF21 to *db/db* mice, which are widely used as obese and type 2 diabetic models[13], and observed its effects on changes in diabetes-related parameters and specifically in renal functional and morphologic changes. I also carried out *in vitro* experiments to investigate the molecular mechanism of direct renal effects of FGF21.

II. MATERIALS AND METHODS

1. Animal experiments

Six-week-old male diabetic *db/db* mice (C57BLKS/J-*lepr*^{db}/*lepr*^{db}) and male nondiabetic *db/m* mice (C57BLKS/J-*lepr*^{db}/+) were purchased from Jackson Laboratory (Sacramento, CA, USA). Recombinant FGF21 was purchased from Phoenix Pharmaceutical, Inc. (Belmont, CA, USA). The mice were treated with FGF21 at the age of 8 weeks, and all mice were kept at controlled temperature (23 ± 2°C) and humidity (55 ± 5%) levels under an artificial light cycle. The mice were divided into three groups. The first group consisted of non-diabetic *db/m* mice as non-diabetic controls (n = 4), the second group was composed of vehicle-treated diabetic *db/db* mice (n = 8), and the third group was made up of diabetic *db/db* mice treated with FGF21 by daily intraperitoneal injection for 3 months at a dose of 25 μg/kg/day (n = 8). I selected this dose of FGF21 because previous studies showed that a minimum dose within 10 to 100 μg/kg/day was required for *in vivo* FGF21 activity in diet-induced obese mice and *ob/ob* mice[6,14]. Food intake, water intake, urine volume, body weight, HbA1c and fasting plasma glucose concentration were measured monthly during the experiment. Plasma glucose levels were measured using a glucose oxidase-based method, HbA1c levels were calculated by the IN2IT system (Bio-Rad Laboratories, Hercules, CA, USA), and creatinine levels were determined by HPLC. Plasma insulin levels and plasma adiponectin levels were measured with an ELISA(enzyme-linked immunosorbent assay) kit (Linco Research, St. Charles, MO, USA). Plasma FGF21 concentration was measured with an ELISA kit (R&D Systems Inc, Minneapolis, MN, USA). The homeostasis model assessment index (HOMA-IR) was calculated using the formula of fasting glucose (mmol/L) × fasting insulin (mU/L)/22.5. Plasma triglyceride and cholesterol analyses were performed using a GPO-Trinder kit (Sigma-Aldrich, St. Louis, MO, USA). Plasma lipoprotein profiles were measured using a fast

protein liquid chromatography (high-performance liquid chromatography) system. Insulin tolerance testing (ITT) was conducted in *db/db* mice following 8-hour fasting, and blood samples were collected through the tail vein. Mice received 0.75 unit/kg regular insulin by intraperitoneal injection and blood glucose was subsequently measured at 0, 30, 60, 90 and 120 min. Plasma and urinary levels of 8-isoprostane were measured with an ELISA kit (Cayman Chemical, Ann Arbor, MI, USA). Lipids from the hepatic, adipose, and renal cortical tissues were extracted as described by Bligh and Dyer[15]. Total cholesterol and triglyceride contents were measured using a commercial kit (Wako Chemicals, Richmond, VA, USA). The extent of peroxidative reaction in the hepatic, adipose tissue and kidneys was determined by directly measuring lipid hydroperoxides (LPOs) using an LPO assay kit (Cayman Chemical) as described previously[16]. To determine urinary albumin excretion, individual mice were caged once per month in a metabolic cage and urine was collected for 24 h. Urinary albumin concentrations were determined by competitive ELISA (Shibayagi, Shibukawa, Japan). After 3 months of treatment, mice were euthanized under anesthesia by intraperitoneal injection of sodium pentobarbital (50 mg/kg). Epididymal fat, liver, and kidney tissues were weighed and subsequently snap frozen in liquid nitrogen. All experiments were conducted in accordance with National Institutes of Health guidelines and with approval of the Institutional Animal Care and Use Committee.

2. Analysis of gene expression by real-time quantitative polymerase chain reaction

Total RNA was extracted from the renal cortical tissues, adipose tissue, and experimental cells with TRIzol reagent and further purified using an RNeasy Mini kit (QIAGEN, Valencia, CA, USA). Primer sequences are listed in Table 1. Quantitative gene expression was performed on a LightCycler 1.5 system (Roche Diagnostics Corp., Indianapolis, IN, USA) using SYBR Green

technology. In brief, 10 μL of SYBR Green master mix were added to 1 μL of RNA (corresponding to 50 ng of total RNA) and 900 nmol/L of forward and reverse primers in a reaction volume of 20 μl. Real-time polymerase chain reaction (PCR) was performed for 10 min at 50ºC, 5 min at 95ºC and a total of 22–30 cycles of 10 sec at 95ºC and 30 sec at 60ºC. Samples were finally heated to 95ºC to verify that a single PCR product had been obtained. The ratio of the expression level of each gene to that of β-actin level (relative gene expression number) was calculated by subtracting the threshold cycle number of the target gene from that of β-actin and raising two to the power of this difference.

Table 1. Primer sequences for real-time quantitative PCR[1]

Target gene[2]	Primer sequence (5' to 3')	Size (bp)
MCP-1, forward	CTGGATCGGAACCAAATGAG	95
MCP-1, reverse	CGGGTCAACTTCACATTCAA	
PAI-1, forward	TCCTCATCCTGCCTAAGTTCTC	365
PAI-1, reverse	GTGCCGCTCTCGTTTACCTC	
TGF-β_1, forward	AGCCCGAAGCGGACTACTAT	96
TGF-β_1, reverse	CTGTGTGAGATGTCTTTGGTTTTC	
Col-IV, forward	GCTCTGGCTGTGGAAAATGT	102
Col-IV, reverse	CTTGCATCCCGGGAAATC	
Adiponectin, forward	TGTTGGAATGACAGGAGCTGAA	121
Adiponectin, reverse	CACACTGAAGCCTGAGCGATAC	
PPARγ, forward	AGACCACTCGCATTCCTTTGACAT	271
PPARγ, reverse	TCCCCACAGACTCGGCACTCAATG	
SREBP-1c, forward	AAGATGTACCCGTCCGTGTC	80
SREBP-1c, reverse	GGCTGTAGGATGGTGAGTGG	
HMG-CoA, forward	AGCCGAAGCAGCACATGAT	201
HMG-CoA, reverse	CTTGTGGAATGCCTTGTGATTG	
ABCA1, forward	CGTTTCCGGGAAGTGTCCTA	246
ABCA1, reverse	GCTAGAGATGACAAGGAGGATGGA	
FAS, forward	CCTGGATAGCATTCCGAACCT	183
FAS, reverse	AGCCATCTCGAAGGCTACACA	
Visfatin, forward	TGCCGTGAAAAGAAGACAGA	143
Visfatin, reverse	ACTTCTTTGGCCTCCTGGAT	
TNF-α, forward	CCGATGGGTTGTACCTTGTC	131
TNF-α, reverse	GGCAGAGAGGAGGTTGACTTT	
FGF21, forward	GTCAAAGCCTCTAGGTTTCTTTGC	75
FGF21, reverse	GGCCTCAGGATCAAAGTGAG	
β-Klotho, forward	CAGGCCCATTGTTACCTTGT	131
β-Klotho, reverse	CTCCAAAGGTCTGGAAGCAG	
FGFR1c, forward	AATACCACCGACAAGGAAATGG	87
FGFR1c, reverse	AGTTACCCGCCAAGCACGTA	
FGFR2c, forward	GTTCTCTATATTCGGAATGTAACTTTTGAG	103
FGFR2c, reverse	AGG CGC TGG CAG AAC TGT	
FGFR3c, forward	ACG CCC TAC GTC ACT GTA CTC A	84
FGFR3c, reverse	GGT GAC ATT GTG CAA GGA CAG A	
FGFR4, forward	GCTCGGAGGTAGAGGTCTTG	95
FGFR4, reverse	GTAGGAAAGGCCGATGGAGT	
β-actin, forward	GGACTCCTATGTGGGTGACG	118
β-actin, reverse	CTTCTCCATGTCGTCCCAGT	

[1] In this experiment, each sample was run in triplicate, and the corresponding non-reverse transcribed mRNA samples were used as negative controls. The mRNA level of each sample was normalized to that of β-actin mRNA.

[2] MCP-1, monocyte chemoattractant peptide-1; PAI-1, plasminogen activator inhibitor-1; TGF-β_1, transforming growth factor-β_1; Col-IV, type IV collagen; PPARγ, peroxisome proliferator-activated receptor γ; SREBP-1c, sterol regulatory element-binding protein-1c; HMG-CoA, cholesterol 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase; ABCA1, ATP-binding cassette transporter-1; FAS, fatty acid synthase; TNF-α, tumor necrosis factor-α; FGF21, fibroblast growth factor 21; FGFR, FGF receptor

3. Histopathological evaluation and immunohistochemistry

Kidney and adipose tissues were fixed for 48 h with 10% paraformaldehyde at 4ºC, dehydrated, embedded in paraffin, cut into 4-μm thick slices, and stained with periodic acid-Schiff and hematoxylin and eosin (H&E). Glomerular mesangial expansion was scored semiquantitatively, and the percentage of mesangial matrix occupying each glomerulus was rated 0–4 as follows: 0, 0%; 1, less than 25%; 2, 25–50%; 3, 50–75%; and 4, more than 75%. For immunohistochemical staining, renal tissues were sliced into 4-μm sections. Slides were transferred to a 10 mmol/L citrate buffer solution at pH 6.0 and heated at 80°C for 30 min for transforming growth factor-β_1 (TGF-β_1), fibronectin, and laminin. For type IV collagen staining, slides were treated with trypsin (one tablet per 1 ml of water) for 20 min for antigen retrieval. To block endogenous peroxidase activity, 3.0% H_2O_2 in methanol was applied for 20 min, followed by incubation at room temperature for 60 min in 3% BSA/3% normal goat serum (type IV collagen, fibronectin and laminin) or 30 min in 20% normal sheep serum (TGF-β_1). Slides were incubated overnight at 4°C with a rabbit polyclonal anti-TGF-β_1 antibody (1:100, Santa Cruz Biotechnology, Santa Cruz, CA, USA), rabbit polyclonal anti-type IV collagen antibody (1:150, BioDesign International, Sarco, ME, USA), rabbit polyclonal anti-fibronectin antibody (1:100, Abcam Plc, Cambridge, MA, USA), or rat monoclonal anti-laminin antibody (1:50, Abcam Plc, Cambridge, MA, USA). Slides were

incubated in secondary antibodies for 30 min, and at room temperature in 0.05% 3,30-diaminobenzidine containing 0.01% H_2O_2 before counterstaining with Mayer's hematoxylin. For evaluation of immunohistochemical staining for type IV collagen, TGF-β_1, fibronectin and laminin, glomerular fields were graded semiquantitatively using a high-power field containing 50-60 glomeruli and an average score was calculated as described previously[17]. A pathologist carried out the histological examinations in a blinded manner.

4. Protein extraction and Western blot analysis

Nuclear and cytoplasmic proteins were extracted from renal cortical tissues and cells using a commercial nuclear extraction kit according to the manufacturer's instructions (Active Motif, Carlsbad, CA, USA). Protein concentrations were determined using the bicinchoninic acid method (Pierce Pharmaceuticals, Rockford, IL). For Western blotting, 40 μg of protein was electrophoresed on a 10% SDS-PAGE minigel. Proteins were transferred onto a polyvinylidene difluoride membrane, and the membrane was hybridized in blocking buffer overnight at 4°C with rabbit polyclonal anti-FGF21 antibody (1:1000; Abcam), mouse monoclonal anti-nuclear factor-κB (NF-κB) p65 antibody (1:1000, Cell Signaling Technology), rabbit polyclonal anti-plasminogen activator inhibitor-1 (PAI-1) antibody (1:500, Santa Cruz Biotechnology, Santa Cruz, CA, USA), sterol-regulatory element-binding protein-1c (SREBP-1c) antibody (1:500, Santa Cruz Biotechnology), rabbit polyclonal anti-adenosine triphosphate-binding cassette transporter A1 (ABCA1) antibody (1:200, Santa Cruz Biotechnology), rabbit polyclonal anti-TGF-β_1 antibody (1:200, Santa Cruz Biotechnology), phospho-specific extracellular signal-regulated kinase (ERK) 1/2, total ERK1/2 (1:1000, New England Biolabs, Inc., Beverly, MA, USA), type IV collagen antibody (1:500, Santa Cruz Biotechnology) or mouse monoclonal anti-TATA binding protein (TBP) antibody (1:1000, Abcam Plc). The membrane was subsequently incubated with

horseradish peroxidase-conjugated secondary antibody (1:1000 dilution) for 60 min at room temperature. Specific signals were detected using the enhanced chemiluminescence method (Amersham, Buckinghamshire, UK.)

5. Mesangial cell culture and stealth RNA interference for FGF21

Because FGF21 was preferentially detected in mesangial cells, I used mesangial cells to elucidate the molecular mechanism of FGF21. A portion of the renal cortex from normal C57BL/6 mice was obtained immediately after surgical nephrectomy and glomeruli were isolated using the differential sieving method. mesangial cells were cultured in DMEM supplemented with 10% FCS. With the use of indirect immunofluorescence staining, the mesangial cells were identified due to their large stellate shape, and the positive staining for α-smooth muscle actin, but negative staining for synaptopodin, common leukocyte antigen and E-cadherin. To evaluate the effect of high glucose on FGF21 synthesis, sub-confluent mesangial cells were serum-starved for 24 hr, and the medium was then exchanged with media containing 30mM D-glucose for 48 h. Since FGF21 showed a beneficial effect in renal function, I next used small interfering RNA (siRNA) directed against FGF21 to investigate whether FGF21 regulated fibrotic molecule synthesis. Mouse FGF21 mRNA was specifically knocked down using commercially available siRNA oligonucleotides. The sequences of stealth siRNAs were designed using BLOCK-iT RNAI Designer (Invitrogen Life Technologies, Gaithersburg, MD): sense strand, 5- CAA GUC CGG CAG AGG UAC CUC UAC A-3; antisense strand, 5- UGU AGA GGU ACC UCU GCC GGA CUU G-3. Mesangial cells were maintained in DMEM with 10% fetal calf serum before transfection with 10 nmol siRNA using Lipofectamine RNAiMax (Invitrogen Life Technologies) according to the manufacturer’s instructions. Mesangial cells were transfected for 24 h with siRNA and cultivated in serum-free and antibiotic-free DMEM. Cells were made quiescent for 24 h, and treated with 30 mM high-glucose medium for 72 h.

Stealth RNAi negative control duplexes were used as controls.

6. Statistical analysis

A nonparametric analysis was used because of the relatively few samples. Results were expressed as the mean ± standard error of the mean (SEM). Multiple comparisons were done using Wilcoxon rank sum tests and Bonferroni correction. A Kruskal-Wallis test compared more than two groups, followed by a Mann-Whitney U-test, using a microcomputer-assisted program with SPSS for Windows 10.0 (SPSS, Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

III. RESULTS

1. Biochemical and physical measurements in experimental animals

Table 2 shows the various biochemical and physical parameters measured in experimental animals. Levels of fasting blood glucose, HbA1c, body weight, urine volume, water and food intake, and fat and liver mass per body weight were significantly higher in diabetic *db/db* than in non-diabetic *db/m* mice. On the other hand, when compared with vehicle-treated *db/db* mice, FGF21-treated *db/db* mice showed significantly reduced mass of kidney, liver, and epididymal fat per body weight, but no significant differences in the levels of fasting blood glucose, HbA1c, urine volume, water and food intake, and plasma concentrations of creatinine. Plasma FGF21 concentrations were elevated 20-fold in diabetic *db/db* mice compared with those in control *db/m* mice. Interestingly, FGF21 treatment significantly decreased plasma FGF21 levels (Table 2).

2. Metabolic parameters in experimental animals

As shown in Figure 1, *db/db* mice had significantly higher levels of plasma insulin and HOMA-IR compared to control *db/m* mice. Interestingly, FGF21 treatment induced a significant improvement in these parameters. ITT confirmed that FGF21 improved the insulin resistance state (Figure 1A). In accordance with these changes, plasma levels of total cholesterol, triglyceride, and LDL cholesterol were significantly higher in diabetic *db/db* than in non-diabetic *db/m* mice, but these increases were significantly ameliorated by FGF21 treatment when compared with vehicle-treated *db/db* mice. In addition, the serum level of adiponectin, an adipokine possessing anti-inflammatory and anti-diabetic properties, was significantly increased by FGF21 treatment (Table 2).

Table 2. Physical and biochemical parameters of experimental animals

Parameters[1]	week	*db/m* control	*db/db* + vehicle	*db/db* + FGF21
Body weight (g)	0	26.25 ± 0.63	37.37 ± 0.82**	38.25 ± 0.83**
	4	28.25 ± 0.85	48.50 ± 1.64***	51.62 ± 2.52***
	8	32.00 ± 1.08	56.00 ± 1.59***	51.25 ± 3.2***
	12	33.25 ± 1.31	61.00 ± 2.05***	52.00 ± 3.83***
Daily Food intake (g)	0	2.37 ± 0.36	5.18 ± 0.67***	5.31 ± 0.28***
	4	2.56 ± 0.18	5.21 ± 0.34***	4.75 ± 0.34***
	8	3.12 ± 0.36	5.00 ± 0.15**	5.62 ± 0.15***
	12	2.87 ± 0.21	5.31 ± 0.13***	5.68 ± 0.20***
Daily water intake (g)	0	5.50 ± 0.43	14.06 ± 1.42***	12.68 ± 1.43***
	4	6.25 ± 0.43	14.68 ± 0.77***	11.62 ± 0.82**
	8	6.37 ± 1.37	14.75 ± 1.23***	15.06 ± 0.28***
	12	6.25 ± 0.72	18.68 ± 0.75***	17.50 ± 0.51***
Fasting blood glucose (mmol/L)	0	9.4 ± 0.4	29.0 ± 0.9***	31.1 ± 0.9***
	4	8.9 ± 0.6	30.9 ± 1.9***	30.3 ± 1.5***
	8	10.7 ± 0.4	32.1 ± 1.8***	35.4 ± 2.9***
	12	7.8 ± 0.1	36.7 ± 1.5***	36.0 ± 1.9***
HbA1c (%)	0	5.40 ± 0.16	8.61 ± 0.25***	9.58 ± 0.41***
	4	5.02 ± 0.25	9.92 ± 0.38***	10.72 ± 0.64***
	8	4.87 ± 0.10	10.31 ± 0.33***	13.03 ± 1.44***
	12	5.05 ± 0.25	12.40 ± 0.31***	12.15 ± 0.45***
UV (ml/day)	0	1.62 ± 0.44	3.97 ± 0.34*	4.44 ± 0.34**
	4	1.16 ± 0.27	3.55 ± 0.39**	4.30 ± 0.80**
	8	0.66 ± 0.14	5.13 ± 0.44***	3.42 ± 0.64**
	12	0.44 ± 0.21	4.00 ± 0.52***	3.52 ± 0.72**
Kidney/100 g BW	12	1.71 ± 0.06	1.38 ± 0.07	1.03 ± 0.15$^{**,\#}$
Heart/100 g BW	12	0.76 ± 0.02	0.46 ± 0.01**	0.45 ± 0.10**
Fat/100 g BW	12	3.43 ± 0.89	8.19 ± 0.41***	5.07 ± 0.36$^{*,\#\#}$
Liver/100 g BW	12	5.87 ± 0.24	9.11 ± 0.63**	5.44 ± 0.23$^{\#\#}$
Adiponectin (μg/mL)	12	2.76 ± 0.13	2.52 ± 0.16*	2.68 ± 0.05$^{\#}$
P-8-isoprostane (pg/mL)	12	475 ± 122	1309 ± 183*	598 ± 186$^{\#}$
P-creatinine (μmol/L)	12	7.0 ± 3.0	8.0 ± 1.0	7.0 ± 3.0
P-FGF21 (pgmL)	12	78.3 ± 12.3	1513 ± 218***	935 ± 156$^{***,\#}$

[1] UV, urine volume; BW, body weight; P, plasma; FGF21, fibroblast growth factor 21. Values are expressed as means ± SEM. Statistical analysis was performed between groups at the same time periods.

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ vs. *db/m* control; $^{\#}P < 0.05$; $^{\#\#}P < 0.001$ vs. *db/db* + vehicle.

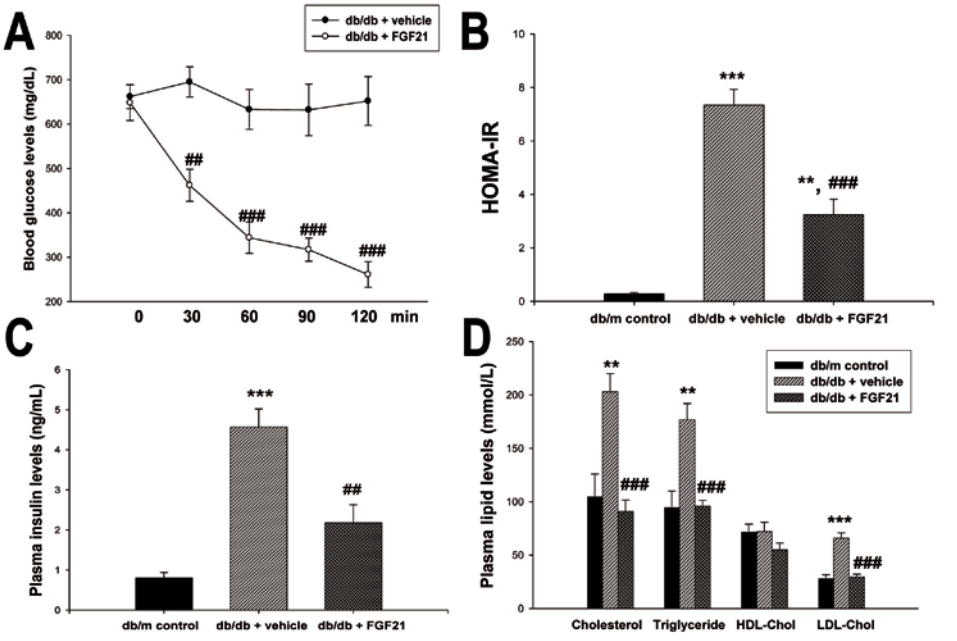

Figure 1. Effect of FGF21 on insulin resistance and lipid parameters at 20 weeks in experimental animals. (A) Insulin tolerance tests (B) HOMA-IR (C) Plasma insulin concentration (D) Plasma lipid concentration. HDL-Chol, HDL-cholesterol; LDL-Chol, LDL-cholesterol; FGF21, fibroblast growth factor 21. Data are expressed as the mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. *db/m* control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. *db/db* + vehicle.

3. Effects of FGF21 treatment on changes in histological and adipocytokine mRNA expression in epididymal adipose tissue in experimental animals

Adipose tissue dysfunction is now considered to play a central role in obesity and its associated disorders and is characterized in part by changes in adipocyte volume and mRNA expression patterns of various adipokines[18]. I therefore investigated whether these changes were influenced by FGF21 treatment. Epididymal adipose tissue of *db/db* mice had larger adipocytes than *db/m* mice, and FGF21 treatment restored their hypertrophic phenotype to the small differentiated phenotype, which was consistent with the finding of reduced epididymal fat mass per body weight after FGF21 treatment (Figure 2A; Table 2). In accordance with this morphological alteration, mRNA expression levels

of proinflammatory and profibrotic adipokines were remarkably upregulated in the fat pad of *db/db* mice compared to *db/m* mice, whereas mRNA expression levels of anti-inflammatory or adipocyte differentiation-related molecules were downregulated (Figure 2B). Among these changes in adipose tissue mRNA levels, expression of both PAI-1 and tumor necrosis factor-α (TNF-α) were significantly reduced in FGF21- compared to vehicle-treated mice. In contrast, mRNA levels of adiponectin and peroxisome proliferator-activated receptor γ (PPARγ) were significantly enhanced by FGF21 treatment (Figure 2B).

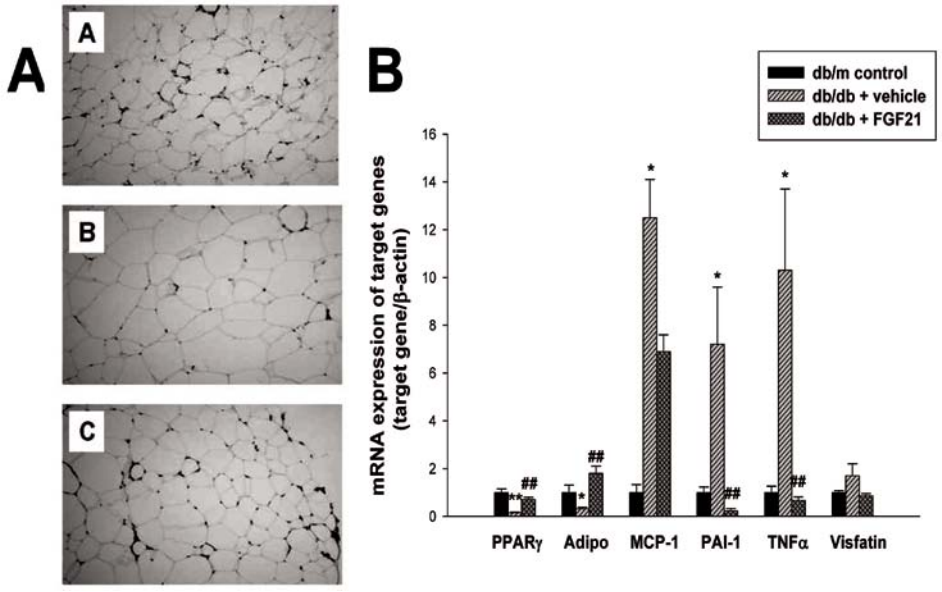

Figure 2. Effects of FGF21 on adipose tissue. (A) Representative histological findings of epididymal adipose tissue (B) mRNA expression of adipocytokines in adipose tissue. A, *db/m* control; B, *db/db* + vehicle; C, *db/db* + FGF21. A H&E stain of adipose tissue. Original magnification X 400. PPARγ, peroxisome proliferator-activated receptor γ; Adipo, adiponectin; MCP-1, monocyte chemoattractant peptide-1; PAI-1, plasminogen activator inhibitor-1; TNF-α, tumor necrosis factor α; FGF21, fibroblast growth factor 21. Data are expressed as the mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. *db/m* control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. *db/db* + vehicle.

4. Effects of FGF21 treatment on renal functional and structural changes in experimental animals

Throughout the experimental period, urinary albumin excretion was significantly increased in *db/db* mice as compared to *db/m* mice. This increase in albuminuria was significantly attenuated by 8 weeks of FGF21 treatment, and the decrease was maintained until 12 weeks of treatment (Figure 3).

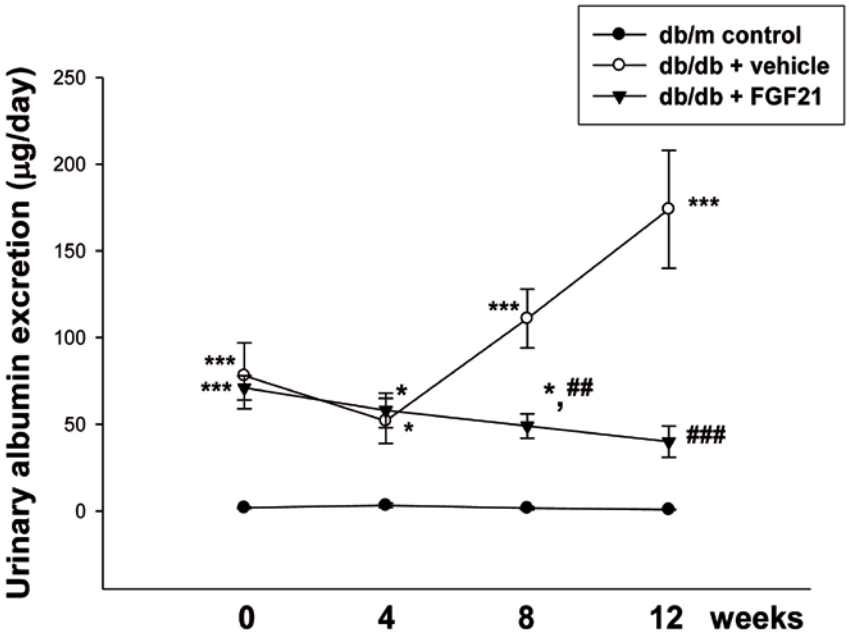

Figure 3. Effects of FGF21 on 24-h urinary albumin excretion in experimental animals. A 24-h urine sample was collected at monthly intervals after FGF21 administration. Statistical analysis was performed between groups at the same time periods. FGF21, fibroblast growth factor 21. Data are expressed as the mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. *db/m* control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. *db/db* + vehicle.

In addition, histological sections of kidneys from vehicle-treated *db/db* mice showed signs of glomerular tuft hypertrophy and mesangial expansion compared to *db/m* mice, and these changes were significantly attenuated by FGF21 treatment (Figure 4, Figure 5). Consistent with these histological

changes, mRNA expression levels of type IV collagen, PAI-1 and TGF-β_1 were increased in vehicle-treated *db/db* compared to *db/m* mice, and this increase in expression of profibrotic cytokine genes was significantly reduced by FGF21 treatment (Figure 5). Immunohistochemical stains for profibrotic markers such as type IV collagen, TGF-β_1, laminin and fibronectin showed similar tendencies. Semiquantitative immunostaining scores for all these markers were significantly increased in vehicle-treated *db/db* compared to *db/m* mice, and all of these profibrotic changes were also significantly ameliorated by FGF21 treatment. These results demonstrated that FGF21 treatment significantly ameliorated functional (urinary albumin excretion) and morphological glomerular abnormalities induced by chronic diabetic injury in *db/db* mice.

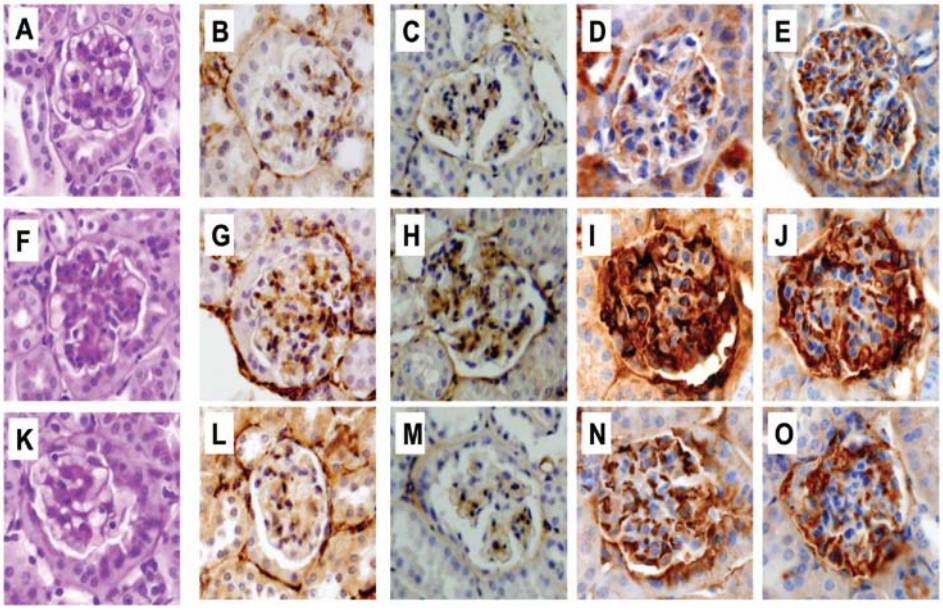

Figure 4. Representative renal histological findings in experimental animals. A, F, K, PAS stain; B, G, L, type IV collagen stain; C, H, M, TGF-β_1 stain; D, I, N, fibronectin stain; E, J, O, laminin stain; A-E, *db/m* control; F-J, *db/db* + vehicle; K-O, *db/db* + FGF21. Original magnification X 400. PAS, periodic acid-Schiff; TGF-β_1, transforming growth factor-β_1.

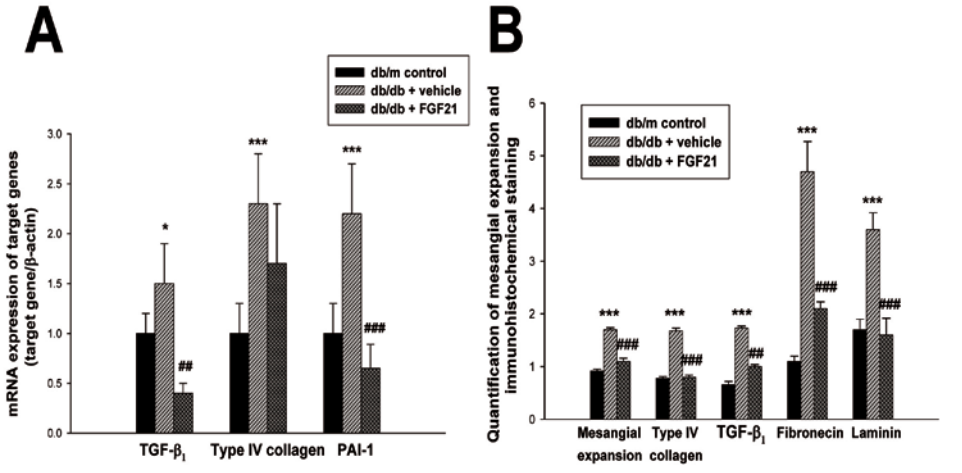

Figure 5. Effect of FGF21 on profibrotic molecule synthesis in renal cortical tissues. (A) mRNA expression of renal cortical tissues (B) Glomerular mesangial expansion score and immunostaining score. TGF-β_1, transforming growth factor-β_1; PAI-1, plasminogen activator inhibitor-1; FGF21, fibroblast growth factor 21. Data are expressed as the mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. *db/m* control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. *db/db* + vehicle.

5. Lipid peroxidation and oxidative stress parameters in experimental animals

Oxidative stress, referred to as an imbalance between reactive oxygen species (ROS) and antioxidants, has been regarded as a major contributor to obesity-related diabetic morbidities[19]. Lipid peroxidation and its bioactive product, isoprostanes, are the most extensively studied markers of diabetes-related free radical attacks[20-21]. Since FGF21 treatment improved insulin resistance and dyslipidemia, and possibly reduced oxidative stress, I next evaluated whether FGF21 treatment also affected these parameters including lipotoxicity in the kidney by measuring the changes in serum and urinary levels of 8-isoprostane, the tissue level of LPO and tissue lipid accumulation. As expected, plasma levels of 8-isoprostane were three times higher in *db/db* mice than in *db/m* mice, and these increases were abrogated by FGF21 treatment (Table 2). FGF 21 treatment also decreased cholesterol and triglyceride accumulation in the

diabetic kidney (Figure 6), which has been proposed to play an important role in the progression of diabetic nephropathy[22-23]. In addition, urinary 8-isoprostane levels and kidney LPO levels were markedly increased in *db/db* compared to *db/m* mice, and these increases were significantly attenuated by FGF21 treatment (Figure 6).

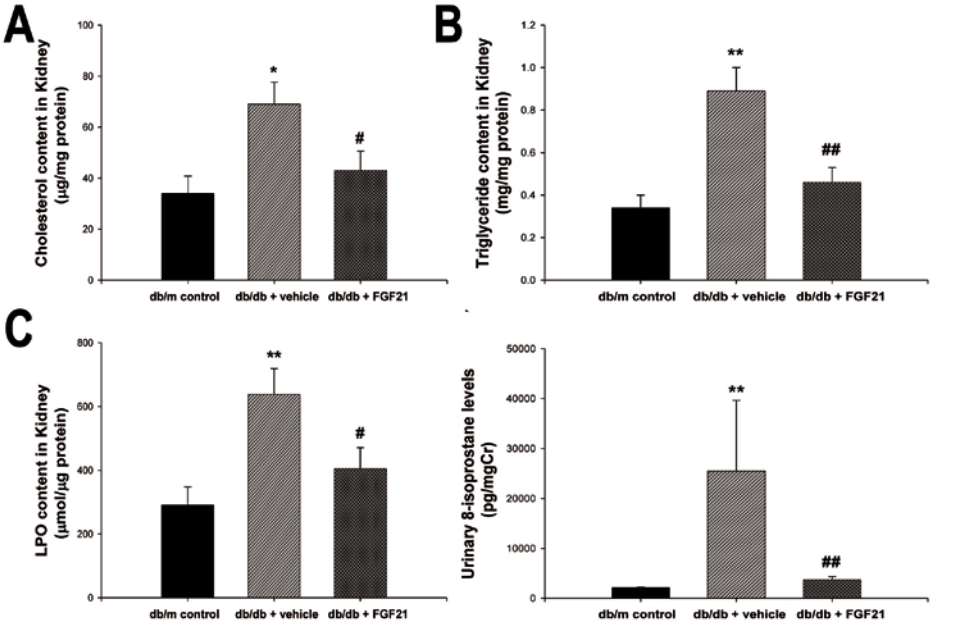

Figure 6. Effects of FGF21 on renal lipid metabolism, lipid peroxidation and oxidative stress in experimental animals. (A) Cholesterol content in renal cortical tissue (B) Triglyceride content in renal cortical tissue (C) LPO content in renal cortical tissue (D) 24-h urinary levels of 8-isoprostane; urinary excretion of 8-isoprostane was corrected by urinary creatinine. LPO, Lipid hydroperoxides; FGF21, fibroblast growth factor 21. Data are expressed as the mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. *db/m* control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. *db/db* + vehicle.

Finally, to explore the mechanism of the beneficial effects of FGF21 treatment on diabetic renal injury, I determined the change in nuclear p65 protein expression, which reflects activity of NF-κB, a key mediator of obesity-

related inflammation. Western blot analysis in extracted renal cortical nuclear proteins demonstrated that there was an increase in p65 protein level in renal tissues of *db/db* mice compared to lean *db/m* mice, and FGF21 treatment markedly abolished this increase (Figure 7A). This finding suggests that the anti-diabetic properties of FGF21 were, at least in part, mediated by suppression of the proinflammatory NF-κB pathway. Because FGF21 treatment improved renal lipid contents and oxidative stress, I next examined whether improvement in renal function originated from the correction of alteration in renal lipid metabolism. Specifically, I measured mRNA expression levels of genes known to be involved in lipid synthesis and cholesterol efflux. As shown in Figure 7B, FGF21 treatment dramatically suppressed gene expression of both SREBP-1c and 3-hydroxy-3-methylglutaryl-coenzyme A (HMG CoA) reductase which induces lipid synthesis. Furthermore, gene expression of ABCA1, which modulates cholesterol efflux, was significantly increased by FGF21 treatment. These findings were in agreement with the results that FGF21 decreased renal cholesterol and triglyceride levels (Figures 6 - 8).

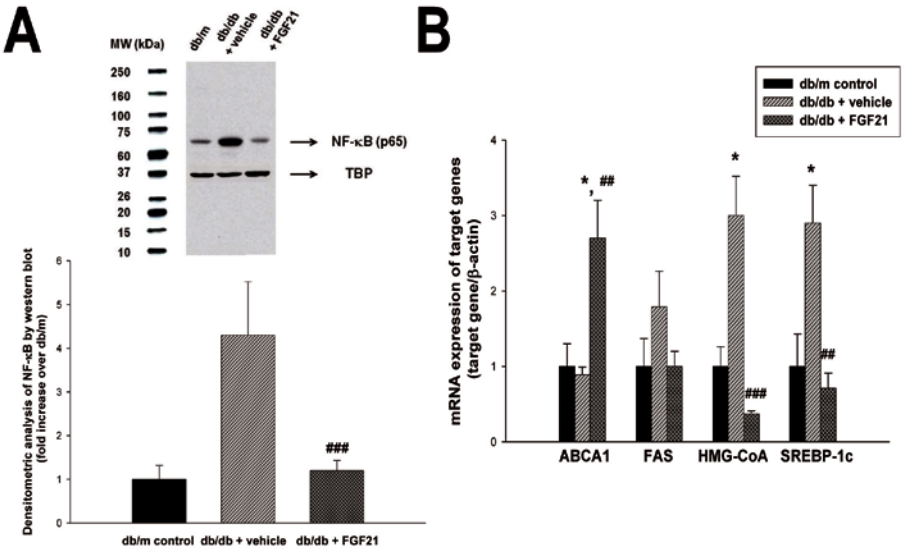

Figure 7. Effects of FGF21 on NF-κB activation and mRNA expression of genes involved in lipid metabolism in renal cortical tissues. (A) Representative Western blot of NF-κB (p65) and densitometric analysis of Western blot results in renal cortical tissues. (B) mRNA expression of genes related to lipid metabolism. TBP, TATA binding protein; NF-κB, nuclear factor-κB; ABCA1, ATP-binding cassette transporter-1; FAS, fatty acid synthase; HMG-CoA, cholesterol 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase; SREBP-1c, sterol regulatory element-binding protein-1; FGF21, fibroblast growth factor 21. Data are expressed as the mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. *db/m* control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. *db/db* + vehicle.

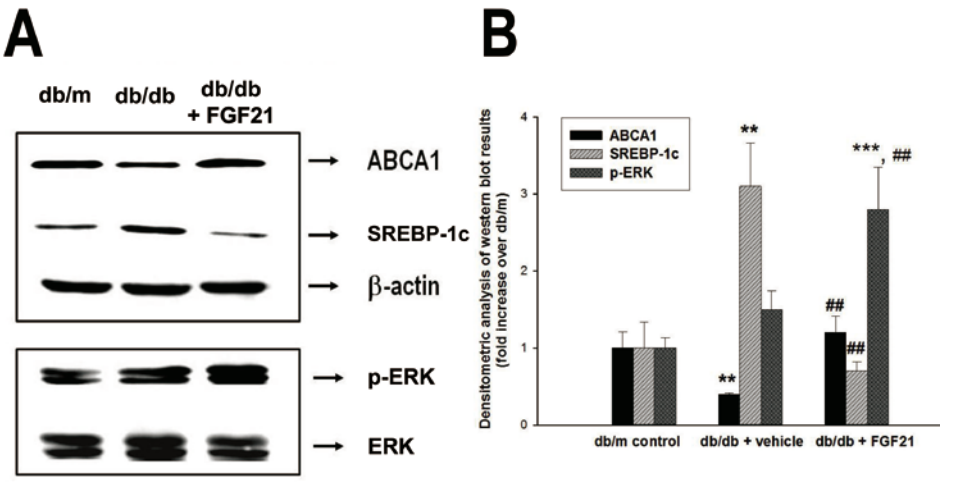

Figure 8. Representative Western blot and densitometric analysis of Western blot results in renal cortical tissues. (A) Representative Western blots for ABCA1, SREBP-1c, and ERK1/2 phosphorylation. (B) Densitometric analysis of Western blot results. ABCA1, ATP-binding cassette transporter-1; SREBP-1c, sterol regulatory element-binding protein-1; ERK 1/2, extracellular signal-regulated kinase 1 and 2; FGF21, fibroblast growth factor 21. Data are expressed as the mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. *db/m* control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. *db/db* + vehicle.

6. Expression of FGF21 in diabetic kidneys and cultured mesangial cells

Since FGF21 has been reported to be expressed in various tissues, I examined whether FGF21 was synthesized by various renal cells. FGF21 expression was investigated in renal cells in the untreated basal state by real-time PCR and Western blot. I tested cultured immortalized podocytes, primary cultured mouse mesangial cells (MMCs), and immortalized human renal proximal tubule cells (HK2 cells) and found that FGF21 was expressed exclusively in mesangial cells and not in podocytes and HK2 cells (Figure 9).

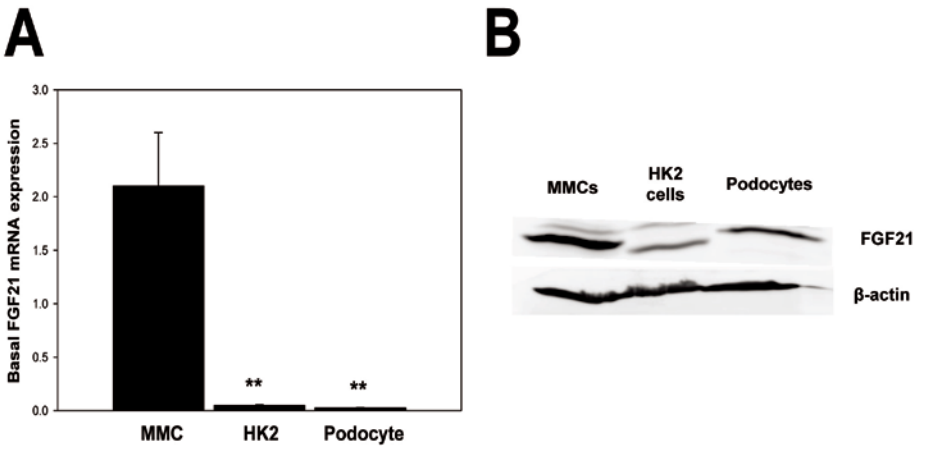

Figure 9. Basal FGF21 expression in various renal cells. (A) mRNA expression of FGF21 in renal cells under basal conditions (B) Representative Western blot for FGF21 in renal cells under basal conditions. MMC, mouse mesangial cell; HK2, human proximal tubule cells; Podocytes, mouse podocytes; FGF21, fibroblast growth factor 21. Data are means ± SEM. $^{**}P < 0.01$ vs. MMC.

Next, I examined the effect of high glucose (30 mM) stimulation on FGF21 synthesis in mesangial cells. As shown in Figure 10, high glucose stimulation did not induce significant change in FGF21 expression. In addition, I performed Western blot analysis from renal lysates to elucidate whether there is altered renal FGF21 signaling in diabetic kidney compared with that in control kidney. As shown in Figure 11, I observed that ERK phosphorylation was decreased in diabetic *db/db* mice compared to *db/m* mice.

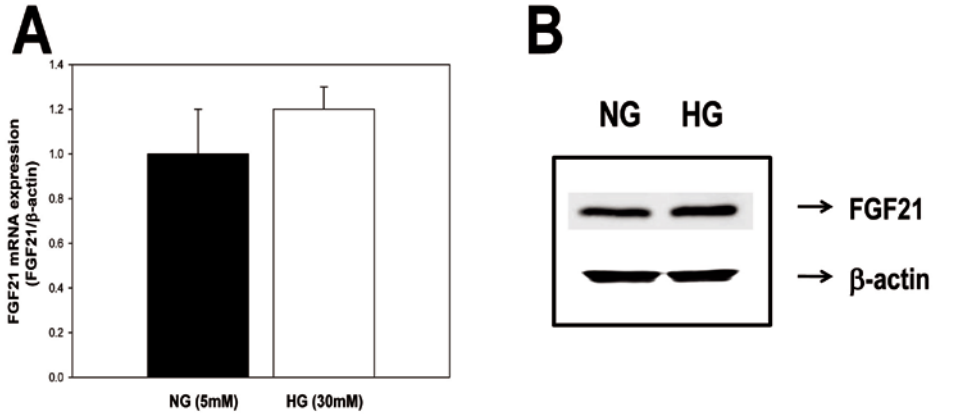

Figure 10. Effects of high glucose (30 mM) stimuli on FGF21 expression in cultured mesangial cells. (A) Effects of HG on FGF21 mRNA expressions. (B) Representative Western blot for FGF21. NG, normal glucose; HG, high glucose; FGF21, fibroblast growth factor 21. Data are expressed as mean ± SEM.

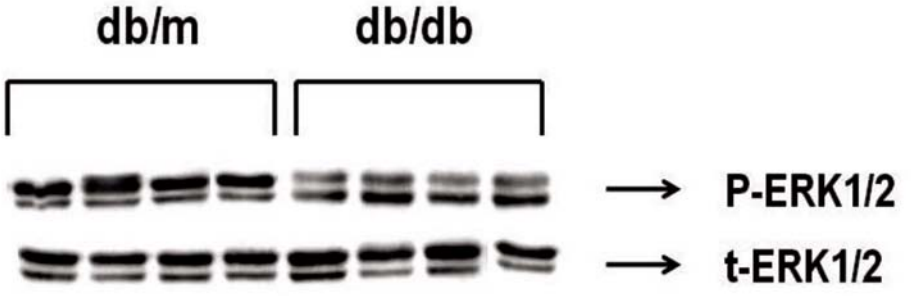

Figure 11. Basal FGF21 signaling as determined by the activation level of ERK1/2 from renal cortical tissues in experimental animals. Representative Western blot for phophospecific-ERK1/2 and total ERK1/2. ERK 1/2, extracellular signal-regulated kinase 1 and 2.

I further evaluated whether FGF21-related components including β-Klotho and FGF21 receptor were changed in the diabetic kidney. As shown in Figure 12, FGF21-related components were significantly upregulated in diabetic *db/db* compared to *db/m* mice. Interestingly, the difference in renal expression of FGF21 was more dramatic at 20 weeks of age (Figure 12B) which suggested that the diabetic kidney was in an FGF21-resistant state. Furthermore, FGF21

treatment significantly suppressed FGF21 components such as β-Klotho, FGFR1c, FGFR2c (Figure 12). Since FGF21 binds to FGF receptors in the presence of co-receptor β-Klotho before leading to activation of ERK1/2, I investigated whether ERK activation occurred in the FGF21-treated diabetic kidney and found that FGF21 treatment significantly activated ERK phosphorylation in the kidney (Figure 8).

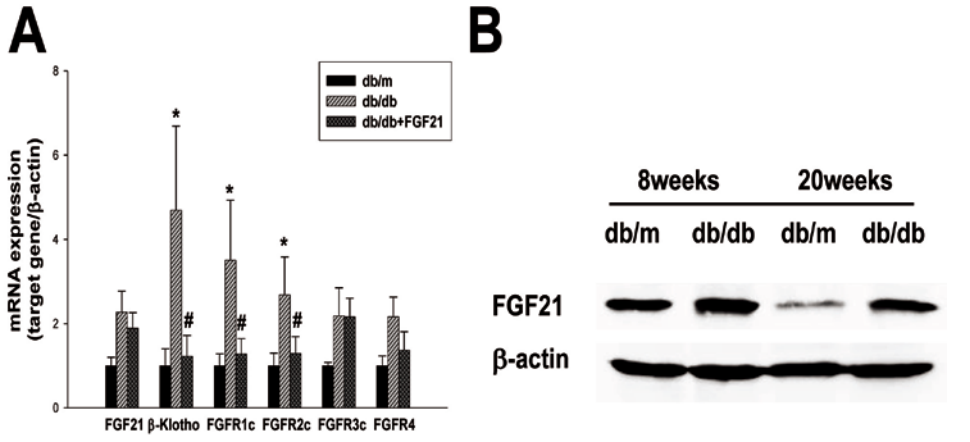

Figure 12. mRNA expression of FGF21 components and representative Western blot for FGF21 in renal cortical tissues. (A) mRNA expression of FGF21, β-Klotho, FGFR1c, FGFR2c, FGFR3c and FGFR4 (B) Representative Western blot for FGF21 at 8 and 20 weeks of age in renal cortical tissues in non-diabetic *db/m* and diabetic *db/db* mice. FGF21, fibroblast growth factor 21; FGFR, fibroblast growth factor 21 receptor. Data are expressed as the mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. *db/m* control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. *db/db* + vehicle.

7. Effects of FGF21 inhibition by FGF21 knockdown on profibrotic molecule synthesis in cultured mesangial cells

Because FGF21 was preferentially activated in mesangial cells, I performed an *in vitro* silencing experiment using mouse mesangial cells. As shown in Figure 13, silencing of FGF21 induced a reduction in FGF21 expression, and further aggravated high-glucose-induced upregulation of the synthesis of profibrotic molecules.

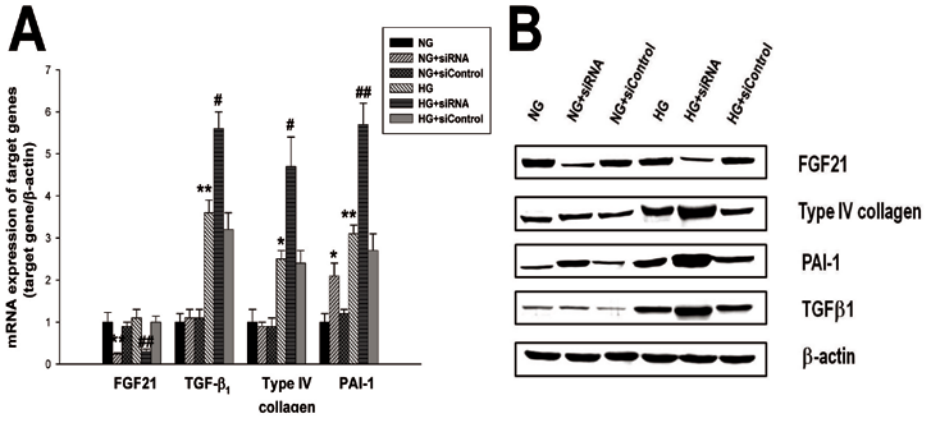

Figure 13. Profibrotic molecule synthesis in response to high-glucose (30 mM) stimulation with or without stealth small interfering (si) RNA of FGF21 in cultured mesangial cells. (A) mRNA expression of profibrotic molecules under high-glucose (30 mM) stimulation with or without gene silencing for FGF21 (B) Representative Western blots for FGF21, TGF-β_1, type IV collagen, PAI-1 with or without transfection with siRNA against FGF21 under high-glucose stimulation. MMCs were treated with 30mM D-glucose medium for 72 h with or without transfection with siRNA for FGF21. NG, normal glucose (5mM); HG, high-glucose (30mM); siControl; Stealth RNAi negative control duplexes; PAI-1, plasminogen activator inhibitor-1; TGF-β_1, transforming growth factor-β_1; FGF21, fibroblast growth factor 21; MMCs, mesangial cells. Data are expressed as the mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. *db/m* control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. *db/db* + vehicle.

IV. DISCUSSION

Since type 2 DM accounts for 90% of all diabetes and is closely related to obesity-related insulin resistance, numerous studies have elaborated on a potential link between obesity and diabetic kidney injury[24-25]. Among the various proposed mechanisms to explain obesity-inducing kidney injury, accumulating evidences support the roles of obesity-related circulating bioactive substances[26]. However, renal protective effects of FGF21 in type 2 diabetic nephropathy have not been fully investigated.

I observed that FGF21 treatment also improved insulin resistance. These results were in agreement with previous reports showing that FGF21 improves obesity-related metabolic alterations in human and experimental animals[8-9]. In this study, I found that FGF21 treatment significantly ameliorated obesity-induced increases in proinflammatory adipocytokines such as PAI-1 and TNF-α in adipose tissue, as well as enhancing anti-inflammatory and antidiabetic molecules such as adiponectin and PPARγ.

In this study I observed that exogenous administration of FGF21 improved dyslipidemia as well as metabolic abnormalities. In addition, FGF21 treatment significantly reduced the obesity-induced increase in fat and liver weight per body weight. This is in agreement with a previous study which showed that FGF21 treatment modulates triglyceride secretion and improvement of hepatosteatosis[8]. More recent research has also demonstrated that FGF21 can augment the action of statin in liver, which ultimately leads to lower blood LDL-cholesterol[27]. On the other hand, the body weight of FGF21-treated mice did not decrease in comparison to vehicle-treated mice. As previously reported, the weight-lowering effect of FGF21 is dependent on the administered dose, which in the literature ranges from 25 μg/kg/day to 8 mg/kg/day, and in this study I used the smallest dose ever attempted for *in vivo* experiments (25 μg/kg/day)[6,8-9]. Therefore, even a low dose of FGF21 appeared to be sufficient

to provide favorable metabolic effects on circulating levels of serum markers, although this dose was not sufficient to produce an expected weight loss.

Hale et al. recently observed that plasma levels of FGF21 are clearly elevated in DIO and *ob/ob* mice, but expression levels of β-Klotho and FGF receptors are markedly downregulated in the white adipose tissues of *ob/ob* and DIO mice[14]. In this study, I observed that all FGF21-related components such as β-Klotho, FGFR1c, FGFR2c were increased in the diabetic kidney compared with normal *db/m* mice, and FGF21 treatment significantly decreased β-Klotho, FGFR1c, FGFR2c components. These results are in contrast with a previous study that found downregulation of β-Klotho and FGFR receptors in liver and fat in DIO mice and *ob/ob* mice[14,25]. However, they did not observe the changes in kidney, and used different animal models.

The above findings such as elevated FGF21 components in the diabetic kidney raised the question of how FGF21 could improve renal function. There is increasing evidence indicating that obesity is an FGF21-resistant state[24-25], and exogenous FGF21 administration can overcome this FGF21 resistance. In this study, I used a dose of 25 μg/kg, and further confirmed that FGF21 treatment activated ERK phosphorylation in the kidney. Taken together, these results suggest that diabetic kidney is also an FGF21-resistant state and FGF21 treatment restores FGF21 sensitivity.

In this study, I observed that renal FGF21 level at 20-week-old *db/m* mice is lower than that at 8-week-old *db/m* mice. The reason for the decreased expression of FGF21 in aged mice is not clear. However, I observed that cholesterol, triglyceride and LPO content in renal cortical tissues was significantly higher in 8-week-old *db/m* mice compared with those in 20-week-old *db/m* mice (Figure 14). There results agree with recent reports that suggest the role of lipotoxicity and oxidative stress in up-regulation of FGF21 synthesis[28-30].

ERK1/2 is one of mitogen activated protein kinases (MAPKs), and ERK

pathway is mainly activated by mitogenic stimuli including growth factors. Although there is a great deal of evidence implicating that activation of ERK1/2 may contribute to the development of human and experimental diabetic nephropathy[31-32], it should be noted that ERK1/2 activation may not always result in tissue injury in the kidney[33]. Furthermore, complex interaction of signaling networks activated in diabetic kidney such as different classes of MAPKs, PI3K/AKT and ERK1/2 pathways. Thus, it is likely that activation of ERK1/2 may induce different protein synthesis and have a different physiological role in disease-specific manner.

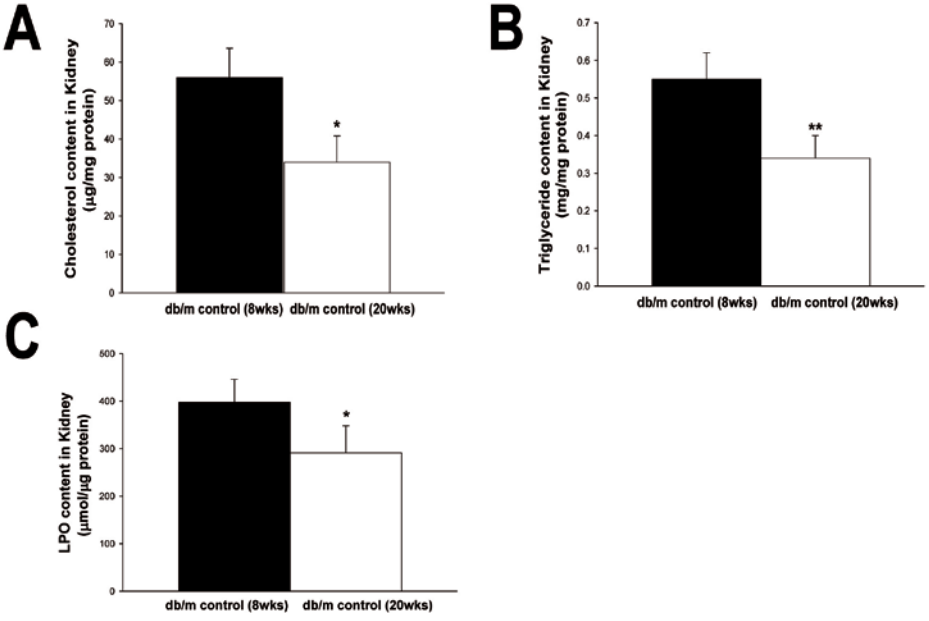

Figure 14. Cholesterol, triglyceride content and lipid peroxidation in renal cortical tissue in control *db/m* mice based on age. (A) Cholesterol content in renal cortical tissue (B) Triglyceride content in renal cortical tissue (C) LPO content in renal cortical tissue. LPO, Lipid hydroperoxides. Data are expressed as the mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. *db/m* control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. *db/db* + vehicle.

Recent studies reported that serum FGF21 concentrations are elevated in patients with renal dysfunction and serum FGF21 concentration correlates with renal function irrespective of the diabetic status[34-35]. The exact mechanism and physiological significance of increased serum FGF21 levels in patients with renal dysfunction are not clear, but there may be several possibilities. First, it may be possible that serum FGF21 concentrations are elevated due to limited clearance of FGF21 in the urine in patients with decreased renal function. Secondly, elevated FGF21 played a causative role in renal injury, but currently there is little evidence to support this hypothesis. Lastly, insulin resistance observed in renal failure leads to compensatory increase in FGF21 levels. However, the relationship between serum FGF-21 concentration and insulin resistance has not been explored in patients with renal disease. Future studies will be needed to determine the physiological significance of increased serum FGF21 levels in patients with renal dysfunction.

In this study, I observed that FGF21 treatment markedly decreased urinary albumin excretion as well as structural changes and oxidative stress in the kidney. Because FGF21 improved systemic insulin resistance and dyslipidemia, the renal protective effects of FGF21 are at least partly responsible for the improvement of systemic metabolic alterations. To further elucidate the direct effects of exogenous FGF21 on renal function, I performed additional *in vitro* experiments. I observed that FGF21 synthesis occurred exclusively in mesangial cells, and silencing of FGF21 in MMC further aggravated synthesis of both high-glucose-induced fibrotic molecules synthesis. Taken together, these results suggest that exogenous FGF21 administration can provide direct renal protective effects in type 2 diabetic nephropathy.

Loeffler et al recently reported TGF-β_1 stimulated mesangial cell proliferation and induced FGF21 expression via activation of MAPK and PI3K/AKT pathways in the absence of type VIII collagen[36]. In addition, FGF21 mediates the shift from TGF-β_1-induced Smad activation to the ERK1/2 and PI3K/AKT

pathway, resulted in mesangial proliferation. It is generally considered that sustained activation of repair mechanisms induces cell cycle arrest and increased extracellular matrix deposition leading to irreversible renal fibrosis in the state of chronic kidney injury. This is further supported by the observation that diabetic p27Kip1 knockout mice have a reduced glomerular matrix expansion and less albuminuria despite an increase in the mesangial cell number compared with diabetic wild-type animals[37]. Collectively, these findings suggest the possibility that increased FGF21 expression may provide renal protective effect against progressive renal fibrosis.

In conclusion, I found that FGF21 treatment provided protective effects against diabetic kidney injuries as well as systemic insulin resistance and obesity-related type 2 diabetes, through both improvement of systemic metabolic alterations and anti-inflammatory mechanisms. These findings suggest that FGF21 treatment could be a novel strategy targeting diabetic nephropathy, in addition to various other diabetic complications.

V. CONCLUSION

1. The purpose of this study was to investigate whether FGF21, which has been known as an important metabolic regulator, has a renoprotective effect and, if it is present, to explore its underlying mechanisms and relationships to changes in insulin resistance.

2. To reveal these associations, I administered recombinant FGF21 to *db/db* mice, which are genetically obese and established models of type 2 diabetes, for 12 weeks, and analyzed various parameters associated with insulin resistance and renal injuries to compare them with lean control *db/m* mice and vehicle-

treated obese control *db/db* mice.

3. After 3 months of experiment, I confirmed that FGF21 treatment led to significantly improved lipid profiles and insulin resistance in obese *db/db* mice. In addition, this treatment also reduced oxidative stress markers. Interestingly, these beneficial effects were not accompanied by a corresponding improvement in blood glucose level. This might be attributed to the dose of administered FGF21 being too low for hypoglycemic effect to be detected, and also suggests that FGF21 might possess anti-diabetic properties independent of glucose lowering. Furthermore, FGF21 treatment ameliorated adipocyte hypertrophy and proinflammatory adipokine secretion in epididymal adipose tissue.

4. Next, I focused on kidney and found that FGF21 treatment improved not only diabetes-induced functional abnormality represented by urinary albumin excretion but also structural abnormality represented by histopathologic changes. These renoprotective effects were found to be mediated by reducing renal lipid contents, oxidative stresses, and proinflammatory signals.

5. Notably, FGF21 and its receptor components in the kidneys were found to be significantly up-regulated in *db/db* mice, whereas ERK phosphorylation, as its downstream signal response, was strongly attenuated. In addition, FGF21 treatment significantly down-regulated FGF21 receptor components, but upregulated ERK phosphorylation. This suggests that kidney in diabetes is under the state of FGF21-resistance, and FGF21 treatment can protect against diabetic renal injuries by overcoming FGF21-resistance.

6. Among cultured renal cells, FGF21 was mainly expressed in mesangial cells and knockdown of FGF21 expression by stealth small interfering RNA further aggravated high-glucose-induced profibrotic cytokine synthesis in mesangial

cells. These findings suggest the possibility that increased FGF21 expression in kidney may provide renal protective effect against progressive renal fibrosis.

7. Collectively, FGF21 improves insulin resistance and protects against renal injury through both improvement of systemic metabolic alterations and anti-fibrotic effects in type 2 diabetic nephropathy. Therefore, targeting FGF21 could provide a potential candidate approach for a therapeutic strategy in type 2 diabetic nephropathy. In this regard, further studies will be needed in the future.

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<국문 요약>

제 2형 당뇨병 모델인 *db/db* 쥐에서 Fibroblast Growth Factor 21의 투여가 인슐린 저항성 및 신손상에 미치는 영향

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김 현 욱

Fibroblast growth factor 21 (FGF21)는 혈청 내에 존재하는 FGF family 중 하나로서 최근의 연구들에 의하면 비만과 관련된 에너지 대사 균형에 있어서 중요한 역할을 하는 것으로 알려져 있으나 신장에 대한 직접적인 영향에 관하여서는 현재까지 연구가 없는 상황이다. 따라서 본 연구에서는 대표적인 제 2형 당뇨병 모델인 8주령 *db/db* 쥐의 복강 내에 유전자 재조합 FGF21을 25 μg/kg의 용량으로 12주간 매일 투여한 후 전신 및 신장에서의 변화를 관찰하였다.

위약을 투여한 대조군 *db/db* 쥐에 비하여 12주간 FGF21을 투여한 *db/db* 쥐에서는 유의하게 혈청 adiponectin 농도가 증가하였으며 이상지질혈증 및 혈청 인슐린 농도, 인슐린 내성시험 및 the homeostatic model assessment 지수로 대변되는 인슐린 저항성이 호전되었고, 혈청 및 소변 내 8-isoprostane의 농도 역시 감소하였다. 뿐만 아니라, 비만과 관련된 제 2형 당뇨병의 특징 중 하나인 부고환 지방조직의 비후 및 염증성 아디포카인의 분비 역시 억제됨을 관찰할 수 있었다. 또한 신장에서의 변화를 분석한 결과, FGF21의 투여는 알부민뇨를 유의하게 감소시켰을 뿐만 아니라 병리조직학적으로는

메산지움의 병적 확장 및 transforming growth factor-β_1, collagen-IV 및 plasminogen activator inhibitor-1 등의 섬유화 인자들의 발현을 유의하게 억제시켰으며 그 기전은 신장 내 지질축적과 산화성 스트레스의 감소 및 대표적인 염증반응 매개인자인 nuclear factor-κB 경로의 차단 등으로 생각된다. 뿐만 아니라 *db/db* 쥐의 신장 조직에서는 FGF21 및 FGF 수용체의 발현이 증가되어 있었고 이들은 FGF21의 외부 투여에 의해서 감소되며 반면에 FGF21의 주요 신호전달 매개인자인 extracellular signal-regulated kinase 1/2는 활성화되는 현상으로 미루어볼 때, 다른 장기들에서와 마찬가지로 비만과 관련된 제 2형 당뇨병에서의 신장은 FGF21 저항성 상태에 있음을 시사한다.

또한 신장 내 세포 중에서는 주로 메산지움 세포에서 FGF21이 발현됨을 관찰하였고 stealth small interfering RNA를 이용하여 메산지움 세포에서 FGF21의 발현을 억제시킨 상태에서 고포도당 자극을 주었을 때 섬유화 인자들의 발현이 유의하게 증가하는 사실을 통하여 신장 내에서, 특히 메산지움 세포에서의 FGF21의 발현은 신보호 효과와 연관 관계가 있음을 알 수 있었다.

결론적으로, FGF21은 비만과 관련된 제 2형 당뇨병에서 전신적으로는 이상지질혈증, 인슐린 저항성 및 지방조직 기능이상을 호전시키고, 신장에서는 항염증 및 항섬유화 효과를 통하여 신보호 효과가 있음을 알 수 있었다. 이에 제 2형 당뇨병성 신증의 새로운 치료 전략으로서 FGF21의 가능성을 확인하였고 이에 대하여 향후 추가적인 심도 있는 연구들이 필요할 것으로 사료된다.

핵심 되는 말: 당뇨병성 신증, fibroblast growth factor 21, 인슐린 저항성