Alcohol ingestion induces pancreatic islet dysfunction and apoptosis via mediation of FGF21 resistance

Bao Chen Yang, Shang Ying Wu, Po Sing Leung

School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China

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Correspondence to: Po Sing Leung, PhD. Room 609A, 6/F, Lo Kwee-Seong Integrated Biomedical Sciences Building, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China. Email: psleung@cuhk.edu.hk.

Background: Disruption of β-cell insulin secretion and viability caused by excessive ethanol consumption increases type 2 diabetes mellitus (T2DM) pathogenesis risk. Fibroblast growth factor 21 (FGF21) plays a significant role in regulating lipid and glucose homeostasis. Recently, FGF21, best known for its role in lipid and glucose homeostasis regulation, and its obligate co-receptor β-klotho have been shown to inhibit ethanol ingestion and metabolism. It remains unclear whether heavy ethanol intake modulates islet FGF21 expression and function. This study investigated the relationship between ethanol exposure, FGF21, and islet function *in vivo*/*ex vivo* islet and *in vitro* cell models.

Methods: Mice were gavaged with 3.5 g/kg ethanol or saline for 1–3 weeks (long-term exposure). Human MIN6 cells and isolated islets were cultured and treated with 80 mM ethanol for 24 h (short-term exposure) to mimic excessive ethanol consumption. We applied the oral glucose tolerance test (OGTT), blood glucometry, enzyme-linked immunosorbent assay (ELISAs) for insulin and FGF21, glucose stimulated insulin secretion (GSIS) testing, reverse-transcription (RT)-polymerase chain reaction (PCR), and western blot experiments.

Results: Long-term ethanol treatment induced FGF21 resistance in mouse pancreatic islets. Moreover, ethanol exposure damaged insulin secretory ability and glucose homeostasis. *In vitro* and *ex vivo* experiments showed that short-term ethanol treatment upregulated the expression of FGF21 signaling pathway-related genes and proteins, without affecting β-cell survival or function.

Conclusions: Long-term ethanol consumption induces FGF21 resistance-mediated pancreatic β-cell dysfunction, and thus diabetes pathogenesis risk.

Keywords: β-klotho; diabetes; ethanol; fibroblast growth factor 21 (FGF21); pancreatic islet cells

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Introduction

Alcohol addiction is a pervasive worldwide problem, causing 3.3 million deaths in 2010, and accounting for 5.9% of all deaths in the world (1). Drinking alcohol regularly increases the risk of adverse health outcome risks, including risks for fatty liver, neurological, cardiovascular, and metabolic diseases. Notably, heavy alcohol drinkers are at an elevated risk of developing type 2 diabetes mellitus (T2DM) relative to non-drinkers. With respect to T2DM pathogenesis, it has been suggested that alcohol use increases risk of islet dysfunction, whereby β-cell apoptosis and hypo-proliferation underlying insulin resistance leads to poor glucose homeostasis (2-4). Human studies have shown that alcohol consumption-induced β-cell disruption is related to mitochondrial dysfunction, oxidative stress, and increased

production of reactive oxygen species in pancreatic islets (5,6).

Fibroblast growth factor 21 (FGF21) is an endocrine factor expressed in liver, adipose, and pancreas tissues. Chronic FGF21 administration has been reported to increase pancreatic islet numbers, increase insulin biosynthesis, and promote β-cell survival in *db/db* mice (7,8). Pharmacological FGF21 doses have been shown to increase glucose uptake, improve glucose clearance, and lower blood glucose and triglyceride levels in high-fat diet-induced obese mice and to reduce glucolipotoxicity in INS-1 cells. Moreover, FGF21 over-expressing transgenic mice exhibit a lower plasma glucagon level than similar mice not induced to over-express FGF21 (9-11).

Several recent studies have described indirect effects of FGF21 on acute ethanol metabolism. Marked increases in serum FGF21 levels triggered by acute and subchronic alcohol consumption in both humans and rodents have been shown to have hepato-protective effects, including suppression of inflammatory reactions, fibrosis, and lipid accumulation (12-15). In addition, FGF21 has been reported to alter gastric emptying rate and early ethanol metabolism, without affecting ethanol dehydrogenase activity or aldehyde dehydrogenase 2 activity (16). On the other hand, FGF21 deficiency can aggravate ethanol-induced liver injury. It has been suggested that FGF21 may regulate drinking behavior by reducing neural release of dopamine in target tissues, similar to the consumption-reducing effects of satiety-related gut peptides, while affecting sympathetic regulation of ethanol-induced adipose lipolysis (17-20).

Although mitochondrial dysfunction and oxidative stress can well explain ethanol-induced β-cell failure, we do not yet have a clear understanding of the relationships among drinking alcohol, islet function, and FGF21 signaling. In the present study, to investigate the impact of long-term ethanol consumption on glucose homeostasis *in vivo*, we applied the oral glucose tolerance test (OGTT), conducted glucometer blood glucose level testing, conducted an enzyme-linked immunosorbent assays (ELISAs) for insulin and FGF21, and performed glucose stimulated insulin secretion (GSIS) testing on islets from mice subjected to chronic ethanol administration via gavage. Reverse-transcription (RT)-polymerase chain reaction (PCR) experiments were conducted to examine the effects of ethanol exposure on the expression of islet function-related genes. We hypothesized that chronic ethanol exposure would lead to FGF21 resistance and β-cell impairment, thereby increasing the risks of T2DM.

Methods

Animal study

Male 10-week-old C57/6J mice were supplied by the Laboratory Animal Service Center of the Chinese University of Hong Kong for this study. The experimental procedures were approved by the Animal Experimentation Ethics Committee of the Chinese University of Hong Kong. The mice were housed at 22±2 ℃ with a 12-h light/dark cycle and free access to food and water. The mice were divided into ethanol and saline control groups. Mice in the ethanol group were gavaged with a dose of 3.5 g/kg bodyweight ethanol (30%) daily in the morning for 1 week, 2 weeks, or 3 weeks. Mice in the saline control group were gavaged with an equivalent volume of saline for 1, 2, or 3 weeks.

Islet isolation and culture

Pancreatic islets were isolated from mice as previously described (21). Subsequently, the intact isolated pancreatic islets were cultured in 5.6 mM glucose-containing RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS; Gibco Laboratories, Grand Island, NY), 100 units/mL penicillin, and 100 μg/mL streptomycin (Invitrogen).

Human islet and cell treatments

Human islets were purchased from Prodo Laboratories Inc. (Irvine, CA) and shipped in PIM(T) medium within 7 d of being isolated. Upon arrival, the islets were left undisturbed overnight in recovery medium at 37 ℃. The islets were placed in human AB serum supplemented with 10 mM HEPES, 1 mM sodium pyruvate, 100 U/mL penicillin/streptomycin, and 10% FBS for 2–4 days before treatment. MIN6 cells were purchased from AddexBio and cultured in DMEM containing 25 mM glucose, 2 mM L-glutamine, and 1 mM sodium pyruvate supplemented with 15% FBS (Thermo Fisher Scientific, USA), 100 U/mL streptomycin, 100 U/mL penicillin (Invitrogen, USA), and 75 μM β-mecaptoethanol (Sigma, USA) in an incubator set to 37 ℃ with 5% CO_2.

Ethanol was diluted with growth medium to a final concentration 80 mM (based on legal driving limits in the USA, UK, and other countries). MIN6 cells and human islets were exposed to this ethanol solution or only vehicle (growth medium without ethanol) for 24 h.

Table 1 Real-time PCR primer sequences

Gene	Forward (5'→13')	Reverse (5'→13')
Mouse *GAPDH*	GCACAGTCAAGGCCGAGAAT	GCCTTCTCCATGGTGGTGAA
Mouse *FGF21*	CQTCTGCCTCAGAAGGACTC	AAGGCTCTACCATGCTCAGG
Mouse *β-klotho*	ACGACCCGACGAGGGCTGTT	GGAGGAGACCGTAAACTCGGGCTTA
Mouse *FGFR1*	TACAAGGTTCGCTATGCCAC	TGCGGAGATCGTTCCACGAC
Mouse *FGFR2*	GACAGGTAACAGTTTCGGCC	TGCCCAGCGTCAGCTTATCT
Mouse *FGFR3*	CGCTTAAGCGACAGGTGTCC	GGCTTACCAAGTGTCAGCCG
Mouse *FGFR4*	ACAGCAAGCACCCTACTGGA	TGTTGGTGGCGCAGCCGAAT
Mouse *Insulin1*	ACAACTGGAGCTGGGTGGAGG	GTTGCAGTAGTTCTCCAGTTGGTAGAG
Mouse *Insulin2*	CCCTGCTGGCCCTGCTCTT	AGGTCTGAAGGTCACCTGCT
Mouse *Insulin receptor 1*	CACCCAGTTTTTCGACACCT	CACCCAGTTTTTCGACACCT
Mouse *Insulin receptor 2*	GCCACCGTGGTGAAAGAGTA	AGCGTTGGTTGGAAACATGC
Mouse *Glut2*	CAACCATTGGAGTTGGCGCTGTAA	AGGTCCACAGAAGTCCGCAATGTA
Mouse *Pdx 1*	GAAATCCACCAAAGCTCACG	AATTCCTTCTCCAGCTCCAG
Mouse *Glucokinase*	GAATCTTCTGTTCCACGGAG	AGTGCTCAGGATGTTAAGGA
Human *FGF21*	GCCTTGAAGCCGGGAGTTATT	GTGGAGCGATCCATACAGGG
Human *β-klotho*	ATGTCAGCAGCACGAATGGTT	GCGTTGGCAACTGTTACTATTCC
Human *FGFR1*	CATCACGGCTCTCCTCCAGT	AGGGGTTTGCCTAAGAC
Human *FGFR2*	GATAAATACTTCCAATGCAGAAGTGCT	TGCCCTATATAATTGGAGACCTTACA
Human *FGFR3*	ATGGGCGCCCCTGCCTG	TCACGTCCGCGAGCCCC
Human *FGFR4*	AGATGCTCAAAGACAACGCCT	CGCACTCCACGATCACGTA

RT-PCR analysis

Total RNA was extracted from islets and from MIN 6 cells with TRIzol reagent (Invitrogen) according to the manufacturer's instructions. RT of first-strand cDNA was performed with a PrimeScript reverse transcriptase master mix kit (Takara Bio Inc., Kusatsu, Japan). Gene expression was measured by conventional or real-time PCR, wherein cDNA samples were mixed with SYBR green QPCR master mix (Applied Biosystems, Walttham, MA) and specific primers (*Table 1*). The fold change in mRNA expression relative to the control group was determined with the $2^{-\Delta\Delta Ct}$ method and normalized to gluceraldehude-3-hosphate dehydrogenase (GAPDH) mRNA levels.

Western blot analysis

Islet and cell proteins were extracted with RIPA. Extracted proteins were separated by 8–12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and transferred to nitrocellulose membranes (Bio-rad Heidemannstraße, Germany), which were blocked with 5% milk and then proved overnight with anti-FGF21 (Abcam, Cambridge, UK), anti-phospho-FRS, 2-anti-FRS2, anti-phospho-ERK1/2, anti-ERK1/2 (Cell Signaling Technology, Boston, MA), or anti-β-actin (Santa Cruz Biotechnology, Santa Cruz, CA) primary antibodies at room temperature (RT). Horseradish peroxide-conjugated secondary antibodies were incubated at RT with membranes for 2 h after washing with phosphate buffered saline with 0.1% Tween-20. Labeled protein bands were visualized on autoradiography films (Fuji Film, Tokyo, Japan) following application of ECL detection reagent (GE healthcare, Chicago, IL). Protein bands were quantitated in Image software (National Institutes of Health, Bethesda, MD) and normalized to β-actin. The primary and secondary antibodies used are listed in *Table 2*.

Table 2 Antibodies used in Western blotting and immunohistochemistry

Antibody	Dilution	Host Species	Supplier
FGF21	1:1,000	Rabbit	Abcam
p-ERK	1:1,000	Rabbit	Cell Signaling
ERK	1:1,000	Rabbit	Cell Signaling
p-FRS2	1:1,000	Rabbit	Cell Signaling
FRS2	1:1,000	Rabbit	Cell Signaling
β-actin	1:1,000	Mouse	Santa Cruz
HRP-anti-rabbit IgG	1:1,000	Donkey	Amersham
HRP-anti-mouse IgG	1:1,000	Sheep	GE Healthcare

Table 3 Antibodies used in immunofluorescent labeling

Antibody	Dilution	Host species	Supplier
FGF21	1:200	Rabbit	Abcam
Insulin	1:200	Guinea pig	Life Technologies

Immunocytochemistry and immunofluorescent examinations

Fresh mouse pancreas was embedded in O.C.T. compound (Tissue Tek, Sakura, Japan) and frozen in nitrogen immediately. Cryostat sections were sliced at 6 μm and fixed in 4% paraformaldehyde (Sigma-Aldrich) at RT for 15 min. After being rinsed with phosphate buffered saline (PBS) three times, the sections were blocked with 2% bovine serum albumin (Sigma-Aldrich) for 1 h and incubated with primary antibodies at 4 ℃ for 12 h. Sections were then incubated for 2 h with secondary antibodies conjugated with Alexa Fluor 568 (1:200; Life Technologies) and Alexa Fluor 488 (1:200; Life Technologies) for 2 h at RT. After counterstaining with 4',6'-diamidino-2-phenylindole (DAPI, 1:40,000, Life Technologies) and rinsing, samples were mounted and examined. Digital images were acquired on an Olympus FV1200 SIM Confocal System and analyzed with FLUOVIEW software. The primary and secondary antibodies used are listed in *Table 3*.

In vivo glucose homoeostasis

In preparation for the OGTT, mice were fasted for 6 h, then gavaged with 2 g/kg glucose. Blood glucose level was measured in blood drawn from the tail vein with a glucometer (Bayer Corporation, Robinson Township, PA) immediately, 15, 30, 60, and 120 min after glucose administration. At the same time, serum insulin levels were measured with a mouse insulin ELISA kit (Antibody and Immunoassay Services, University of Hong Kong).

GSIS

Isolated islets were allowed to equilibrate in Krebs-Ringer bicarbonate buffer (KRBB, supplemented with 0.1% NEFA-free BSA and 10 mM HEPES) with 1.7 mM glucose for 1 h. The islets or cells were then incubated in KRBB containing 1.7 mM glucose for 1 h, and in KRBB with 16.7 mM glucose for an additional 1 h. KRBB solution samples were subjected to insulin quantification with an ELISA kit (University of Hong Kong, China)

Plasma FGF21 concentration

Immediately upon collection, tail vein blood samples were placed on ice and then separated by centrifugation for 15 min at 3,000 rpm. Plasma FGF21 concentrations were measured with an FGF21-specific mouse ELISA kit (Antibody and Immunoassay Services, University of Hong Kong).

Cell viability assay

Metabolic activity of MIN6 cells was measured with a 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium (MTT) bromide assay immediately after a 24-h ethanol treatment. The cells were seeded in a 96-well cell culture plate at 4×10^4 cells/well for 1 day, then incubated in 2% FBS media with or without ethanol for 24 h. Subsequently, 0.15 mg MTT was added to each well. After the medium was discarded, the cells were allowed to incubate for 3 h at 37 ℃ and then 100 μL dimethyl sulfoxide was added to each well and the cells were left for 15 min at RT. Finally, optical density at 490 nm was read in a microplate reader (SpectraMax i3x Multi-Mode Detection Platform, Molecular Devices, CA). Cell viability was expressed as a percentage of the quantity of cells observed in the non-ethanol medium control group.

Cell death rate

MIN 6 cells were seeded at a density of 4×10^4 cells/well in a 96-well cell culture plate and cultured under experimentally indicated conditions. Cell death rate was determined by a cell death detection ELISA plus kit (Roche Applied Science)

according to the manufacturer's instructions.

Statistical analysis

Results are displayed as means ± standard errors (SEs). Groups were compared with two-tailed Student's t test or one-way analyses of variance (ANOVA) followed by Tukey's post hoc test or with a two-way ANOVA. In all cases, $P<0.05$ was considered statistically significant.

Results

Chronic ethanol consumption impairs glucose metabolism and insulin secretion ability

Young adult mice did not show significant changes in blood glucose or insulin content relative to (equivalent-volume) saline controls after 1 week of being gavaged with ethanol (*Figure 1A*), but did show a reduced insulin peak after 2 weeks of ethanol gavage (*Figure 1B*) and reduced levels of insulin overall together with reduced glucose tolerance, as evidenced by OGTT results, after 3 weeks of ethanol gavage (*Figure 1C*). Meanwhile, GSIS testing showed that insulin secretory ability, which was similar between the ethanol and saline control groups after one-week ethanol gavage, became impaired in the ethanol group, relative to the saline control group, after 2 weeks and this impairment became more pronounced after 3 weeks (*Figure 1D*).

Chronic ethanol consumption induces FGF21 resistance

A progressive reduction in mRNA levels of the FGF21 receptor co-factor β-klotho and FGF receptors was seen with increasing ethanol gavage time (*Figure 2A*,*B*). The mRNA levels of β-klotho and FGF receptor genes were reduced in the ethanol group, relative to the control group, at the 2- and 3-week treatment time points. Meanwhile, serum FGF21 levels were shown to increase slightly in the first and second weeks of ethanol administration, reaching approximately double control levels by the 3-week time point (*Figure 2C*). Correspondingly, FGF21 protein levels were upregulated in isolated islets from mice in the ethanol group (*Figure 2D*). Similar results were also found as demonstrated by immunostaining data (*Figure S1*).

Long-term ethanol drinking reduces expression of islet function related genes

Compared to saline control group levels, mRNA expression of the insulin encoding genes Insulin1 (*Ins1*) and Insulin2 (*Ins2*) began to decrease after 1 and 2 weeks, respectively, of ethanol administration, whereas mRNA expression of the insulin receptor substrate genes Insulin receptor1 Insulin receptor 1 (*Irs1*) and Insulin receptor 2 (*Irs2*) showed an apparent upregulation at the 1-week time point, followed by a steady decrease through weeks 2 and 3 (*Figure 3A*). The expression patterns over the analyzed time points of the islet function related genes [pancreatic and duodenal homeobox 1 (*Pdx-1*)] and glucose transporter 2 (*Glut2*) were similar to those of *Ins1* and *Ins2*, whereas that of glucokinase (*Glk*) was similar the patterns observed for *Irs1* and *Irs2* (*Figure 3B*).

Ethanol exposure for 24 h alters human β-cell function

As shown in *Figure 4A*, human islet and MIN6 cells were subjected to 24-h ethanol exposure exhibited a robust insulin secretory response to glucose treatments in an *in vitro* GSIS experiment. The 24-h ethanol exposure treatment did not significantly affect cell death rate (*Figure 4B*) or cell viability (*Figure 4C*). After the 24-h ethanol treatment, however, we observed obvious increases in the mRNA expression of *FGF21*, the FGF receptor genes *FGFR1* and *FGFR3*, and the FGF receptor cofactor β-klotho, which showed the most pronounced increase (*Figure 5A*). Notably, in a parallel experiment with isolated human pancreatic islets, 24-h ethanol exposure resulted in significantly increased mRNA expression of *FGF21*, all four FGF receptor genes (*FGFR1*, *FGFR2*, *FGFR3*, and *FGFR4*), and the FGF receptor cofactor β-klotho (*Figure 5B*). Western blot protein analysis of these MIN6 cell groups showed significantly increased levels of phosphorylated FRS2 (fibroblast growth factor receptor substrate 2; an FGF21 signaling reporter) and phosphorylated ERK (extracellular-signal-regulated kinase) compared to the vehicle control group (*Figure 5C*).

Discussion

Although exogenous FGF21 has well-known pharmaceutical effects, including weight loss, increased insulin sensitivity, and improved blood glucose clearance (22,23), and clinical studies have correlated alcohol consumption with insulin resistance and glucose intolerance (5,24,25), little is known about FGF21 involvement in alcoholism effects on pancreatic islets and T2DM pathogenesis. In the current study, we report, for the first time, that ethanol exposure impairment of pancreatic islet glucose metabolism and insulin secretion ability is associated with FGF21 resistance.

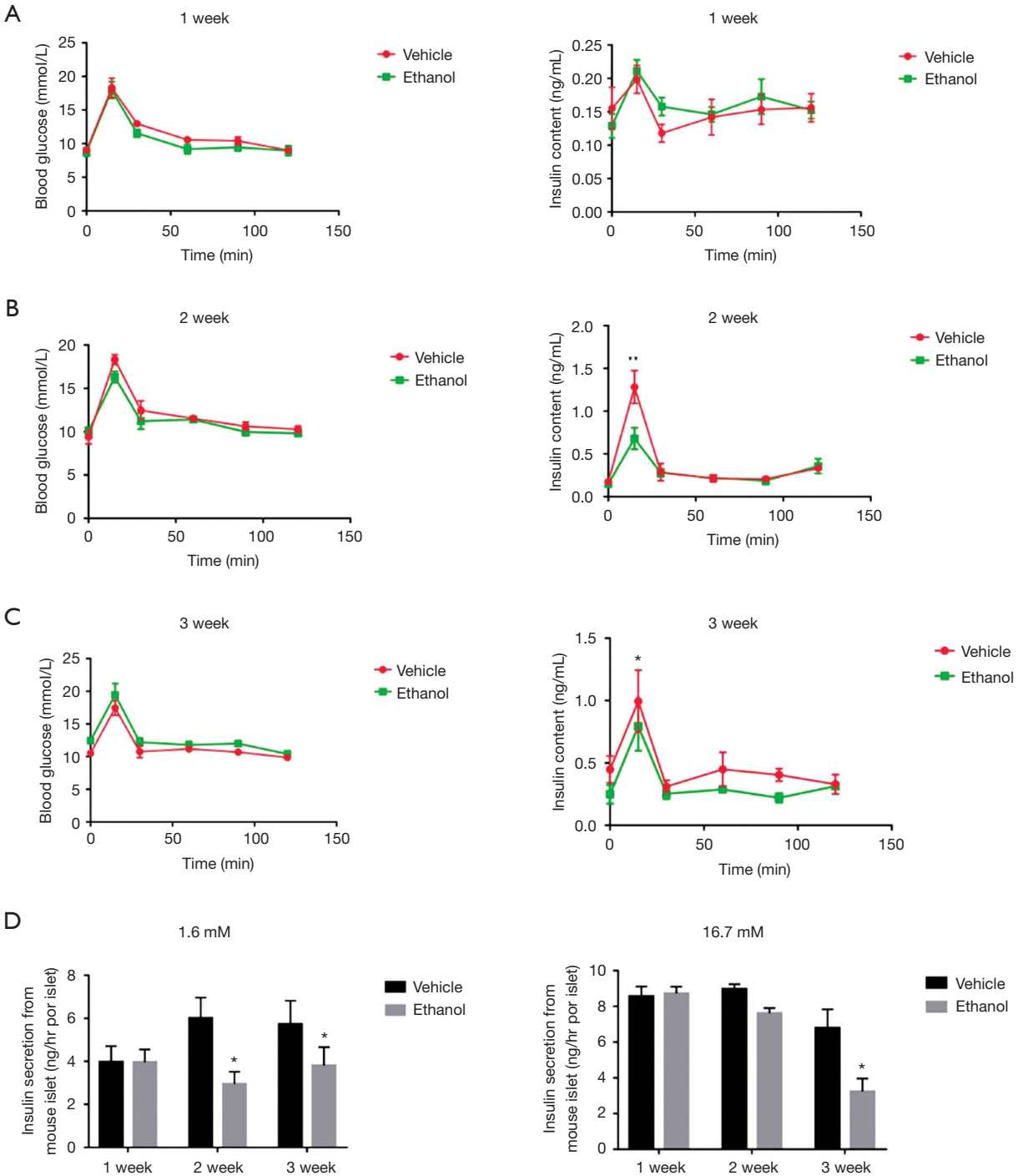

Figure 1 Chronic ethanol exposure impaired glucose metabolism. Over a period of weeks, daily gavaged ethanol led to reduced plasma insulin levels, which were not yet demonstrable after 1 week (A), but that continued to decrease and become significant through week 2 (B) and week 3 (C). GSIS tests were performed on islets isolated from the gavaged mouse groups at low (1.6 mM) and high (16.7 mM) glucose concentrations. (D) Graphic summary of ethanol treatment effects on insulin secretion by time point. Data are means ± SEs; *P<0.05, **P<0.01 *vs.* matched vehicle group (N=5 per treatment-time group).

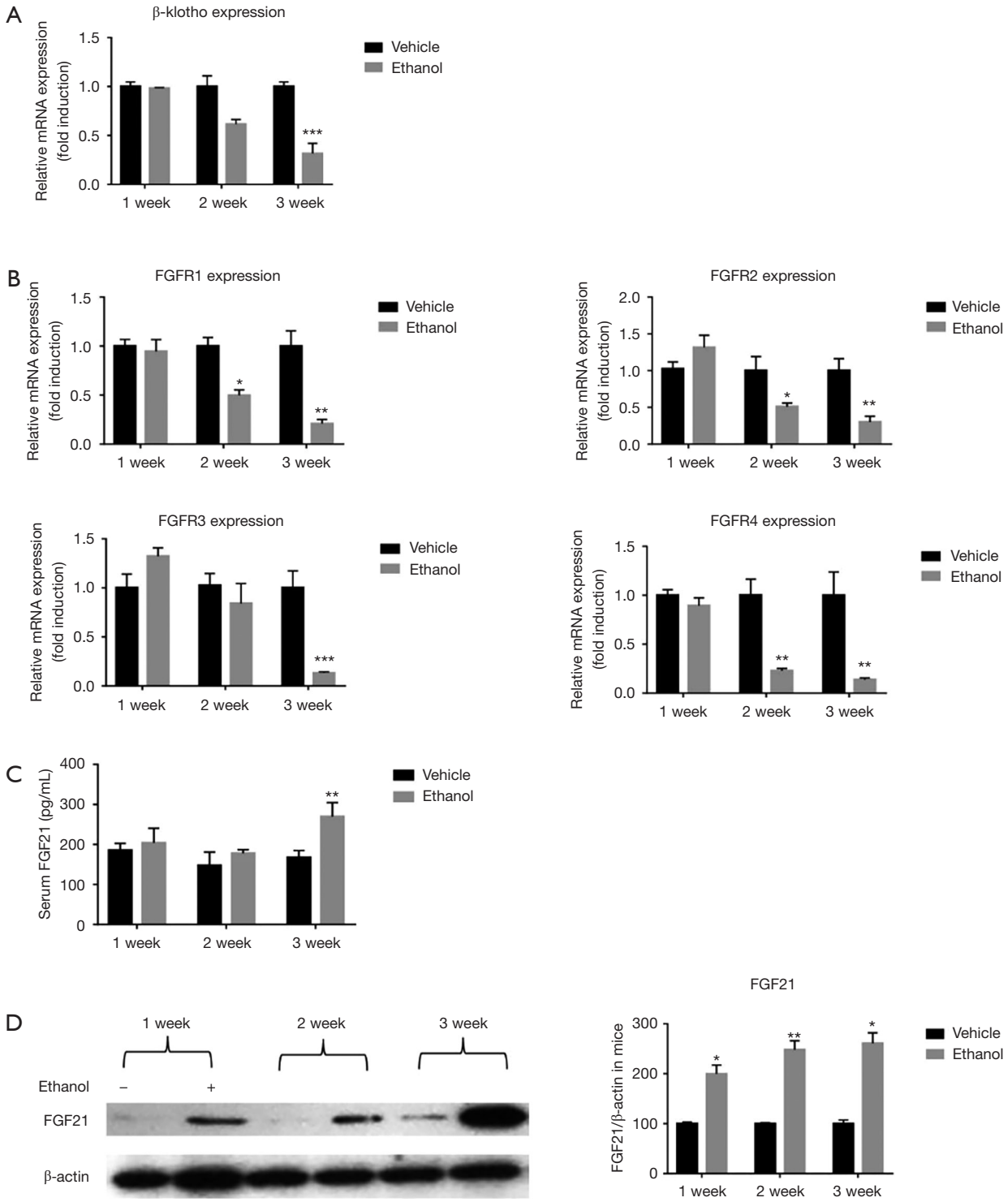

Figure 2 Chronic ethanol exposure induced FGF21 resistance. Quantitative RT-PCR determined relative β-klotho (A) and FGF receptor (FGFR) (B) mRNA levels in islets isolated from mice after 1–3 weeks of being gavaged with or without ethanol. (C) Increased levels of serum FGF21 levels in terminal bleed blood, determined by ELISAs, after 3 weeks of ethanol exposure. (D) Western blot, with β-actin densitometry loading control, demonstrating ethanol exposure-induced elevation of FGF21. Data are means ± SEs; *P<0.05, **P<0.01, ***P<0.001 *vs.* time-matched saline gavaged group (N=5 per treatment-time group).

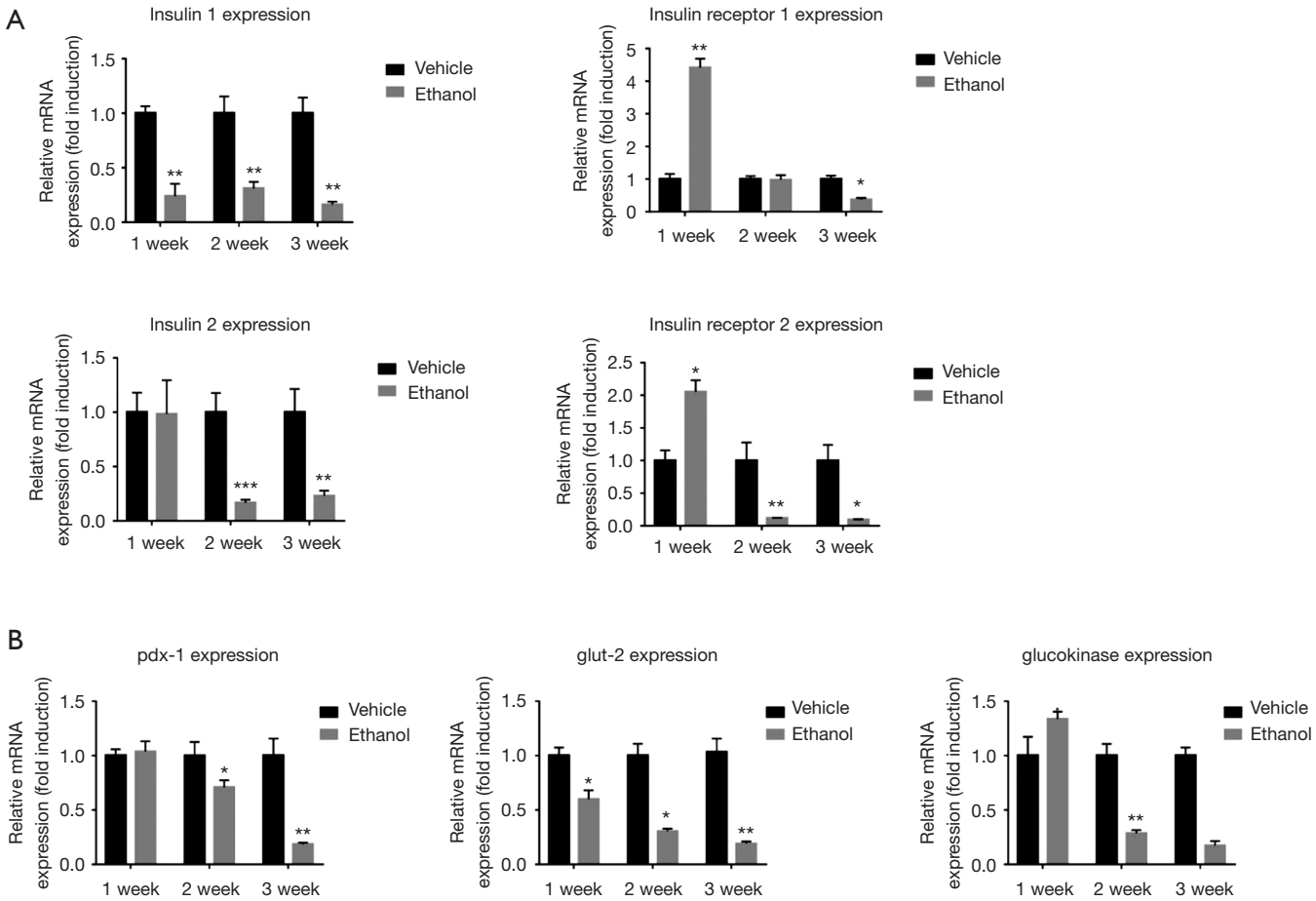

Figure 3 Long-term ethanol exposure reduced transcription levels of islet function-related genes. The mRNA expression of *Ins1*, *Ins2* and Insulin receptors (A) and function related genes *pdx-1*, *glut2*, *glk* (B) in isolated islets were analyzed after 1, 2, or 3 weeks of daily alcohol (or saline vehicle) gavage. Data are means ± SEs; *P<0.05 and **P<0.01 *vs.* saline gavage group with same administration time (N=3 per treatment-time group).

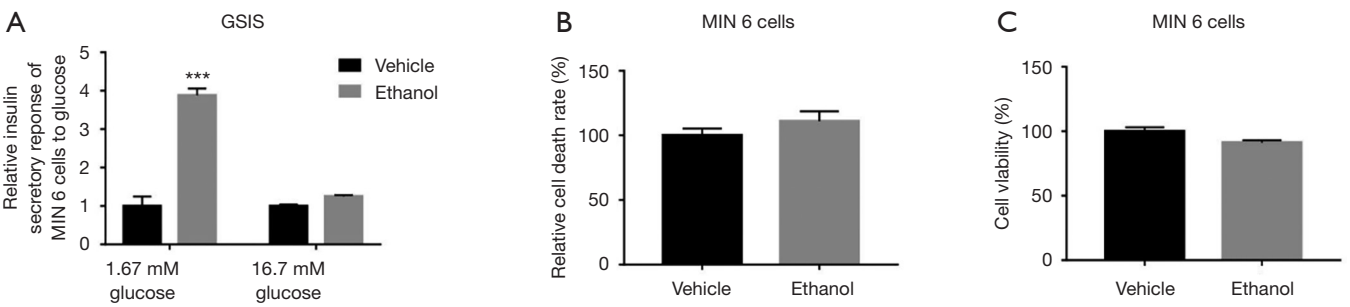

Figure 4 Short-term ethanol exposure did not impair pancreatic islet function and survival. GSIS, relative cell death rate, and cell viability (as determined by an MTT assay) were determined after 24-h exposure to ethanol or vehicle (control group). (A) Relative to MIN6 cells exposed to only vehicle, MIN6 cells exposed to ethanol showed an elevated response to 1.67 mM glucose and a response to 16.7 mM glucose similar to that seen in the control group. (B) Cell death rate and (C) cell viability were similar between the ethanol-exposed and vehicle control MIN6 cell groups. Data are means ± SEs; N=6 per group; *P<0.05 and ***P<0.001 *vs.* vehicle group.

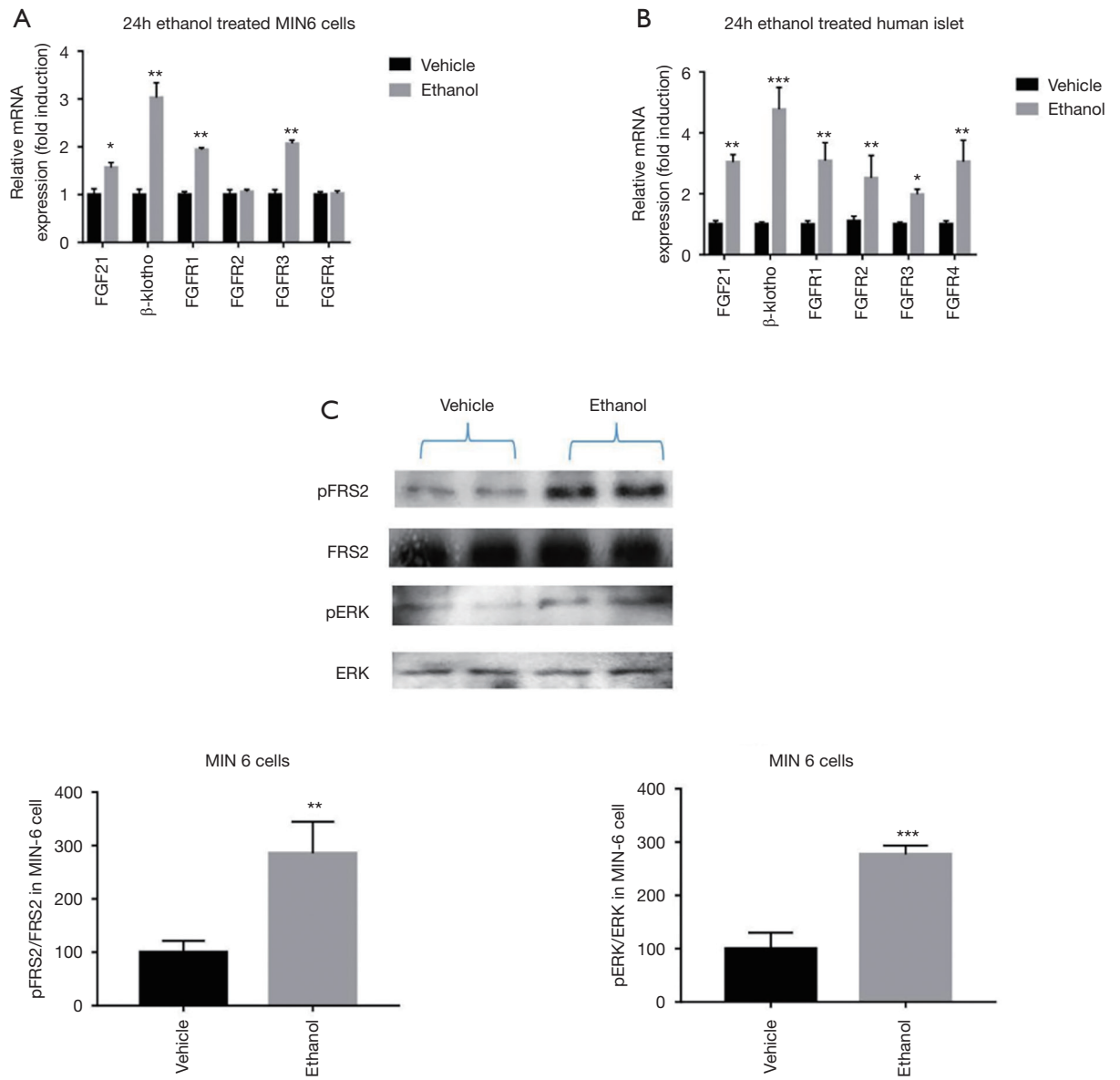

Figure 5 Alcohol exposure altered the expression of FGF21 pathway-related molecules in human islets and MIN6 cells. After 24-h exposure to ethanol (or vehicle), MIN6 cells or human islets were subjected to RT-PCR analysis of *FGF21*, *KLB* (encodes β-klotho), and *FGFR1–4* mRNAs as well as Western blot protein analysis of unphosphorylated and phosphorylated (p) forms of FRS2 and ERK. (A,B) Standard quantitative RT-PCR showed that the ethanol group had elevated *FGF21*, *KRB*, *FGFR1*, and *FGFR3* mRNA levels, compared to the vehicle control group. (C) Densitometric western blot analysis showed very significantly elevated levels of activated FRS2 (pFRS2/FRS2 ratio) and of activated ERK (pERK/ERK ratio) in the ethanol group compared to vehicle control cells. Data are means ± SEs of 3–6 separate experiments; *P<0.05, **P<0.01, and ***P<0.001 *vs.* vehicle group.

The development of FGF21 resistance in pancreatic islets may interfere with FGF21's ability to act a potent endocrine regulator of glucose and lipid metabolism and thus increase T2DM pathogenesis risk.

Our OGTT and GSIS results showed that long-term ethanol exposure reduced insulin levels and insulin secretary ability, consistent with prior studies reporting that chronic ethanol exposure can reduce insulin secretion, increase β-cell

apoptosis and decrease βcell mass (26,27). Islets isolated from mice subjected to long-term ethanol exposure showed marked reductions in *Ins1*, *Ins2*, *Irs1*, and *Irs2* mRNA levels at the 2- and 3-week exposure time points. Chronic ethanol exposure also led to reductions in the mRNA expression of the islet function related genes *Pdx-1*, *Glut2*, and *Glk* within the 3-week experimental period (28,29). These data support the notion that chronic ethanol consumption induces pancreatic β-cell dysfunction and apoptosis (30).

Because previous studies showing that subchronic and acute ethanol drinking can cause substantial increases in serum FGF21 levels in both humans and mice (31,32), we postulated that FGF21 resistance development may be a mediator of ethanol-induced pancreatic islet dysfunction. We confirmed in this study that long-term alcohol consumption (3 weeks) did indeed result in increased serum FGF21 levels in mice *in vivo*. Moreover, in isolated human islets, 24-h ethanol exposure increased FGF21, *FGFR1–4*, and β-klotho mRNA levels. Given that FGF21 resistance is characterized by increased levels of circulating FGF21 levels concomitant with decreased FGF21 receptor expression (33-35), these findings suggest that our ethanol treatments produced a state of FGF21 resistance. Meanwhile, our GSIS, cell death rate, and cell viability data showing that short-term ethanol exposure did not impair β-cell function or survival support the hypothesis that FGF21 may protect pancreatic islets from ethanol-induced pancreatic islet damage similar to the way that it has been shown to be protective against ethanol-induced hepatic damage and the progression of alcoholic liver disease in an acute alcohol drinking model (36-38).

The results of our analysis of ethanol exposure effects on FGF21 signaling pathway component expression support the notion that FGF21 resistance has a strong relationship with pancreatic islet dysfunction (39-41). Islets from mice exposed to our long-term binge drinking model had upregulated levels of phosphorylated FRS2 and phosphorylated ERK, which mediate essential steps in FGF21 signaling (42), leading to downstream changes in gene expression (43,44), in addition to increased mRNA expression of FGF21, β-klotho, and FGF receptors.

Duration is an important factor in alcoholism-associated pancreatic disease (45,46). Thus, we investigated the effects of ethanol exposure on pancreatic islets over multiple time points. After 1 week of ethanol administration (short-term exposure), GSIS and OGTT results remained quite similar between the ethanol exposed and control groups and the expression of FGF21, FGF receptors, and the FGF receptor co-factor β-klotho were only slightly or negligibly raised in the ethanol exposed group. However, substantial changes in these variables were readily apparent after 2 weeks of ethanol administration, and these changes showed further progression by the 3-week time point. Thus, our data indicate that islet function is unlikely to be disrupted by short-term ethanol consumption, at least in the range of the ethanol concentrations used here (47,48). Notwithstanding, an apparent pre-FGF21 resistance islet state induced by short-term ethanol exposure suggests that with additional exposure time, complete FGF21 resistance and consequent pancreatic islet dysfunction will develop if the ethanol exposure becomes a long-term influence.

Conclusions

In summary, the present study demonstrates a new link between pancreatic islet ethanol metabolism and FGF21 resistance, complementing prior work reporting that diabetic hyperglycemia leads to FGF21 resistance in pancreatic islets (49). Our findings suggest that short-term ethanol exposure may induce a pre-FGF21 resistance state, which, although not islet impairing itself, evolves into clinically significant islet FGF21 resistance and functional impairment if there is continued ethanol exposure. These results support the notion that the development of FGF21 resistance in response to chronic alcohol consumption could have an increased risk of developing T2DM. In this sense, FGF21 levels are proposed to serve as a measurement for the pancreatic cell function. We cannot, however, exclude the possibility for the effects of ethanol consumption on the dedifferentiation and apoptosis of pancreatic cells. In fact, dedifferentiation and apoptosis are very important factors for the determination of pancreatic cells dysfunction. Future work should be focused on the detailed mechanism(s) whereby the dedifferentiation and apoptosis of pancreatic alcoholism might be involved.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: All authors are accountable for all aspects of the work, including full data access, integrity of the data and the accuracy of the data analysis. All the experimental procedures were approved by the relevant institutional committee on the use of animals of The Chinese University of Hong Kong.

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Supplementary

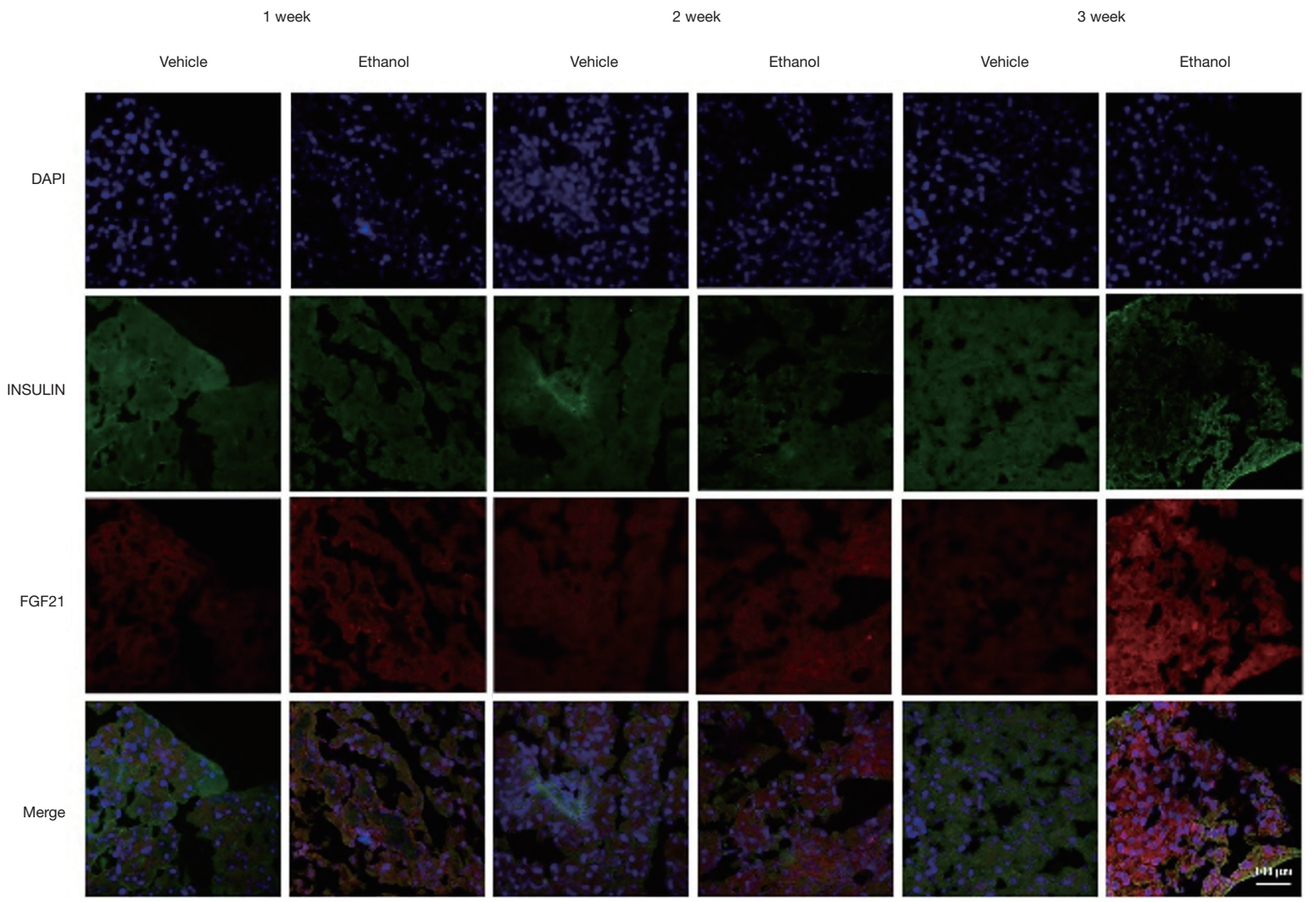

Figure S1 Immunocytochemical examination for the chronic ethanol effects on the expression of insulin and FGF21 in pancreatic islets. Representative images of islets were labeled for DAPI (blue), insulin (green) and FGF21 (red) Scale bar =100 μm.