Master of Science

Alterations of serum FGF21 concentration and its receptor expression in subjects with obesity

비만 환자에서의 혈 중 **FGF21** 농도와 수용체의 변화

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Alterations of serum FGF21 concentration and its receptor expression in subjects with obesity

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ABSTRACT

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Fibroblast growth factor (FGF) 21 is a hepatokine that mainly produced by as well as secreted from liver; act as an endocrine factor. Main target tissue of circulating FGF21 is adipose tissues. Adipose tissues express its receptor FGF receptor 1 (FGFR1) and co-receptor β-Klotho. Through these receptors in adipose tissues, FGF21 acts as a potent metabolic regulator of glucose and lipid homeostasis. Nevertheless, serum FGF21 concentrations are increased in obese and type 2 diabetes (T2D) animal models and patients. Besides, serum FGF21 concentrations are positively correlated with body mass index (BMI) and insulin resistance-associated metabolic parameters in obese patients. Thus, researchers hypothesize that obesity is FGF21-resistance state. On the contrary to this hypothesis, exogenous administrate of FGF21 in obesity and T2D animal models shows beneficial effects on glucose and lipid metabolisms including weight loss as well as reduction of blood glucose. Thus, this issue is remained controversial. Moreover, despite the regulations on expression and action of FGF21 have been extensively studied in animals, less is known in humans.

We recruited total 74 women including 27 non-diabetic obese, 15 obese with T2D, and 32 normal-weight control women. Serum FGF21 concentrations were significantly

increased in obese women, regardless of the association with T2D. In obese women, FGFR1 mRNA and β-Klotho mRNA expressions in adipose tissues are not changed in visceral adipose tissues, but those were significantly suppressed in subcutaneous adipose tissues compared with that of normal-weight control women. Serum FGF21 concentrations and β-Klotho mRNA expressions in subcutaneous adipose tissues were significantly correlated with obesity and insulin resistance-associated metabolic parameters as well as endoplasmic reticulum (ER) stress and inflammation in adipose tissues. In obese patients, serum FGF21 concentrations were significantly reduced after Roux-en-Y Gastric Bypass (RYGB) surgery and percent reductions in waist circumference and BMI were positively correlated with pre-operative serum FGF21 concentrations. When differentiated human adipocytes and HepG2 cells (human hepatocyte cell line) were cultured with similar tisseue conditions in obesity (elevated free fatty acids, ER stress and inflammation in liver and adipose tissues), FGF21 mRNA expressions in HepG2 cell were remarkably elevated whereas β-Klotho mRNA expressions in differentiated human adipocytes were dramatically suppressed. Anti-diabetic agents such as pioglitazone and metformin can reduce ER stress-induced FGF21 mRNA expressions in HepG2 cells.

In conclusion, our data show that these obesity-induced alterations of serum FGF21, FGFR1 mRNA, and β-Klotho mRNA expressions in adipose tissues can be caused by the responses of hepatocytes and adipocytes to common pathophysiological conditions associated with obesity (e.g. increased free fatty acids, ER stress, and chronic inflammation). These data also support the hypothesis that obesity is an FGF21 resistant state with compensatory overproduction of FGF21. In addition, serum FGF21 concentrations are reduced after RYGB surgery and pre-operative serum FGF21 concentrations can be an indicator for weight loss after RYGB surgery. Lastly, beneficial effect of pioglitazone and metformin include regulation of ER stress-induced hepatic FGF21 mRNA expressions.

Keywords: FGF21, obesity, RYGB, hepatocytes, adipocytes

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ABBREVIATIONS

BMI	Body Mass Index
BP	Blood pressure
CD68	Cluster of differentiation 68
CHOP	CCAAT/Enhancer-Binding Protein Homologous Protein
CT	Computerized tomography
ELISA	Enzyme-linked immunosorbent assay
ER	Endoplasmic reticulum
FBS	Fatal bovine serum
FGF	Fibroblast growth factor
FGFR	Fibroblast growth factor receptor
GLUT	Glucose transporter
HDL	High density lipoprotein
HFD	High fat diet
HOMA-IR	Homeostasis Model Assessment for Insulin Resistance Index
hsCRP	High-sensitivity C-reactive protein
IBMX	3-isobutyl-1-methylxanthine
IL-1β	Interleukin-1β
LDL	Low density lipoprotein
LPS	Lipopolysaccharide
NAFLD	Nonalcoholic fatty liver disease
OB	Obesity
RYGB	Roux-en-Y gastric bypass
RT-PCR	Reverse transcription polymerase chain reaction
SAT	Subcutaneous adipose tissue

SVF	Stromal vascular fraction
T2D	Type 2 diabetes
TAT	Total adipose tissue
TNFα	Tumor necrosis factor α
TRIB3	Tribbles homolog 3
VAT	Visceral adipose tissue
VSR	The ratio of visceral adipose tissue area to subcutaneous adipose tissue area

Introduction

Epidemics of obesity has been existed since 1980s [1]. Obesity is characterized by increased adiposity, elevated free fatty acids, systemic low grade chronic inflammatory state, and insulin resistance [2]. Moreover, researchers found that endoplasmic reticulum (ER) stress and inflammation are elevated in adipose tissues [3, 4] and liver [5, 6] under obese state. These pathophysiologic conditions are also related with development of other metabolic diseases such as type 2 diabetes (T2D) and nonalcoholic fatty liver disease (NAFLD) [2]. Therefore, searching a good therapeutic agent for obesity is a major issue to many researchers.

Fibroblast growth factor (FGF) 21 is a hepatokine that mainly produced by and secreted from the liver [7]. Basically, FGFs are the growth factors that involved in cell proliferations and differentiations by exerting autocrine or paracrine effects [8, 9]. They share FGF receptors (FGFRs) for their signaling transduction. They are a heparin binding proteins, so they has a heparin binding domains and it help to bind their receptors [9]. However, one of the FGF19 subfamily members, FGF21 has different properties. Because of lack of heparin-binding domain, it can be released to circulation rather than binding to their receptors where they produced. Therefore, FGF21 can acts as an endocrine factor [8]. Main target tissue of circulating FGF21 is adipose tissues. Adipose tissues express its receptor FGFR1 and co-receptor β-Klotho that is essential for binding to FGFR1 and actions of FGF21. In adipose tissues, β-Klotho is mainly expressed in adipocytes than stromal/vascular fraction (SVF) [10]. If β-Klotho is absent, FGF21 signaling cannot be transmitted. In fact, when β-Klotho in the adipocytes is suppressed by siRNA, even though FGFR1 is existed, FGF21 cannot exert their beneficial effects [11]. That is, regulation of β-Klotho in adipose tissues is relatively important for FGF21 signaling in adipose tissues because FGFRs also can be used by other FGFs; β-Klotho is more specific to FGF21 signaling. As binding to these receptors, FGF21 exert beneficial effects on adipose tissues

including induction of glucose transporter 1 (GLUT1), synergistic with effect of insulin [12], and regulation of lipolysis [10, 13]. Like this, FGF21 is a potent metabolic regulator that has beneficial effects on glucose and lipid homeostasis.

However, circulating FGF21 is increased in obese animal models [14] and human with obesity [15]. In addition, serum FGF21 concentrations show positive correlation with body mass index (BMI) and insulin-resistance related metabolic parameters in human with obesity [16]. Therefore, researchers thought that obesity is an FGF21 resistance state [14]. Nevertheless, several studies using obese animal models show that administration of exogenous FGF21 decreases glucose levels, increases insulin sensitivity, and improves adverse lipid profiles [17, 18]. Besides, treatment of FGF21 analog also shows beneficial effects to T2D patients [19]. As these discrepancies, induction mechanisms of circulating FGF21 in obese states are not fully understood until this time. Moreover, the results from the reports that measured serum FGF21 concentrations after RYGB surgery are controversial [20-23]. Thus, many researches on the regulation of FGF21 are done, but most of them are performed by using animal models whereas studies on FGF21 in human are very limitedly preformed.

We thus examined the alterations of serum FGF21 concentrations and its receptor expressions in human with obesity, and searched the factors that trigger these alterations in obese state. We recruited 27 non-diabetic obese, 15 obese with T2D, and 32 normal-weight control women. In these subjects, we measured serum FGF21 concentrations, FGFR1 mRNA, and β-Klotho mRNA expressions in adipose tissues. For finding the factors that associated with these alterations, we performed the correlation analysis of serum FGF21, FGFR1 mRNA, and β-Klotho mRNA expressions in adipose tissues with metabolic parameters and other gene expressions in adipose tissues. In addition, we measured serum FGF21 concentrations and metabolic parameters before and after RYGB surgery. Based on our human studies, we performed the in vitro experiments by using human hepatocyte cell

line and differentiated human adipocytes for confirming these factors indeed cause alterations of FGF21 and it receptors in human models. We also checked the effects of anti-diabetic drugs (pioglitazone and metformin) on ER stress-induced FGF21 mRNA expressions in human hepatocyte cell line as well as FGFR1 mRNA and β-Klotho mRNA expressions in differentiated human adipocytes.

PART1: Elevated serum FGF21 concentrations, and suppressed FGFR1 mRNA and β-Klotho mRNA expressions in adipose tissues in obese patients

We measured serum FGF21 concentrations as well as FGFR1 mRNA and β-Klotho mRNA expressions in adipose tissues from our subjects. After that, we focused on investigating the associations between these and metabolic parameters or other gene expressions in adipose tissues, for finding the factors that associated with these alterations. In addition, we measured serum FGF21 and metabolic parameters before and after RYGB surgery.

Methods

Study subjects

The main study subjects consisted of 27 non-diabetic obese (BMI ≥ 30 kg/m^2), 15 obese with T2D, and 32 normal-weight control women (BMI ≤ 25 kg/m^2). Recruited normal-weight control women were undergone elective abdominal surgery for benign conditions at the Gynecology Unit of the Asan Medical Center (Seoul, Korea). Non-diabetic obese and obese with T2D subjects were undergone laparoscopic Roux-en-Y gastric bypass (RYGB) surgery at the Obesity Center of the Inha University Hospital (Incheon, Korea). Among these patients who received RYGB patients, only 19 patients completed their recording of pre-operative and post-operative metabolic information. Post-operative information was recorded after 7.3 ± 1.5 months. Those 19 subjects consisted of 10 non-diabetic obese and 9 obese with T2D subjects (age = 38.6 ± 11.6 years, pre-operative BMI = 37.5 ± 6.4 kg/m^2, post-operative BMI = 28.5 ± 5.1 kg/m^2).

SVF samples from adipose tissues are limited. Thus, we use another group when measuring FGFR1 mRNA and β-Klotho mRNA expressions in SVF and adipocytes from visceral adipose tissue (VAT). These groups consisted of 11 non-diabetic obese, 9 obese

with T2D, and 14 normal-weight control subjects. Mean age of non-diabetic obese subjects is 36.7 ± 9.4 years and mean BMI is 39.2 ± 3.2 kg/m^2. Mean age of obese with T2D subjects is 45.0 ± 8.9 years and mean BMI is 38.1 ± 10.2 kg/m^2. Mean age of normal-weight control subjects is 43.6 ± 8.9 years and mean BMI is 22.6 ± 1.9 kg/m^2. But this group includes several main study subjects (normal-weight control, n = 8; non-diabetic obesity, n = 8; obesity with T2D, n = 7). Because samples are limited, we cannot measure all the mRNA expressions in these subjects. Numbers of the subjects are described in the figure legends.

Four days before surgery, these patients were admitted and underwent routine physical examinations, systematic fasting biochemical analyses, and abdominal computerized tomography (CT). Women with secondary causes of obesity, those who were pregnant or lactating, and those with evidence of malignancy or severe hepatic or renal disease were excluded. All subjects provided written informed consent at enrollment. The study protocol was approved by the Institutional Review Boards of the Asan Medical Center and the Inha University Hospital. All applicable institutional regulations on the ethical use of human volunteers were followed during this research

Assessment of blood pressure (BP) and anthropometric measurements

BP was measured in the morning. Each patient was seated in a quiet room for 10 minutes. Thereafter, three successive BP measurements using a standard mercury sphygmomanometer were obtained at 5 minutes intervals. The results were averaged. Anthropometric measurements were taken while the subjects were dressed in light clothing but without shoes. Height to the nearest 0.1 cm and weight to the nearest 0.1 kg were measured by using an automatic height-weight scale. BMI was calculated as weight in kilograms divided by the square of height in meters (kg/m^2).

Measurements of metabolic parameters

The subjects discontinued medications for diabetes and hypertension 3 days before blood withdrawal. The blood samples were obtained after a 12 hours fast and plasma and serum were immediately separated by centrifugation. Plasma glucose concentrations were measured by the glucose oxidase method, and insulin concentrations were assessed by using a human insulin radioimmunoassay kit (TFB, Tokyo, Japan). The homeostasis model assessment for insulin resistance index (HOMA-IR) was calculated by using the formula: fasting plasma insulin in μU/mL × fasting plasma glucose in mg/dL ÷ 405, as previously described [24]. Total cholesterol was determined by enzymatic procedures using an autoanalyzer (Hitachi-747; Hitachi, Tokyo, Japan). High sensitivity C-reactive protein (hsCRP) was measured by immunoturbidimetric assay using Roche/Hitachi cobas c702 system.

Estimation of abdominal fat distributions

The distribution of abdominal fat was assessed by CT using a Siemens Somatom Scanner (Erlangen, Germany), as previously described [25, 26]. The subjects were placed in a supine position, and a 10 mm thick cross-sectional scan centered on the L4-L5 vertebral disc space was obtained by using a skeletal radiograph as a reference to establish the position of the scan to the nearest millimeter. The area of total abdominal adipose tissue (TAT) was measured by delineation with a graphic pen, followed by computation of the area using an attenuation range of -190 to 30 Hounsfield units. The VAT area was measured by drawing a line within the muscle wall surrounding the abdominal cavity. The subcutaneous adipose tissue (SAT) area was calculated by subtracting the VAT area from the TAT area. In addition, the ratio of the VAT area to the SAT area (VSR), which is an index of visceral obesity [27, 28], was calculated.

Enzyme-linked immunosorbent assay (ELISA)

Serum adiponectin (AdipoGen, Incheon, Korea), leptin (R&D Systems, Abingdon, Oxfordshire, UK), and FGF21 (R&D Systems) concentrations were measured by using commercial ELISA kits according to the manufacturer's instructions. The assay sensitivity for adiponectin was 100 pg/mL, and the intra- and inter-assay coefficients of variance were 3.0 - 3.8% and 2.8 – 5.5%, respectively. The assay sensitivity for leptin was 7.8 pg/mL, and the intra- and inter-assay coefficients of variance were 3.0 - 3.3% and 3.5 – 5.4%, respectively. The assay sensitivity for FGF21 was 1.61 - 8.69 pg/mL, and the intra- and inter-assay coefficients of variance were 2.9 - 3.9% and 5.2 - 10.9%, respectively.

Calculation of percent reduction in parameters after RYGB surgery

Alterations of parameters after RYGB surgery are represented as percent reduction. Percent reductions are calculated [(pre-operative value – post-operative value)/pre-operative value]*100. Percent reduction is shown as delta (Δ).

Human adipose tissue sampling

During surgery, 2~5 g of VAT and SAT samples were collected. VAT was obtained from the distal portion of the greater omentum (i.e., the epiploon), and SAT was taken from the site of surgical incision (the lower abdomen). The samples were immediately taken to the laboratory in ice-cold 0.9% (w/v) saline, frozen in liquid nitrogen, and stored at -80 ℃ for subsequent analyses.

Fractionation of human adipocytes and SVF

VAT was used for this experiment because it is enough to be divided into two factions. First, we washed the human adipose tissues with α-MEM (gibco, USA) in 100 mm dish. At this time, we removed the connective tissues. Then, tissues were washed one more

time with new α-MEM. After that, we chopped the washed adipose tissues in the new dish that contained α-MEM + 10% collagenase. The chopped tissues were moved to new beaker, and then fill with α-MEM + 10% collagenase (347 U/mg; Sigma, St. Louis, USA) that 1.5 fold volume of the tissue. That beaker was shaken for 1 hour in 37 ℃ of water bath. After 1 hour, we assembled the cell strainer and coke with 50 ml syringe, and then slowly filter the shook tissues into the syringe by using cell strainer and scraper. After that, the syringe filled with α-MEM until 50 ml, and wait for 10 - 15 minutes. Next, we set the filtered syringe above the new 50 ml conical tube and open the coke until 5 ml medium was remained. After refill the syringe until 50 ml with α-MEM, we wait 10 - 15 minutes and then move the medium to the new tube same as before. After that, we refill the syringe with 30 - 40 ml of α-MEM and then fill the 50 ml tube that previously filled with medium using refilled syringe to matching the volume balance. In this step, for isolating adipocytes, after syringe's medium was removed, we collected the yellow layer, adipocytes. For collecting stromal/vascular cells, we centrifuged the 50 ml conical tube that filled with medium for 15 minutes at 250 g. After removed the supernatant, pellet was mixed with the red blood cell lysis buffer [10 mM $KHCO_3$, 1 mM ethylenediaminetetraacetic acid (EDTA), and 154 mM NH_4Cl in 1 L of TDW] and transport to new tube for mixing again. Last, we centrifuged one more time and removed the supernatant. Then, pellet, stromal/vascular cell, was collected; SVF.

RNA isolation and real-time quantitative RT-PCR

For extracting RNA, cell was collected in the e-tube with 1ml trizol (TRIsure, bioline, UK) and followed these steps. We added 200 μl chloroform and mix well. Then mixed samples were centrifuged for 15 minutes at 12000 g (4 ℃). After centrifuge, we took upper clear solution to new tube and added same amount of isopropanol. After isopropanol was added, tube was gently inverted 50 times and waited 20 minutes on ice. 20 minutes later, tube was centrifuged for 15 minutes at 12000 g (4 ℃), and isopropanol was carefully

removed. We inserted 1 ml of 70% ethanol that made by nuclease free water and centrifuged for 15 minutes at 12000 g (4 ℃). Next, we removed an ethanol and dried on room temperature. Then, we added the 20 μl nuclease free water for melting RNA. RNA concentrations were calculated by using NanoDrop spectrophotometer and compared with result of 1% agarose gel electrophoresis of RNA for confirming RNA concentrations. The mRNA was made to cDNA by using iScript^{TM} cDNA synthesis kit (bio-rad, USA) and oligo-dT primers (Bionics, Korea). For the real-time quantitative RT-PCR, lightcycler® 480 SYBER green I master (Roche diagnostics, Germany) was used. The primers are presented in table 1.

Statistical analysis

The data that did not present normal distribution such as fasting glucose, fasting insulin, HOMA-IR, triglyceride, hsCRP, serum leptin, serum adiponectin, cluster of differentiation 68 (CD68) mRNA, CCAAT/Enhancer-Binding Protein Homologous Protein (CHOP) mRNA, tribbles homolog 3 (TRIB3) mRNA, tumor necrosis factor α (TNFα) mRNA , Interlukin-1β (IL-1β) mRNA , FGFR1 mRNA, and β-Klotho mRNA were log-transformed before statistical analysis to achieve normal distribution. A student's paired or unpaired *t*-test was performed as indicated for pairwise group comparisons, and one-way analysis of variance (ANOVA) followed by a Tukey *post hoc* test for significance was used for multiple comparisons. The correlation coefficients between the measures were calculated using Pearson's correlation or Spearman's correlation. The data that present normal distribution were shown as the mean ± standard deviation (SD) and the data that did not present normal distribution were shown as the mean ± errors (SE). For all tests, $p < 0.05$ was regarded as statistically significant results. All analyses were performed by using SPSS (version 20 for IBM; SPSS, Chicago, IL).

Results

1-1. Clinical characteristics of the study subjects

We recruited 27 non-diabetic obese, 15 obese with T2D, and 32 normal-weight women as our study subjects. Clinical characteristics of the study subjects are presented in table 2.

1-2. Metabolic parameters of the study subjects

Metabolic parameters of the study subjects are presented in the table 3. The non-diabetic obesity group (n = 27; age = 31.3 ± 8.5 years, ranged 22 - 53 years; BMI = 39.9 ± 4.7 kg/m^2, ranged 29.8 - 51.4 kg/m^2) was significantly younger, but their BMI was significantly higher than the normal-weight control (n = 32; age = 42.8 ± 6.2 years, ranged 27 - 57 years; BMI = 22.5 ± 1.6 kg/m^2, ranged 18.5 – 24.9 kg/m^2) and obesity with T2D (n = 15; age = 43.9 ± 8.4 years, ranged 32 - 57 years; BMI = 36.2 ± 6.5 kg/m^2, ranged 29.8 - 54.4 kg/m^2) groups. Systolic BP (SBP), diastolic BP (DBP), fasting insulin, HOMA-IR, triglyceride, hsCRP, serum leptin, TAT area, VAT area, and SAT area were significantly elevated in non-diabetic obese and obese with T2D subjects compared with the normal-weight control subjects. Fasting glucose was significantly elevated in the obese with T2D subjects compared with normal-weight control and non-diabetic obese subjects. Total cholesterol was significantly elevated in obese with T2D subjects compared with normal-weight control subjects. High density lipoprotein-cholesterol (HDL-cholesterol) was significantly reduced obese with T2D subjects in comparison to the normal-weight control and non-diabetic obese subjects. Serum adiponectin was significantly lower in obese subjects than normal-weight control subjects.

1-3. Serum FGF21 concentrations of the study subjects

At first, we measured the serum FGF21 concentrations in total 74 subjects (Figure 1). As previously reported [15], serum FGF21 concentrations were significantly elevated in non-diabetic obese (180.7 ± 39.9 pg/mL, ranged 126.2 – 268.5 pg/mL) and obese with T2D subjects (201.8 ± 30.2 pg/mL, ranged 160.5 – 274.8 pg/mL) compared with the normal-weight control subjects (132.8 ± 19.7 pg/mL, ranged 92.7 – 168.5 pg/mL). But serum FGF21 concentrations between non-diabetic obese and obese with T2D subjects were not statistically different ($p = 0.088$).

1-4. The mRNA expressions of FGFR1 and β-Klotho in adipose tissues of the study subjects

Next, we examined the mRNA expressions of FGFR1 and β-Klotho in VAT and SAT. FGFR1 mRNA and β-Klotho mRNA expressions in VAT showed no difference between three groups whereas FGFR1 mRNA and β-Klotho mRNA expressions in SAT showed significant reduction in non-diabetic obese and obese with T2D subjects, compared with normal-weight control subjects (Figure 2). Moreover, obesity-induced alterations of β-Klotho mRNA expressions in SAT showed more dramatic reduction compared with that of FGFR1.

1-5. The mRNA expressions of FGFR1 and β-Klotho in SVF and adipocytes from VAT

FGFR1 mRNA and β-Klotho mRNA expressions in VAT were not significantly changed by obesity. Therefore, we estimated the alterations of FGFR1 mRNA and β-Klotho mRNA expressions by fractionating VAT into SVF and adipocytes. Because of samples were limited, different group of subjects were employed this experiment. These groups information is presented in the method section. FGFR1 mRNA expressions in SVF and adipocytes were similar (Figure 3A), and not changed by obesity (data not shown). β-Klotho

mRNA expressions were predominant in adipocytes, confirming adipocytes are main target cells of the circulating FGF21, in the adipose tissues (Figure 3A). However, β-Klotho mRNA expressions in adipocytes also showed no difference between three groups (Figure 3B)

1-6. Correlations of serum FGF21 concentrations with metabolic parameters and abdominal fat distributions

For investigating the potential factors associated with alterations of the serum FGF21 concentrations, we checked the correlations of serum FGF21 concentrations with metabolic parameters (Table 4). As previously reported, serum FGF21 concentrations were significantly correlated with most of the obesity and insulin resistance-associated metabolic parameters we examined. BMI, SBP, DBP, fasting glucose, fasting insulin, HOMA-IR, total cholesterol, LDL-cholesterol, triglyceride, hsCRP, serum leptin, TAT area, and VAT area showed significant positive correlations with serum FGF21 concentrations. In case of HDL-cholesterol and serum adiponectin, these displayed significant negative correlations with serum FGF21 concentrations.

1-7. Correlations of serum FGF21 concentrations with gene expressions in adipose tissues

We could not detect FGF21 mRNA expressions in human adipose tissues (data not shown). However, serum FGF21 concentrations showed significant correlation with obesity and insulin resistance-associated metabolic parameters. In addition, these metabolic parameters were closely associated with ER-stress and inflammation in adipose tissues. Thus, we investigated whether ER stress and inflammation-associated gene expressions in adipose tissues are correlated with serum FGF21 concentrations (Table 5).

The well-known ER stress marker, CHOP mRNA expressions in both VAT and SAT were

positively correlated with serum FGF21 concentrations. ER stress induced TRIB3 mRNA expressions in adipocytes [29]. This ER stress-inducible gene, TRIB3 mRNA expressions in both VAT and SAT were also positively correlated with serum FGF21 concentrations. TNFα and IL-1β is inflammatory cytokine and CD68 is macrophage marker. They were closely associated with inflammation. TNFα mRNA and IL-1β mRNA expressions in VAT as well as CD68 mRNA expressions in both VAT and SAT were positively correlated with serum FGF21 concentrations.

1-8. Correlations of FGFR1 and β-Klotho mRNA expressions in adipose tissues with metabolic parameters and abdominal fat distributions

Next, for finding which factors are associated with alterations of FGFR1 mRNA and β-Klotho mRNA expressions in adipose tissues, we checked the correlations of FGFR1 mRNA and β-Klotho mRNA expressions in adipose tissues with metabolic parameters and abdominal fat distributions (Table 6). At first, FGFR1 mRNA and β-Klotho mRNA expressions in VAT were nearly not correlated with metabolic parameters and abdominal fat distributions compared with that of SAT. Besides, β-Klotho mRNA expressions in SAT showed more significance with metabolic parameters and abdominal fat distributions than FGFR1 mRNA expressions in SAT. In SAT, FGFR1 mRNA expressions were negatively correlated with fasting glucose (r = -0.316, p = 0.006), fasting insulin (r = -0.293, p = 0.011), and HOMA-IR (r = -0.348, p = 0.002). β-Klotho mRNA expressions in SAT are negatively correlated with BMI (r = -0.349, p = 0.002), SBP (r = -0.302, p = 0.002), DBP (r = -0.352, p = 0.002), fasting glucose (-0.459, $p < 0.001$), fasting insulin (r = -0.409, $p < 0.001$), HOMA-IR (r = -0.491, $p < 0.001$), total cholesterol (r = -0.257, p = 0.027), triglyceride (r = -0.361, p = 0.002), serum leptin (r = -0.353, p = 0.002) and VAT area (r = -0.331, p = 0.016) whereas positively correlated with serum adiponectin (r = 0.441, $p < 0.001$).

1-9. Correlations of FGFR1 and β-Klotho mRNA expressions in adipose tissues with other gene expressions in adipose tissues

In obese state, ER stress and inflammation in adipose tissues were elevated, and these are closely associated with development of insulin resistance. In addition, insulin resistance-associated metabolic parameters showed significant negative correlation with FGFR1 mRNA and β-Klotho mRNA expressions in SAT. Therefore, we thought that ER stress-associated and inflammation-associated gene expressions in SAT are correlated with FGFR1 mRNA and β-Klotho mRNA expressions in SAT. As we suspected, β-Klotho mRNA expressions in SAT were negatively correlated with CHOP mRNA and TRIB3 mRNA expressions as well as IL-1β mRNA expressions in SAT (Table 7). However, FGFR1 mRNA expressions in SAT and FGFR1 mRNA and β-Klotho mRNA expressions in VAT are not correlated with these gene expressions (Table 7).

1-10. Alterations of serum FGF21 concentrations and correlations with metabolic parameters after RYGB surgery

We checked the alterations of serum FGF21 concentrations after RYGB surgery in our subjects. In addition, beneficial effects of RYGB surgery are accompanied by significant weight loss. In our study, BMI shows marked correlation with serum FGF21 concentrations. Thus, we wonder whether beneficial effects of RYGB surgery are associated with serum FGF21 concentrations. We performed the correlation analysis between serum FGF21 concentrations and alteration of metabolic parameters after RYGB surgery. Alterations of the metabolic parameters after RYGB surgery are presented as Δ; the percent reduction of the metabolic parameters after RYGB surgery.

First of all, most of the parameters that we measured were significantly restored after RYGB surgery. BMI, waist circumference, SBP, fasting glucose, fasting insulin, HOMA-IR, total cholesterol, LDL-cholesterol, triglyceride, hsCRP, and serum Leptin were

significantly reduced whereas HDL-cholesterol and serum adiponectin were significantly elevated after RYGB surgery (Table 8). In our non-diabetic obese and obese with T2D subjects, Serum FGF21 concentrations are significantly reduced after RYGB surgery (pre-operative serum FGF21 concentrations = 190.7 ± 32.1 pg/mL, post-operative serum FGF21 concentrations = 143.9 ± 58.4 pg/mL) (Figure 4A). Besides, Δ waist circumference shows significant positive correlation with pre-operative serum FGF21 concentration (Figure 4B). In addition, Δ BMI shows positive correlation with marginal *p*-value ($p = 0.056$) (Figure 4C)

Discussion

Differently from the previous report [15], serum FGF21 concentrations of our obese with T2D subjects were not different with that of non-diabetic obese subjects. By the way, when compared with subjects of previous reports [15], our obese with T2D subjects showed almost 2-fold higher level of fasting glucose than their obese with T2D subjectst. Thus, we wonder whether glucose can change serum FGF21 concentrations by modulating FGF21 mRNA expressions in hepatocytes. Because we don't have primary human hepatocytes, we performed this experiment by using human hepatocyte cell line (HepG2 cells), and that is described in the next section.

β-Klotho mRNA expressions of several non-diabetic obese and obese with T2D subjects were undetectable (VAT of non-diabetic obese subjects = 3, SAT of non-diabetic obese subjects = 4, SAT of obese with T2D subjects = 5). But, we could not see undetectable β-Klotho mRNA expressions in adipose tissues from normal-weight control subjects. Therefore, we thought that these suppressions of β-Klotho mRNA expressions in adipose tissues represent so called FGF21-resistance state in adipose tissues. If so, these subjects have more severe FGF21 resistance state in adipose tissues. Thus, we wanted to find the pathophysiological difference between these β-Klotho undetectable and detectable obese subjects. However, we cannot find the reason why β-Klotho mRNA expressions are undetectable in these obese subjects.

Our results showed that serum FGF21 concentrations are elevated whereas FGFR1 mRNA and β-Klotho mRNA expressions in SAT are suppressed in obese subjects. The previous study [15] also described the alterations of FGF21 and its receptors in obese subjects with/without T2D. However, their data on FGFRs mRNA expressions in adipose tissues are not enough to explain about alterations of FGFRs mRNA expressions in adipose tissues from obese subjects, because their data are based on the low subjects numbers (almost groups are lower than 10). In that study, FGFR1 mRNA expressions in SAT and VAT are not

significantly changed by obesity but only β-Klotho mRNA expressions in VAT are significantly suppressed by obesity. However, in our study, both FGFR1 mRNA and β-Klotho mRNA expressions were significantly decreased but these alterations were restricted in SAT. In addition, β-Klotho mRNA expressions in SAT of control subjects were significantly much higher than that of VAT. This discrepancy might be occurred by difference of study subjects such as difference of race. But we thought our results indicate some other interesting points. FGF21 is an important regulator of lipid homeostasis and SAT is a main tissue where lipids are stored. If accumulations of lipids in SAT are over their capacity, these lipids are accumulated at other organs such as VAT, liver, and muscle. These lipid accumulations cause various adverse effects. Therefore, lipid homeostasis in SAT must be regulated, appropriately. In this context, if beneficial effects of FGF21 on lipid homeostasis are mainly exerted by regulating lipid metabolism in SAT, the higher β-Klotho mRNA expressions in SAT of control subjects are reasonable. Moreover, because hormone resistance are easy to occur in their target tissues (e.g. insulin resistance in adipose tissues and muscles), if SAT is a main target tissue of FGF21, the reason why only β-Klotho mRNA expressions in SAT (not in VAT) are more sensitively responded to obesity are also understood. Thus, we suspect that SAT is a main target tissue of circulating FGF21 for regulating lipid homeostasis.

As previously reported [15], serum FGF21 concentrations of our subjects also showed significant correlation with obesity-associated (e. g. BMI), insulin resistance-associated metabolic parameters, and circulating lipids. Because of FGF21 are mainly produced by liver, these results suggest that hepatic FGF21 mRNA expressions is associated with these parameters. In addition, ER stress-associated and inflammation-associated gene expressions in adipose tissues (CHOP, TRIB3, TNFα, IL-1β, and CD68) showed significant correlation with serum FGF21 concentrations. ER stress and inflammation in adipose tissues are elevated in obese state, and these adipose tissues secret various factors such as inflammatory

cytokines and free fatty acids. Taken together, these data imply the possibility that obese adipose tissues that suffer from ER stress and inflammation secret inflammatory cytokines and free fatty acids to circulation, and these factors may affect to hepatic FGF21 mRNA expressions. Therefore, we check the effects of free fatty acids and inflammation on the hepatic FGF21 mRNA expressions. In addition, ER stress is elevated in liver under obese state [5]. Thus, we also treated the ER stressors in hepatocytes, and observed the alterations of hepatic FGF21 mRNA expressions. These results are described in the next section.

In correlation analysis, β-Klotho mRNA expressions in SAT showed significant correlation with obesity-associated and insulin resistance-associated metabolic parameters. In addition, ER stress-associated and inflammation-associated gene expressions in adipose tissues were significantly correlated with β-Klotho mRNA expressions in SAT. These patterns were not observed in VAT, but show similar pattern with that of serum FGF21. It indicates that alteration of hepatic FGF21 mRNA expressions and β-Klotho mRNA expressions in adipose tissues are affected by same factors. Therefore, we cultured differentiated human adipocytes with the same factors that was treated in HepG2 cells, then measured the alteration of FGFR1 mRNA and β-Klotho mRNA expressions (described in the next section).

However, even though FGFR1 mRNA expressions in SAT are also suppressed by obesity, FGFR1 mRNA expressions in SAT could not show significant correlation with others as much as that of β-Klotho mRNA expressions in SAT. FGFR1 is used by other FGFs but, β-Klotho is more relatively specific to FGF21 signaling than FGFR1. Therefore, regulatory mechanisms of FGFR1 in adipose tissues can be different with that of β-Klotho.

Alterations of serum FGF21 concentrations after RYGB surgery are still controversial. Some reports indicate that serum FGF21 concentrations are increased after RYGB surgery [22, 23]. However, in other reports, serum FGF21 concentrations are unchanged or decreased after RYGB surgery [20, 21]. In our non-diabetic obese and obese with T2D

subjects, most of the obesity and insulin resistance-associated metabolic parameters were significantly restored after RYGB surgery and serum FGF21 concentrations were significantly reduced after RYGB surgery. It indicates that serum FGF21 concentrations are very sensitive parameter to obesity-induced metabolic alterations. Our correlation analysis shows that pre-operative serum FGF21 concentrations were positively correlated with Δ BMI and Δ waist circumference. That is, the person who had a higher serum FGF21 concentration lost more weight after RYGB surgery. The prominent effect of RYGB surgery was weight loss and it was accompanied by other beneficial effects. It means pre-operative serum FGF21 concentrations can be used as a predictor for efficiency of RYGB surgery. The suggestion of the U.S. National Institutes of Health helps to determine the person who received bariatric surgery. Our results suggest that the prediction of efficiency of RYGB surgery by pre-operative serum FGF21 concentrations also can help to determine the person who received this operation.

In this section, we checked the obesity-induced alteration of serum FGF21 concentrations as well as FGFR1 mRNA and β-Klotho mRNA expressions in adipose tissues. In obese human, serum FGF21 concentrations were elevated, but FGFR1 mRNA and β-Klotho mRNA expressions in SAT were suppressed. Serum FGF21 concentrations and β-Klotho mRNA expressions in SAT showed close correlations with obesity-associated and insulin resistance-associated metabolic parameters. Besides, Serum FGF21 concentrations and β-Klotho mRNA expressions in SAT were significantly correlated with ER stress-associated and inflammation-associated gene expressions in adipose tissues. Therefore, we thought these factors could affect to hepatic FGF21 mRNA expressions as well as FGFR1 mRNA and β-Klotho mRNA expressions in adipose tissues. Thus, we performed the in vitro experiments by using HepG2 cells and differentiated human adipocytes, and these results are described in the next section.

PART2: Regulation of FGF21, FGFR1, and β-Klotho expressions; in vitro studies

Consistently with previous reports [16], our data showed that serum FGF21 concentrations were elevated in obese subjects. In addition, FGFR1 mRNA and β-Klotho mRNA expressions in SAT showed significant suppression in human with obesity. Serum FGF21 concentrations and β-Klotho mRNA expressions in SAT were significantly correlated with obesity-associated and insulin resistance-associated metabolic parameters as well as ER stress-associated and inflammation-associated gene expressions in adipose tissues. Therefore, we checked whether these various factors that show significant correlation affect to the mRNA expressions of FGF21, FGFR1, and β-Klotho. In addition, effect of anti-diabetic drugs on ER stress-induced FGF21 mRNA, FGFR1 mRNA, and β-Klotho mRNA expressions were evaluated.

Methods

Cell line culture

HepG2 cells were used for studying hepatocytes. 3 x 10^5/well HepG2 cells were grown in 6-well plate with DMEM (gibco) + 10% fatal bovine serum (FBS) (biowest, USA) + 1% Antibiotic-Antimycotic (gibco). Before cell was treated some chemicals or drugs, cells were washed with serum free DMEM and filled with 1ml serum free DMEM. Then cells were treated some chemicals or drugs.

Thapsigargin, tunicamycin, homocysteine, insulin, vehicle for free fatty acids, lipid mixture, palmitate, oleate, pioglitazone, and metformin were purchased from sigma and treated to cells as indicated in each figure legends. TNFα (Biosource, USA), IL-1β (R&D Systems), and lipopolysaccharide (LPS) (sigma) were used to inducing inflammation. For normal glucose experiment, HyClone™ Dulbecco's low glucose Modified Eagles Medium (GE healthcare, Life Sciences, UK) was used because that containing similar glucose

concentration of normal physiological condition, 5.5 mM glucose. For high glucose experiment, cell was incubated with DMEM contain high glucose (25 mM) (gibco).

Isolation and differentiation of human preadipocytes

Discarded subcutaneous tissues were obtained from the transverse rectus abdominis musculocutaneous flap of three separately recruited women (BMI = 21.3 ± 0.5 kg/m^2; age = 44 ± 11.1 years) undergoing breast reconstruction surgery. All protocols were approved by the Ethical Committees of Asan Institute for Life Sciences (Seoul, Korea) and the subjects provided written informed consent. For isolation of human preadipocytes, stromal/vascular cells (these were collected as described in the PART1) were suspended in DMEM supplemented with 10% FBS and cultured until passage 2 to eliminate the cell contamination by non-preadipocytes. The isolated preadipocytes were cultured on 6-well culture dishes. After attaining confluence, the cells were differentiated into adipocytes by culture for 3 day in the medium containing 1 μg/ml insulin, 1 μM dexamethasone (sigma), 0.5 mM 3-isobutyl-1-methylxanthine (IBMX, sigma), 33 μM biotin (sigma), 17 μM pantothenate (sigma), and 10 μM rosiglitazone (sigma). After that, cells were culture for additional 9 - 11 days in the same medium, but without IBMX. Treated chemicals or drugs for in vitro experiments are same as that of treated in hepatocyte cell lines.

RNA isolation and real-time quantitative RT-PCR

This method was described in the PART1, and the primers are presented in table 1.

Statistical analysis

A Student's paired or unpaired *t*-test was performed as indicated for pairwise group comparisons, and one-way ANOVA followed by a Tukey *post hoc* test for significance was used for multiple comparisons. The correlation coefficients between the measures were

calculated using Pearson's correlation. r = Pearson correlation coefficient. For all tests, $p < 0.05$ was regarded as statistically significant information. All analyses were performed by using SPSS (version 20 for IBM; SPSS, Chicago, IL).

Results

2-1. Alterations of FGF21 mRNA expressions in HepG2 cells by various stressors

Circulating lipids as well as ER stress [5] and inflammation [6] in liver are elevated under obese state and these factors are closely related with obesity and insulin resistance. In our results, obesity-associated and insulin resistance-associated metabolic parameters were significantly correlated with serum FGF21 concentrations. Therefore, we checked the effects of these factors on FGF21 mRNA expressions in human hepatocyte cell line, HepG2 cells. For this experiment, we used various stressors including ER stressors (thapsigargin, tunicamycin, and homocysteine [30-32]), free fatty acids (lipid mixture, palmitate, and oleate), inflammatory cytokines (TNFα and IL-1β), and LPS. ER stressors (thapsigargin, tunicamycin), free fatty acids (lipid mixture, palmitate, and oleate), and inflammatory response inducers (TNFα, IL-1β, and LPS) significantly elevate FGF21 mRNA expressions in HepG2 cells (Figure 5). Among them, effects of ER stressors and palmitate were prominent.

2-2. Effects of various stressors on FGFR1 mRNA and β-Klotho mRNA expressions in differentiated human adipocytes

In our subjects with obesity, circulating lipids were increased compared with normal-weight control subjects. Not only that, in obese animal models, ER stress and inflammation in adipose tissues are elevated [3, 4]. According to our correlation analysis, these factors were significantly correlated with β-Klotho mRNA expressions in SAT (Table 7). Therefore, we checked the effects of these factors on the FGFR1 mRNA and β-Klotho mRNA expressions in differentiated human adipocytes. In addition, obese adipose tissues are thought as hypoxic state. Thus, we also checked the effect of chemical hypoxia inducer, cobalt chloride ($CoCl_2$). FGFR1 mRNA expressions in differentiated human adipocytes were

suppressed by thapsigargin, tunicamycin, and palmitate whereas elevated by TNFα and IL-1β (Figure 6A). However, β-Klotho mRNA expressions in differentiated human adipocytes were markedly suppressed by these all stressors (Figure 6B).

2-3. Effects of insulin and glucose on hepatic FGF21mRNA expressions

Fasting insulin level was positively correlated with serum FGF21 concentrations in our subjects. Thus, we checked the effect of insulin on the FGF21 mRNA expressions in HepG2 cells. As observed in rat hepatocytes [33], insulin induces FGF21 mRNA expressions in HepG2 cells (Figure 7A).

In rat hepatocytes, high glucose induced FGF21 mRNA expressions [34, 35]. In addition, our obese with T2D subjects showed higher glucose levels than normal-weight control and non-diabetic obese subjects. However, different with previous report [15], serum FGF21 concentrations of our obese with T2D subjects were not different with non-diabetic obese subjects. When compared with subjects of previous report [15], our obese with T2D subjects show almost 2-fold higher level of glucose. Therefore, we speculated high glucose might suppress hepatic FGF21 mRNA expressions in human. In comparison to normal glucose (similar with normal physiological glucose level, 5.5 mM), high glucose (25 mM) markedly suppressed FGF21 mRNA expressions in HepG2 cells (Figure 7B). These data suggest that effects of glucose on hepatic FGF21 mRNA expressions in human may be different with that of observed in rat.

2-4. Effects of anti-diabetic drugs on the ER stress-induced FGF21 mRNA expressions in HepG2 cells

One of the anti-diabetic drugs, rosiglitazone can restore ER stressor-induced suppression of β-Klotho mRNA expressions in 3T3-L1 adipocytes [36]. It suggests that anti-diabetic drugs may improve β-Klotho mRNA expressions in adipose tissues under obese

state. However, the effects of anti-diabetic drugs on ER stress-induced FGF21 mRNA expressions in hepatocytes and ER stress-induced reduction of FGFR1 mRNA and β-Klotho mRNA expression in human adipocytes are uncertain. Therefore, we checked the effects of anti-diabetic drugs on ER stress-induced FGF21 mRNA expressions in HepG2 cells as well as FGFR1 mRNA and β-Klotho mRNA expressions in differentiated human adipocytes. Pioglitazone and metformin induceed FGF21 mRNA expressions, but significantly suppressed ER stress-induced FGF21 mRNA expressions in HepG2 cells (Figure 8A). Pioglitazone and metformin did not affect to FGFR1 mRNA and β-Klotho mRNA expression in differentiated human adipocytes (Figure 8B, C). Thapsigargin-induced suppressions of FGFR1 mRNA expressions in differentiated human adipocytes were significantly elevated by pioglitazone (Figure 8B). Tunicamycin-induced suppressions of β-Klotho mRNA expressions in differentiated human adipocytes were significantly elevated by metformin (Figure 8C).

Discussion

Most of the stressors used in these experiments induced FGF21 mRNA expressions in HepG2 cells whereas markedly suppressed β-Klotho mRNA expressions in differentiated human adipocytes. Serum FGF21 concentrations were significantly elevated and β-Klotho mRNA expressions in SAT were dramatically suppressed in obese subjects. In addition, circulating lipids as well as ER stress-associated and inflammation-associated gene expressions in adipose tissues were closely related with serum FGF21 and β-Klotho mRNA expressions in SAT. These results indicate that common pathophysiological conditions that accompanied by obesity (e.g. ER stress, elevated free fatty acids, inflammation) may elevate serum FGF21 concentrations by inducing hepatic FGF21 mRNA expressions, and suppress β-Klotho mRNA expressions in adipose tissues by reducing β-Klotho mRNA expressions in adipocytes.

ATF4 is thought as a main factor that triggers ER stress-induced FGF21 mRNA expressions. Moreover, thapsigargin, tunicamycin, and homocysteine are known as an inducer of ATF4. However, homocysteine cannot induce FGF21 mRNA expressions in HepG2 cells. It suggests that there is another factor that associated with ATF4-mediated FGF21 mRNA expressions in hepatocytes. It requires further investigation.

Indeed, regulation of FGFR1 mRNA expression showed the difference from that of β-Klotho in differentiated human adipocytes. FGFR1 can be used by other FGFs. Because of this reason, regulatory mechanisms of FGFR1 and β-Klotho can be different. In addition, our data indicate that β-Klotho mRNA expressions in adipose tissues are damaged severely by various stresses that elevated in obese state (e.g. ER stress, elevated free fatty acids, and inflammation). However, β-Klotho mRNA expressions in VAT were not changed by obesity. In fact, ER stress-associated and inflammation-associated gene expressions were also elevated in VAT (data not shown). But, β-Klotho mRNA expressions in VAT were unchanged in obese subjects comparison to normal weight control subjects. Previously, we mentioned

about the possibility that SAT is a main target tissue of circulating FGF21. If this assumption is right, SAT has some difference with VAT. Therefore, we think this difference made different regulation of β-Klotho mRNA expressions in VAT and SAT. Even though our data are not enough to proof this assumption, it is worth to be more identified.

Our data showed high glucose suppressed FGF21 mRNA expressions in HepG2 cells. It suggests that high glucose level may suppress serum FGF21 concentrations. Thus, we speculated that serum FGF21 concentrations of our obese with T2D subjects were suppressed by high glucose concentrations, so their serum FGF21 concentrations did not show difference with that of non-diabetic obese subjects. In addition, we found effects of glucose on hepatic FGF21 mRNA expressions could be different between human and animal model. Therefore, even though some mechanisms are already identified in animal model, it needs to be validated in human.

Pioglitazone and metformin significantly suppressed ER stress-induced hepatic FGF21 mRNA expressions. However, the effects of these anti-diabetic drugs on FGFR1 mRNA and β-Klotho mRNA expressions in differentiated human adipocytes were not effective. Nevertheless, thapsigargin-induced suppressions of FGFR1 mRNA expressions were significantly elevated by pioglitazone and tunicamycin-induced suppressions of β-Klotho mRNA expressions were significantly increased by metformin. These results indicate that regulations of hepatic FGF21 mRNA expressions are a one of the beneficial effects of pioglitazone and metformin. In addition, it also suggests a possibility that these anti-diabetic drugs partly affect to ER stress-induced suppressions of FGFR1 mRNA and β-Klotho mRNA expressions in human adipocytes.

In summary, together with our human data, our in vitro study shows that ER stress, free fatty acids, and inflammation are potent factors that trigger alterations of FGF21 mRNA expressions in human hepatocytes as well as FGFR1 mRNA and β-Klotho mRNA expressions in differentiated human adipocytes. But not all ER stressor can induce hepatic

FGF21 mRNA expressions. In addition, similarly to the correlation analysis, insulin and glucose changed FGF21 mRNA expressions in HepG2 cells. Taken together, these results show that common pathophysiological conditions in obesity can trigger alterations of serum FGF21 concentrations by elevating hepatic FGF21 mRNA expressions as well as suppress β-Klotho mRNA expressions in adipose tissues by reducing β-Klotho mRNA expressions in adipocytes. In addition, we showed one of the effects of anti-diabetic drugs are regulation of ER stress-induced hepatic FGF21 mRNA expressions. Moreover, regulation of FGF21 in human can be different with that observed in animal models. Therefore, more intensive human studies about FGF21 are needed to confirm that established in animal models.

Conclusion

In obese human, serum FGF21 was significantly increased whereas FGFR1 mRNA and β-Klotho mRNA expressions in adipose tissues were significantly suppressed. Our data show that these can be caused by the responses of hepatocytes and adipocytes to common pathophysiological conditions associated with obesity (e.g. increased free fatty acids, ER stress, and chronic inflammation). However, these data also support the hypothesis that obesity is an FGF21-resistance state with compensatory overproduction of FGF21.

Results from obese animal models and T2D patients support the therapeutic potential of FGF21 [17-19]. In addition, several researchers already try to develop FGF21 as therapeutic agents. Nevertheless, there is a reason why still studies about regulatory mechanisms of FGF21 and its receptors are important.

In obese animal models, excessive FGF21 administration exerts various beneficial effects including weight loss, improve glucose tolerance [37]. However, in these improved metabolic conditions, endogenous hepatic FGF21 mRNA expressions are decreased and FGFRs mRNA as well as β-Klotho mRNA expressions in adipose tissues are still suppressed. Endogenous FGF21 signaling is important to maintaining glucose and lipid homeostasis including regulation of blood glucose and body weight [14]. Taken together, these results indicate that exogenous FGF21 administration cannot be a fundamental therapy for obesity because endogenous FGF21 signaling is not restored. Therefore, together with exogenous FGF21 administration, therapeutic strategies that regulate endogenous FGF21 and its receptors in adipose tissues are needed. Thus, human studies about regulation of FGF21 and its receptors in adipose tissues are useful, and our studies present informative results for that.

FGF21 is rapidly converted to bio-inactive form in circulation by losing their C-terminus that is important to binding with β-Klotho [38]. It means that considerable part of increased circulating FGF21 in obese human may contain considerable parts of inactive form of FGF21 and elevated circulating FGF21 in obese state may not important to FGF21

signaling in adipose tissues. Moreover, increased bio-inactive form of FGF21 may trigger competitions with active form of FGF21 against suppressed FGFR1 and β-Klotho. In this situation, receptor specificity of bioactive form of FGF21 may more important to FGF21 signaling in adipose tissues. FGF21 cannot directly bind to FGFR1, and β-Klotho is needed for this binding [38]. Therefore, appropriate ratio of bioactive form of FGF21 as well as β-Klotho may important to exerting beneficial effects of endogenous FGF21. Taken together, for that appropriate regulation of these, human studies about regulatory mechanisms of FGF21 and it receptors are needed to be preceded. In these contexts, our studies indicate RYGB surgery and anti-diabetic drugs can use for this regulation.

Our studies have some limitations. Our study subjects were restricted in Korean female subjects. Thus, our results may not be matched to other population. In addition, because we did not have human primary hepatocytes, we used HepG2 cells for representing human hepatocytes. But results from cell lines can be different from real human physiological state. In conclusion, our results will help to understand regulation of FGF21 and its receptors expressions in obese human, and suggest a target for increasing responsiveness to endogenous FGF21 under obese state. This study will be an informative guide line for overcoming obesity.

Table 1. Real-time quantitative RT-PCR primers

	Forward	Reverse
FGF21	5’-GGGAGTCAAGACATCCAGGT-3’	5’-GCCTTCGGACTGGTAAACAT-3’
FGFR1	5’-AAGGACAAACCCAACCGTGT-3’	5’-GTTGCCCTTGGAGGCATACT-3’
β-Klotho	5’-AACTTACAACACATACCATTAA-3’	5’-GTTTACATCCAAGAACTGAGT-3’
CHOP	5’-AGGGAGAACCAGGAAACGGAAACA-3’	5’-TCCTGCTTGAGCCGTTCATTCTCT-3’
TRIB3	5’-GACCGTGAGAGGAAGAAGCTGG-3’	5’-TGCCTTGCCCGAGTATGAGG-3’
TNFα	5’-GAGCTGAACAATAGGCTGTTCCCA-3’	5’-AGAGGCTCAGCAATGAGTGACAGT-3’
IL-1β	5’-ACAGCTGGAGAGTGTAGATCC-3’	5’-CTTGAGAGGTGCTGATGTACC-3’
CD68	5’-CTACATGGCGGTGGAGTACAA-3’	5’-ATGATGAGAGGCAGCAAGATGG-3’
β-actin	5’-GACGGGGTCACCCACAC-3’	5’-GTGGTGGTGAAGCTGTAGCC-3’
36B4	5’-CCTGAGTGATCTGCAGCTG-3’	5’-CACCTGCTGGATGACCAGC-3’

Table 2. Clinical characteristics of the non-diabetic obesity, obesity with type 2 diabetes, and the normal-weight control groups (numbers of patients are shown)

	Control	Obesity	Obesity with diabetes
N	32	27	15
Gynecologic surgery patients			
Myoma	21		
Leiomyoma	4		
Teratoma			
Cystadenoma			
Others	7		
Duration of diabetes			
< 1 year			1
2–9 years			7
≥ 10 years			7
Receiving treatment			
Hypertension	1	2	10
Beta-receptor blocker			3
Angiotensin receptor blocker	1	2	9
Calcium channel blocker		1	3
Diuretics		2	3
Diabetes			12
Metformin			9
Sulfonamides			7
Insulin			6
Glucosidase inhibitor			2
Dyslipidemia			3
Statins			3

Table 3. Metabolic parameters and abdominal fat distributions of the non-diabetic obesity, obesity with type 2 diabetes, and the normal-weight control groups

	Control	Obesity	Obesity with diabetes
N	32	27	15
Age (year)[a]	42.8 ± 6.2	**31.3 ± 8.5**[*]	**43.9 ± 8.4**[†]
BMI (kg/m^2)[a]	22.5 ± 1.6	**39.9 ± 4.7**[*]	**36.2 ± 6.5**[*, †]
Systolic BP (mmHg)[a]	113 ± 15	**141 ± 15**[*]	**136 ± 14**[*]
Diastolic BP (mmHg)[a]	70 ± 10	**85 ± 9**[*]	**84 ± 9**[*]
Fasting glucose (mg/dL)[b]	102 ± 3	96 ± 3	**204 ± 14**[*, †]
Fasting insulin (μU/mL)[b]	4.4 ± 0.5	**24.7 ± 2.1**[*]	**22.0 ± 3.8**[*]
HOMA-IR[b]	1.1 ± 0.1	**5.9 ± 0.5**[*]	**11.7 ± 2.4**[*]
Total cholesterol (mg/dL)[a]	160 ± 36	183 ± 30	**190 ± 50**[*]
HDL-cholesterol (mg/dL)[a]	47 ± 10	49 ± 9	**39 ± 8**[*,†]
LDL-cholesterol (mg/dL)[a]	101 ± 27	115 ± 26	116 ± 38
Triglyceride (mg/dL)[b]	73 ± 5	**150 ± 23**[*]	**269 ± 65**[*,†]
hsCRP (mg/dL)[b]	0.08 ± 0.02	**0.60 ± 0.10**[*]	**0.69 ± 0.18**[*]
Serum leptin (ng/mL)[b]	5.7 ± 0.62	**40.9 ± 3.42**[*]	**23.2 ± 2.72**[*,†]
Serum adiponectin (μg/mL)[b]	6.71 ± 0.64	**2.75 ± 0.29**[*]	**3.09 ± 1.19**[*]
Abdominal CT			
TAT area (cm^2)[a]	227 ± 73	**675 ± 146**[*]	**611 ± 201**[*]
VAT area (cm^2)[a]	67 ± 30	**140 ± 56**[*]	**235 ± 55**[*,†]
SAT area (cm^2)[a]	159 ± 60	**535 ± 141**[*]	**376 ± 167**[*,†]
VSR[a]	0.48 ± 0.25	**0.29 ± 0.17**[*]	**0.68 ± 0.22**[*,†]

a, Data are shown as the mean ± SD.

b, Log-transformed for statistical analysis. Data are shown as mean ± SE on the original (back-transformed) scale. [*], $p < 0.05$ *vs.* control; [†], $p < 0.05$ *vs.* obesity.

Abbreviations: BMI, body mass index; BP, blood pressure; HOMA-IR, homeostasis model assessment of insulin resistance; HDL, high density lipoprotein; LDL, low density lipoprotein; hsCRP, high-sensitivity C-reactive protein; CT, computerized tomography; TAT, total adipose tissue; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; VSR, the ratio of visceral adipose tissue area to subcutaneous adipose tissue area.

Table 4. Correlations of serum FGF21 concentrations with metabolic parameters and abdominal fat distributions (n = 74)

	r	*p*
Age (year)	–0.104	0.376
BMI (kg/m^2)	**0.560**	< 0.001
Systolic BP (mmHg)	**0.534**	< 0.001
Diastolic BP (mmHg)	**0.476**	< 0.001
Fasting glucose (mg/dL)	**0.293**	0.011
Fasting insulin (μU/mL)	**0.604**	< 0.001
HOMA-IR	**0.622**	< 0.001
Total cholesterol (mg/dL)	**0.235**	0.044
HDL-cholesterol (mg/dL)	**–0.250**	0.032
LDL-cholesterol (mg/dL)	**0.275**	0.018
Triglyceride (mg/dL)	**0.359**	0.002
hsCRP (mg/dL)	**0.438**	< 0.001
Serum leptin (ng/mL)	**0.481**	< 0.001
Serum adiponectin (μg/mL)	**–0.525**	< 0.001
Abdominal CT		
TAT area (cm^2)	**0.341**	0.011
VAT area (cm^2)	**0.394**	0.004
SAT area (cm^2)	0.249	0.072
VSR	0.075	0.591

r = Pearson correlation coefficient.

Abbreviations: BMI, body mass index; BP, blood pressure; HOMA-IR, homeostasis model assessment of insulin resistance; HDL, high density lipoprotein; LDL, low density lipoprotein; hsCRP, high-sensitivity C-reactive protein; CT, computerized tomography; TAT, total adipose tissue; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; VSR, the ratio of visceral adipose tissue area to subcutaneous adipose tissue area.

Table 5. Correlations of serum FGF21 concentrations with gene expressions in adipose tissues (n = 74)

	VAT		SAT	
	r	*p*	*r*	*p*
CHOP mRNA	**0.387**	0.001	**0.476**	< 0.001
TRIB3 mRNA	**0.487**	< 0.001	**0.355**	0.002
TNFα mRNA	**0.318**	0.006	0.197	0.092
IL-1β mRNA	**0.364**	0.001	0.157	0.183
CD68 mRNA	**0.377**	0.001	**0.374**	0.001

r = Pearson correlation coefficient.

Abbreviations: VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; CHOP, CCAAT/Enhancer-Binding Protein Homologous Protein; TRIB3, Tribbles homolog 3; TNFα, Tumor necrosis factor α; IL-1β, interleukin-1β; CD68, cluster of differentiation 68

Table 6. Correlations of FGFR1 mRNA and β-Klotho mRNA expressions in adipose tissues with metabolic parameters and abdominal fat distributions (n = 74)

	FGFR1 mRNA		**β-Klotho mRNA**	
	VAT	SAT	VAT	SAT
	r	*r*	*r*	*r*
Age (year)	0.104	–0.086	0.143	–0.036
BMI (kg/m^2)	–0.048	–0.123	–0.189	**–0.349****
Systolic BP (mmHg)	–0.074	–0.148	–0.095	**–0.302****
Diastolic BP (mmHg)	–0.042	–0.145	–0.154	**–0.352****
Fasting glucose (mg/dL)	–0.130	**–0.316****	–0.187	**–0.459****
Fasting insulin (μU/mL)	–0.135	**–0.293***	–0.203	**–0.409****
HOMA-IR	–0.156	**–0.348****	**–0.233***	**–0.491****
Total cholesterol (mg/dL)	0.001	–0.177	–0.126	**–0.257***
HDL-cholesterol (mg/dL)	–0.064	0.100	–0.016	0.153
LDL-cholesterol (mg/dL)	0.037	–0.147	0.054	–0.224
Triglyceride (mg/dL)	–0.060	–0.116	**–0.343***	**–0.361****
hsCRP (mg/dL)	–0.161	–0.162	–0.202	–0.181
Serum leptin (ng/mL)	–0.096	–0.161	**–0.252***	**–0.353****
Serum adiponectin (μg/mL)	–0.012	0.197	0.170	**0.441****
Abdominal CT				
TAT area (cm^2)	–0.046	0.070	–0.107	–0.123
VAT area (cm^2)	–0.008	–0.106	–0.172	**–0.331***
SAT area (cm^2)	–0.050	0.123	–0.057	–0.013
VSR	0.095	–0.184	–0.026	–0.199

r = Pearson correlation coefficient. *, $p < 0.05$; **, $p < 0.01$.

Abbreviations: BMI, body mass index; BP, blood pressure; HOMA-IR, homeostasis model assessment of insulin resistance; HDL, high density lipoprotein; LDL, low density lipoprotein; hsCRP, high-sensitivity C-reactive protein; CT, computerized tomography; TAT, total adipose tissue; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; VSR, the ratio of visceral adipose tissue area to subcutaneous adipose tissue area

Table 7. Correlations of FGFR1 mRNA and β-Klotho mRNA expressions with other gene expressions in adipose tissues

	VAT FGFR1 mRNA		VAT β-Klotho mRNA	
VAT	*r*	*p*	*r*	*p*
CHOP mRNA	–0.005	0.966	–0.124	0.293
TRIB3 mRNA	–0.044	0.713	–0.049	0.676
TNFα mRNA	0.185	0.115	0.010	0.934
IL-1β mRNA	–0.141	0.231	–0.096	0.416
CD68 mRNA	0.061	0.603	–0.106	0.369

	SAT FGFR1 mRNA		SAT β-Klotho mRNA	
SAT	*r*	*p*	*r*	*p*
CHOP mRNA	–0.125	0.289	**–0.317**	0.006
TRIB3 mRNA	–0.166	0.157	**–0.356**	0.002
TNFα mRNA	–0.038	0.750	–0.154	0.189
IL-1β mRNA	–0.071	0.550	**–0.286**	0.014
CD68 mRNA	–0.077	0.515	–0.212	0.070

r = Pearson correlation coefficient.

Abbreviations: VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; CHOP, CCAAT/Enhancer-Binding Protein Homologous Protein; TRIB3, Tribbles homolog; TNFα, Tumor necrosis factor α; IL-1β, interleukin-1β; CD68, cluster of differentiation 68.

Table 8. Metabolic parameters after RYGB surgery

	Pre-op	Post-op	Δ (%)
N	19		
Age (year)	38.6 ± 2.7		
BMI (kg/m^2)	37.5 ± 1.5	**28.5 ± 1.2****	23.8 ± 1.4
Waist circumference (cm)	108.7 ± 2.2	**97.5 ± 2.4****	10.3 ± 1.2
Systolic BP (mmHg)	142.2 ± 3.8	**121.6 ± 2.9****	13.6 ± 2.8
Diastolic BP (mmHg)	84.6 ± 2.1	77.3 ± 2.7	7.6 ± 4.1
Fasting glucose (mg/dL)	147.6 ± 14.2	**104.6 ± 6.6****	21.2 ± 5.9
Fasting insulin (μU/mL)	26.4 ± 3.3	**7.9 ± 0.7****	61.3 ± 5.6
HOMA-IR	9.8 ± 1.9	**2.0 ± 0.2****	66.7 ± 7.7
Total cholesterol (mg/dL)	185.5 ± 9.8	**164.3 ± 7.9****	8.0 ± 5.8
HDL-cholesterol (mg/dL)	42.1 ± 2.0	**46.8 ± 2.2****	-13.1 ± 4.2
LDL-cholesterol (mg/dL)	116.0 ± 7.4	**92.1 ± 6.8***	15.1 ± 8.5
Triglyceride (mg/dL)	221.1 ± 52.7	**104.6 ± 14.7***	41.0 ± 6.1
hsCRP (mg/dL)	0.76 ± 0.18	**0.27 ± 0.10****	46.3 ± 25.1
Serum Leptin (ng/mL)	31.0 ± 4.5	**8.6 ± 1.8****	62.1 ± 8.8
Serum adiponectin (μg/mL)	3.3 ± 0.6	**11.0 ± 1.5****	-732.77 ± 268.1

Data are shown as the mean ± SE. Δ value = [(pre-value - post-value)/pre-value]*100 (%). The significance between pre-op and post-op was analyzed paired t-test.

*, $p < 0.05$ *vs.*pre-op; **, $p < 0.01$ *vs.* post-op.

Abbreviations: RYGB, Roux-en-Y Gastric Bypass; Pre-op, pre-operative; Post-op, Post-operative; BMI, body mass index; BP, blood pressure; HOMA-IR, homeostasis model assessment of insulin resistance; HDL, high density lipoprotein; LDL, low density lipoprotein; hsCRP, high-sensitivity C-reactive protein;

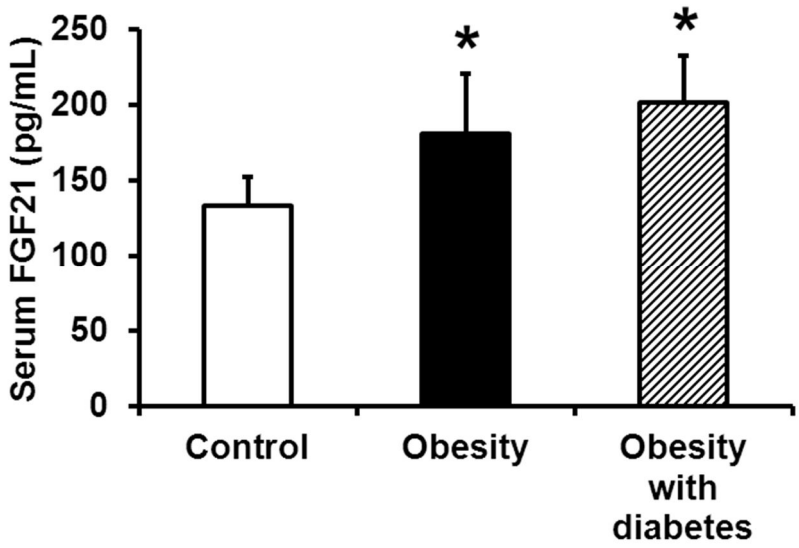

Figure 1. Serum FGF21 concentrations of the study subjects.

Serum FGF21 concentrations were measured by ELISA. Results are represented by means ± SD. The significances of the results between control, obesity, and obesity with diabetes groups were analyzed by ANOVA with a Tukey *post hoc* test.

*, $p < 0.05$ *vs*. control.

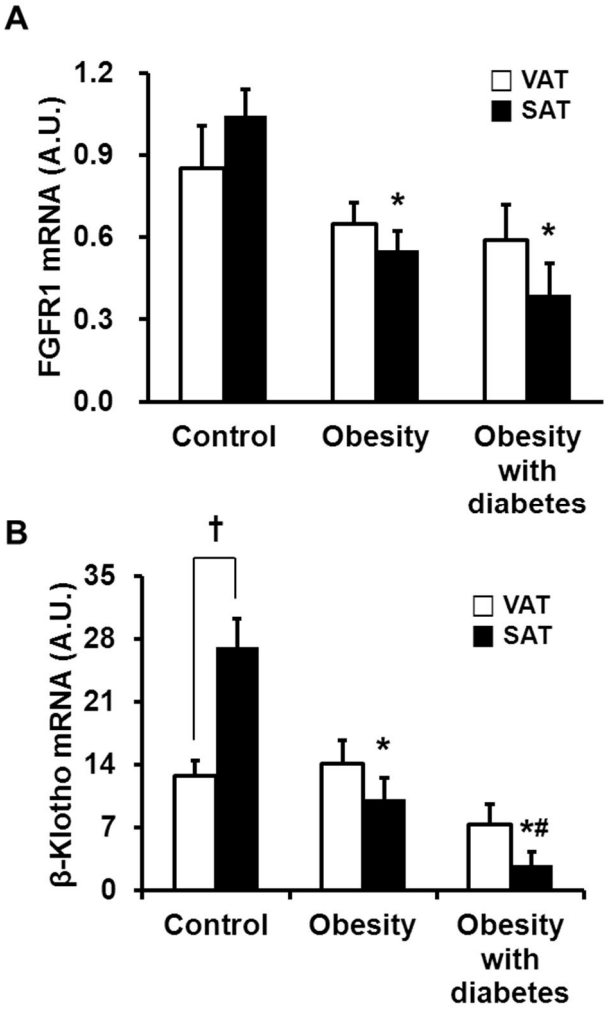

Figure 2. The mRNA expressions of FGFR1 and β-Klotho in adipose tissues of the study subjects.

FGFR1 mRNA and β-Klotho mRNA expressions in VAT and SAT were measured by real-time quantitative RT-PCR. The mRNA expressions were normalized by that of β-actin. The mRNA expressions are presented by means ± SE. The significances of the mRNA expressions between three groups were analyzed by ANOVA with a Tukey *post hoc* test. Significance of the mRNA expressions between VAT and SAT was analyzed by paired t-test. *, $p < 0.05$ *vs*. control of the same tissue. #, $p < 0.05$ *vs*. obesity. †, $p < 0.05$.

Abbreviations: VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue

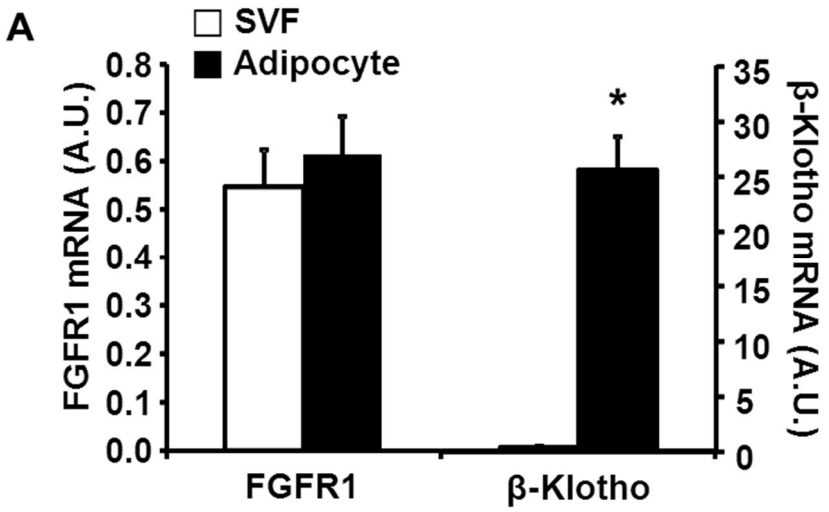

Figure 3. The mRNA expressions of FGFR1 and β-Klotho in SVF and adipocytes from VAT.

These subjects are different from main study subjects. A, FGFR1 mRNA and β-Klotho mRNA expressions in the SVF and adipocytes from VAT of normal-weight control subjects (n = 14). B, β-Klotho mRNA expressions in adipocytes from VAT of normal-weight control (n = 14), non-diabetic obese (n = 11), and obese with diabetes subjects (n = 9). FGFR1 mRNA and β-Klotho mRNA expressions were measured by real-time quantitative RT-PCR, and normalized by that of β-actin. FGFR1 mRNA and β-Klotho mRNA expressions are represented by means ± SE. A, Significances between mRNA expressions of SVF and adipocytes were analyzed by paired t-test. B, Significances between three groups were analyzed by ANOVA with a Tukey *post hoc* test.

*, $p < 0.05$ *vs.* SVF.

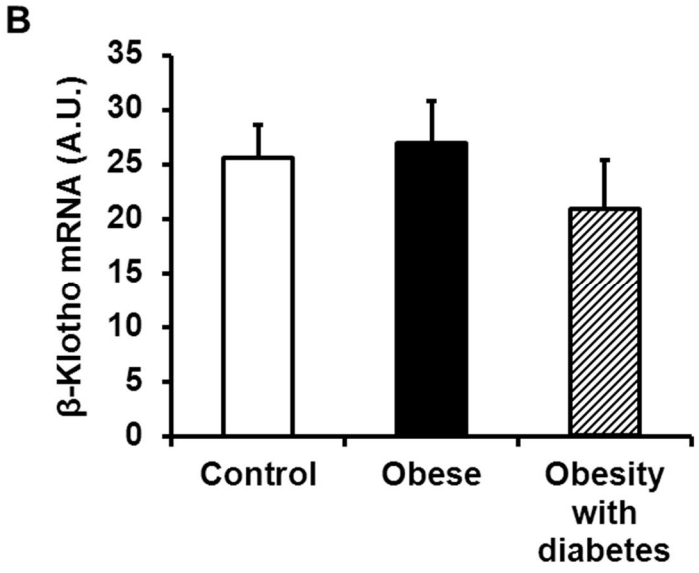

Figure 3. (Continued).

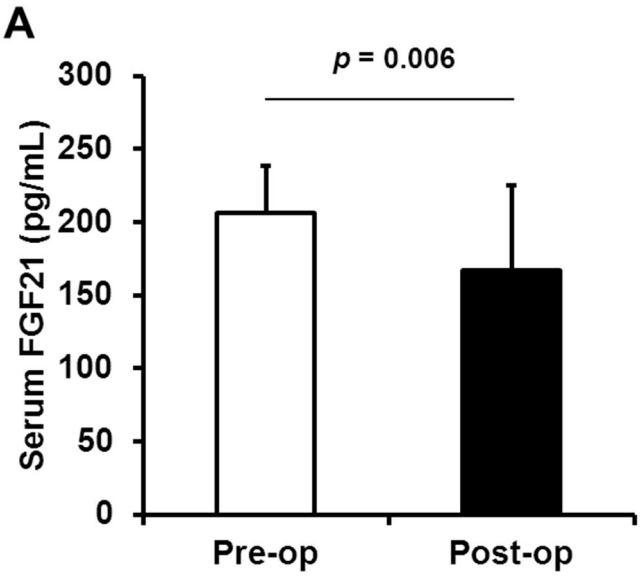

Figure 4. Alterations of serum FGF21 concentrations and correlations with metabolic parameters.

Serum FGF21 concentrations were measured by ELISA. Results are represented by means ± SD. Δ value = [(pre-value - post-value)/pre-value]*100 (%). The significance between pre-op and post-op was analyzed paired t-test. *r* = Spearman's correlation coefficient.

Abbreviations: BMI, Body mass index; RYGB, Roux-en-Y gastric bypass; Pre-op, Pre-operative; Post-op, Post-operative.

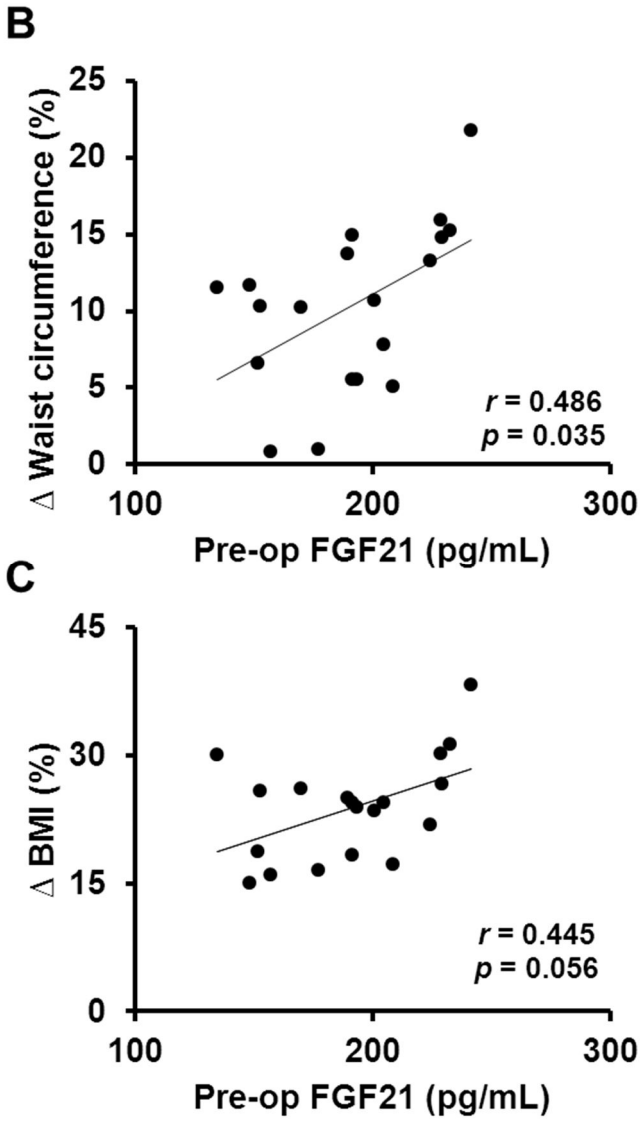

Figure 4. (continued)

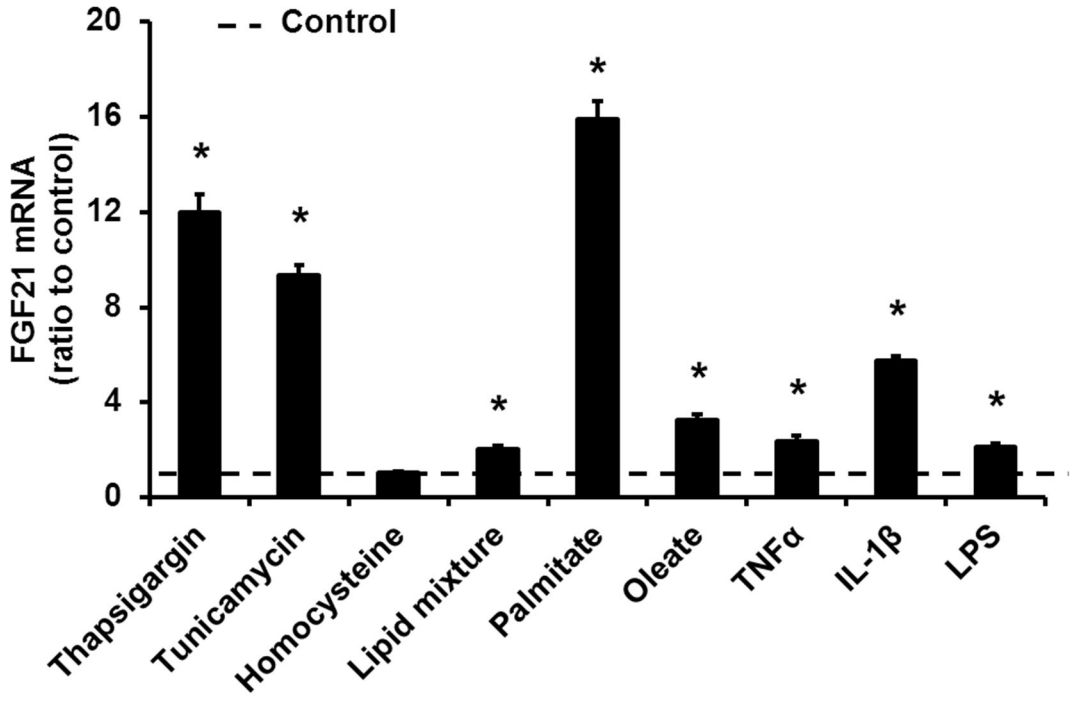

Figure 5. Alterations of FGF21 mRNA expressions in HepG2 cells by various stressors. ER stressor (thapsigargin, 500 nM; tunicamycin, 2 μg/ml; homocysteine, 4 mM), Lipid mixture (100 μM), free fatty acids (palmitate, 500 μM, oleate 500 μM), inflammatory cytokine (TNFα, 10 ng/ml; IL-1β, 10 ng/ml), and LPS (1 μg/ml) were treated 15 hours in HepG2 cells (n = 4). FGF21 mRNA expressions were measured by real-time quantitative RT-PCR, and normalized by 36B4 mRNA expressions. FGF21 mRNA expressions are presented by means ± SE. The significance versus control was analyzed by unpaired t-test. *, $p < 0.05$ *vs*. control

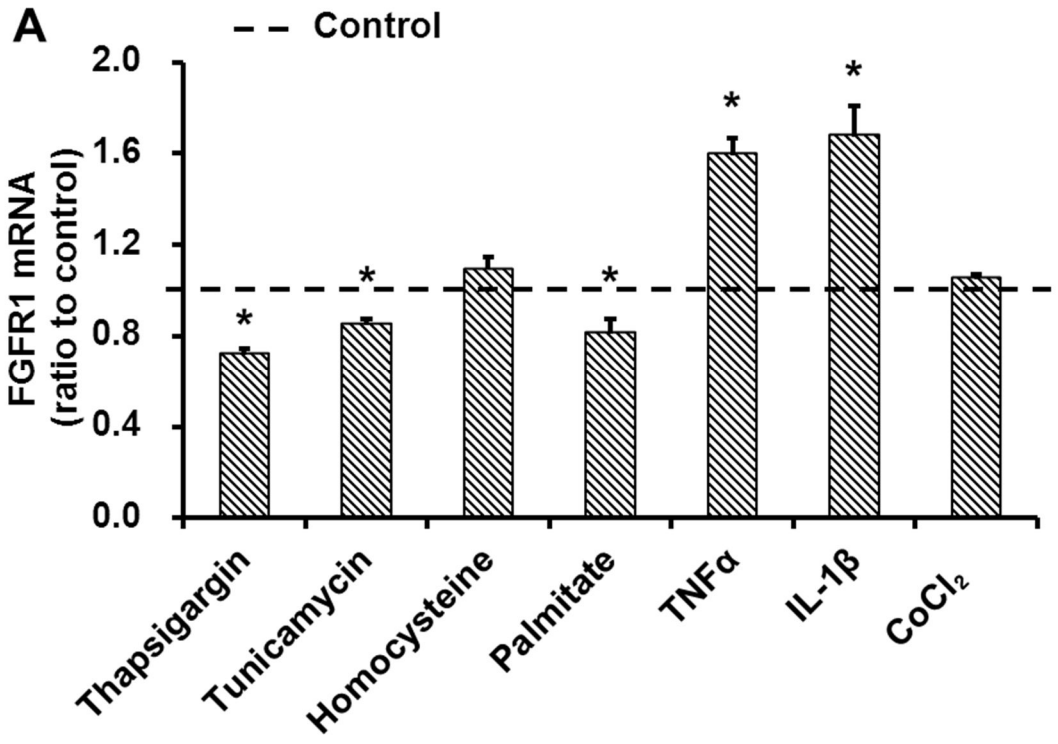

Figure 6. Effects of various stressors on FGFR1 mRNA and β-Klotho mRNA expressions in differentiated human adipocytes.

ER stressor (thapsigargin, 500 nM; tunicamycin, 2 μg/ml; homocysteine, 4 mM), free fatty acid (palmitate, 500 μM), inflammatory cytokine (TNFα, 10 ng/ml; IL-1β, 10 ng/ml), and chemical hypoxia inducer ($CoCl_2$, 100 μM) were treated 24 hours in differentiated human adipocytes (n = 3). FGFR1 mRNA and β-Klotho mRNA expressions were measured by real-time quantitative RT-PCR, and normalized by β-actin mRNA expressions. The mRNA expressions are presented by means ± SE. The significance versus control was analyzed by unpaired t-test. *, $p < 0.05$ *vs*. control.

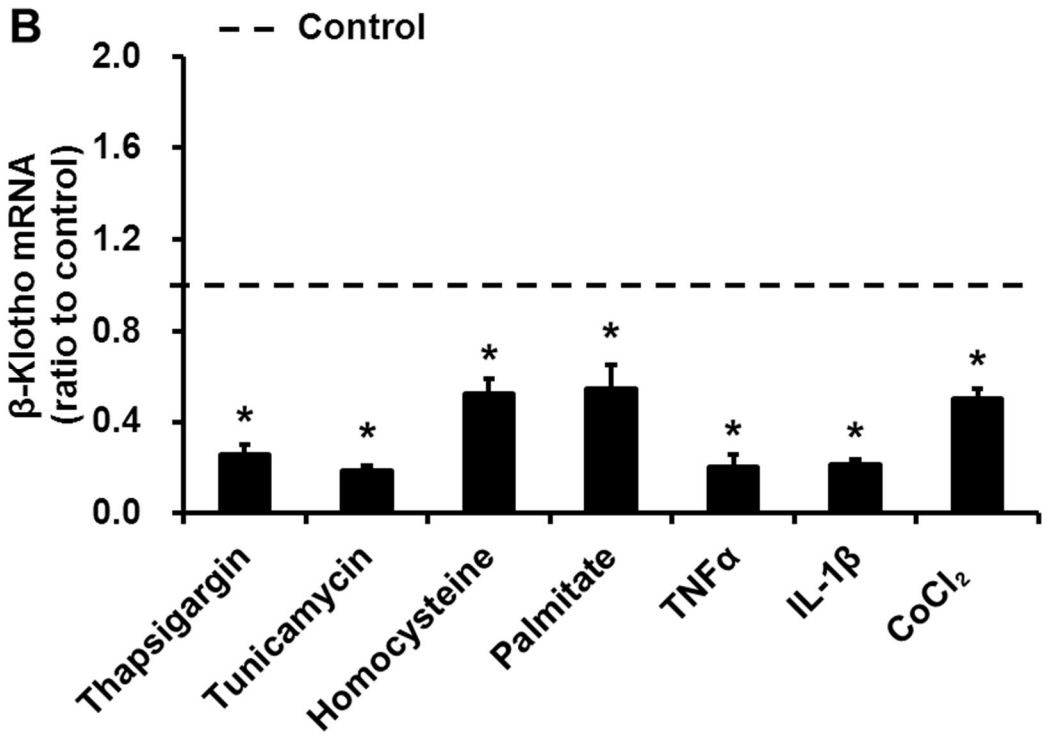

Figure 6. (continued).

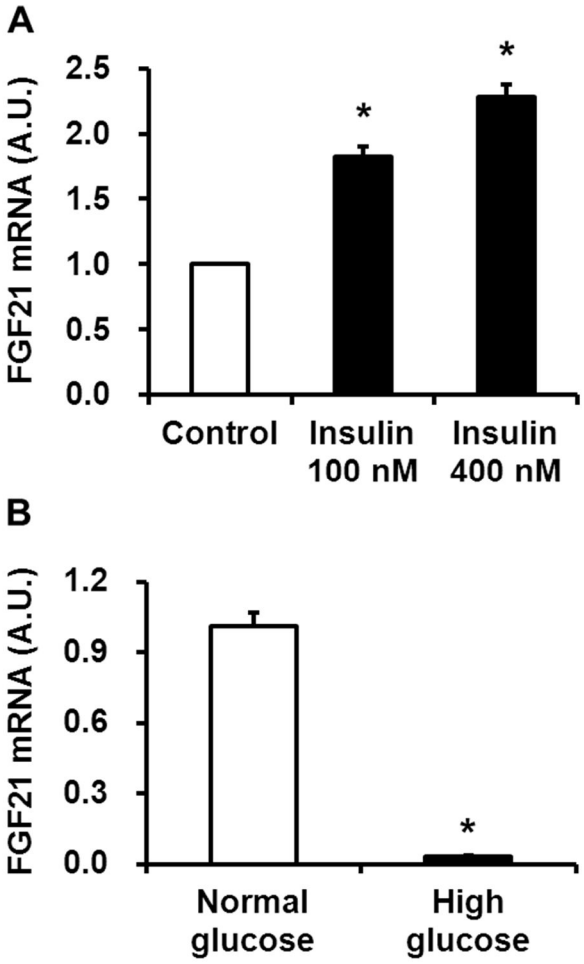

Figure 7. Effects of insulin and glucose on the FGF21 mRNA expressions in HepG2 cells.

A, Insulin was treated 15 hours in HepG2 cells (n = 4). B, Normal glucose medium contained 5.5 mM glucose and high glucose medium contained 25 mM glucose. With these medium, HepG2 cells were incubated 15 hours (n = 4). FGF21 mRNA expressions were measured by real-time quantitative RT-PCR, and normalized by 36B4 mRNA expressions. FGF21 mRNA expressions are presented by means ± SE. The significance versus control or normal glucose was analyzed by ANOVA with a Tukey *post hoc* test or unpaired t-test, respectively. *, $p < 0.05$ *vs*. control or normal glucose.

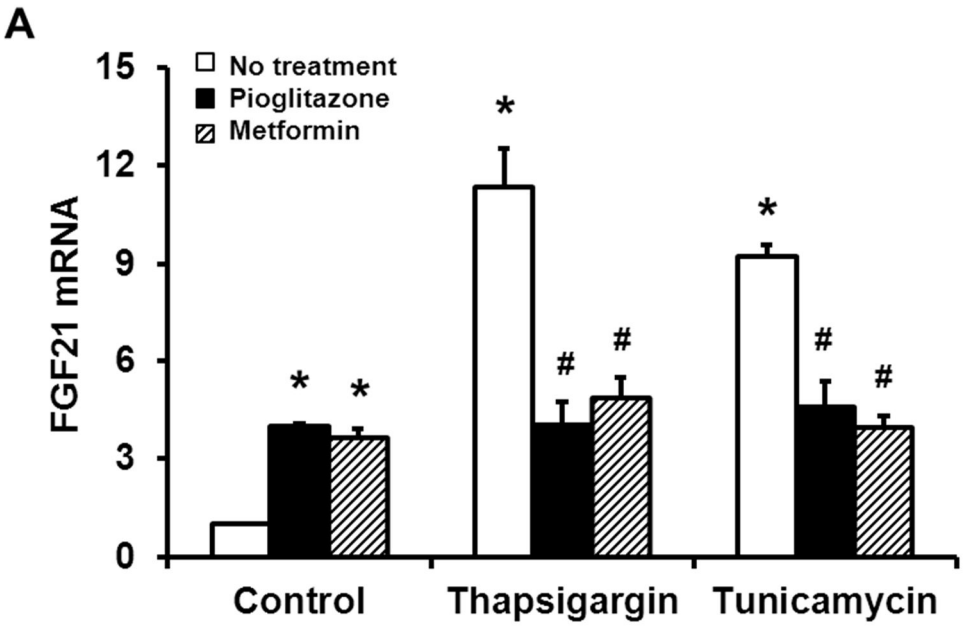

Figure 8. Effects of anti-diabetic drugs on the ER stress-induced FGF21 mRNA, FGFR1 mRNA, and β-Klotho mRNA expressions.

A, Pioglitazone (10 μM) or metformin (4 mM) was treated 15 hours with ER stressor (thapsigargin, 500 nM; tunicamycin, 2 μg/ml) in HepG2 cells (n = 4). B and C, Pioglitazone (10 μM) or metformin (4 mM) was treated 24 hours with ER stressor (thapsigargin, 500 nM; tunicamycin, 2 μg/ml) in differentiated human adipocytes (n = 3). The mRNA expressions were measured by real-time quantitative RT-PCR, and normalized by 36B4 mRNA expressions. The mRNA expressions are presented by means ± SE. The significances versus control or no treatment were analyzed by ANOVA with a Tukey *post hoc* test.

*, $p < 0.05$ *vs*. control with no treatment. #, $p < 0.05$ *vs*. same ER stressor with no treatment.

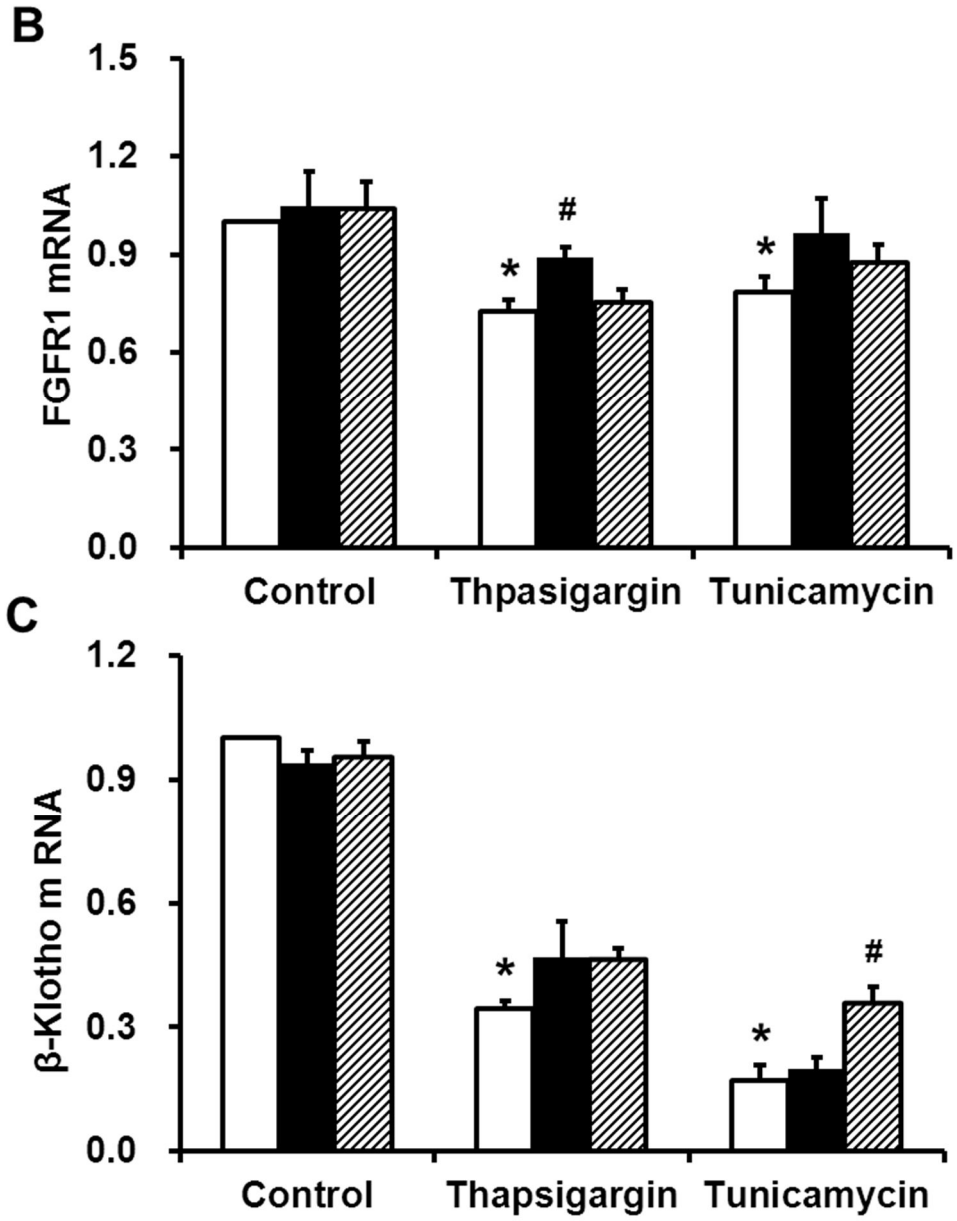

Figure 8. (continued)

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국문초록

비만 환자에서의 혈 중 FGF21 농도와 수용체의 변화

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비만은 현대 사회에서 인류의 건강을 위협하는 주된 질환 중 하나로 좋은 비만 치료제의 개발이 현재 많은 연구자들이 추구하는 주요 연구 과제로 대두되어 있다. Fibroblast growth factor (FGF) 21은 여러 FGF 중 내분비 경로를 통해 세포간 신호전달을 하는 FGF19 subfamily에 속하며 간에서 주로 생산된다. 다른 FGF들이 주로 paracrine 혹은 autocrine 경로를 통해 세포의 증식과 분화를 촉진하는 반면 FGF21은 지방조직을 주된 표적기관으로 하여 당과 지질대사의 항상성유지에 관여한다고 알려져 있다. 그러나 비만, 제2형 당뇨병 동물모델 및 환자에서 혈중 FGF21 농도가 정상상태에 비해 유의하게 증가되어있음이 관찰되어 비만은 "FGF21 저항성 상태"일 것이라는 가설이 제안되었다. 하지만 이러한 가설에도 불구하고, 비만과 제2형 당뇨병 동물모델에 FGF21을 투여 시 체중감소, 혈당감소를 비롯하여 당과 지질 대사에 있어서 여러 가지 이로운 효과가 관찰되었다. 따라서 이에 대한 많은 연구가 수행되었으나 상반되는 결과들이 관찰되어 아직까지 비만에서 혈 중 FGF21 농도 증가의 기전을 확실히 설명하지 못하고 있는 상태이다. 또한, RYGB 수술 후 혈 중 FGF21 농도 변화를 측정한 논문들에서 상반되는 결과들이 관찰되어 RYGB 수술 후 혈 중 FGF21 농도가 어떻게 변화되는지 확실히 알려져 있지 않다. 현재까지 FGF21에 대한 많은 연구는 실험동물을 이용하여 수행되었으며 사람에서는 혈 중 농도 측정 외의 다른 연구는 매우 제한적으로 수행되었다.

본 연구에서는 27명의 비-당뇨성 비만 여성, 15명의 제2형 당뇨병을 동반하는 비만 여성, 32명의 정상 체중 여성으로 구성된 총 74명의 여성을 대상으

로 혈 중 FGF21 농도를 측정하고, 지방조직에서 FGF21 신호전달에 관여하는 FGF receptor 1 (FGFR1)과 co-receptor인 β-Klotho의 mRNA 발현 정도를 복부 피하지방과 내장지방에서 측정하였다. 당뇨병 동반 여부에 상관없이 비만환자의 혈중 FGF21 농도는 정상인에 비해 유의하게 증가되어 있었으며, FGFR1과 β-Klotho의 mRNA 발현은 내장지방조직에서는 변화가 없었지만 피하지방조직에서는 정상인에 비해 유의하게 감소하였다. 혈 중 FGF21 농도와 피하지방조직의 β-Klotho의 mRNA 발현은 비만지표 관련, 인슐린저항성 관련 대사 지표들과 유의한 상관관계를 보였으며, 지방조직의 염증-관련 유전자 및 소포체 스트레스-관련 유전자의 발현과 유의한 상관관계를 보였다. 비 당뇨성-비만 및 제 2형 당뇨병을 동반한 비만여성의 혈 중 FGF21 농도는 RYGB 수술 후 유의하게 감소하였으며, 수술 전 혈 중 FGF21 농도는 RYGB 수술 후에 감소한 허리둘레 및 BMI 와 양의 상관관계를 보였다. 비만이나 제2형 당뇨병 환자의 조직에서 세포가 접하는 환경과 유사한 조건 (염증성 사이토카인, 유리지방산, 소포체-스트레스 증가 등) 에서 인간 간세포주인 HepG2 세포와 분화한 인간지방세포를 배양하였더니, 대체로 HepG2 세포에서 FGF21 mRNA는 증가하고 지방세포에서의 FGFR1 mRNA와 β-Klotho mRNA의 발현은 감소하였다. 또한 제2형 당뇨병 치료제로서 사용되는 pioglitazone과 metformin은 소포체 스트레스에 의해 증가된 간 세포의 FGF21 mRNA 발현을 감소시킬 수 있었다.

본 연구의 결과는 이와 같은 현상이, 비만과 이에 동반되는 제2형 당뇨병에서 나타나는 염증, 소포체 스트레스 증가 등의 세포 주위 환경 변화에 대해 FGF21을 생산하는 간세포와 이의 표적세포인 지방세포가 각기 특이적으로 반응하여 나타나는 현상일 가능성을 제시하며, 지방조직의 FGF21 저항성 상태에 대한 보상반응으로 FGF21이 과다 생산 되었다는 가설로도 설명될 수 있다. 게다가 비-당뇨성 비만 및 제2형 당뇨병을 동반한 비만여성의 혈 중 FGF21 농도는 RYGB 수술 후 유의하게 감소하였으며, 수술 전 혈 중 FGF21 농도는 RYGB 수술 후 체중감소를 예측하는 요인이 될 수 있고, 제2형 당뇨병 치료제인 pioglitazone과 metformin의 이로운 효과는 간세포의 FGF21 mRNA 발현조절을 포함함을 알 수 있다.

중심단어: FGF21, 비만, RYGB, 간세포, 지방세포