

High Glucose Represses β -Klotho Expression and Impairs Fibroblast Growth Factor 21 Action in Mouse Pancreatic Islets

Involvement of Peroxisome Proliferator–Activated Receptor γ Signaling

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Circulating fibroblast growth factor 21 (FGF21) levels are elevated in diabetic subjects and correlate directly with abnormal glucose metabolism, while pharmacologically administered FGF21 can ameliorate hyperglycemia. The pancreatic islet is an FGF21 target, yet the actions of FGF21 in the islet under normal and diabetic conditions are not fully understood. This study investigated the effects of high glucose on islet FGF21 actions in a diabetic mouse model by investigating *db/db* mouse islet responses to exogenous FGF21, the direct effects of glucose on FGF21 signaling, and the involvement of peroxisome proliferator–activated receptor γ (PPAR γ) in FGF21 pathway activation. Results showed that both adult *db/db* mouse islets and normal islets treated with high glucose *ex vivo* displayed reduced β -klotho expression, resistance to FGF21, and decreased PPAR γ expression. Rosiglitazone, an antidiabetic PPAR γ ligand, ameliorated these effects. Our data indicate that hyperglycemia in type 2 diabetes mellitus may lead to FGF21 resistance in pancreatic islets, probably through reduction of PPAR γ expression, which provides a novel mechanism for glucose-mediated islet dysfunction. *Diabetes* 62:3751–3759, 2013

Type 2 diabetes mellitus (T2DM), a chronic debilitating disease, results when insulin resistance develops in association with dysregulated insulin secretion and loss of β -cell mass (1). However, emerging physiologic and genetic data suggest that dysfunction of the pancreatic β -cell is the key determinant of whether an insulin-resistant individual will progress to frank hyperglycemia and diabetes (2–4). Previous studies have identified fibroblast growth factor 21 (FGF21) as a potent metabolic regulator; it is a distinctive member of the FGF family that acts through a canonical FGF receptor (FGFR) with four isoforms in the presence of the cofactor β -klotho (5–7). Binding of FGFs to FGFRs leads to receptor dimerization and autophosphorylation, which phosphorylate

the tyrosine residues of FGF receptor substrate 2 (FRS2) by tyrosine kinase. Phosphorylated FRS2 acts as a docking protein forming a complex with Grb2/Sos, which in turn activates the extracellular signal–regulated kinase (ERK) pathway (6,8,9). Nuclear translocation of phosphorylated ERK1/2 triggers rapid transcription of immediate early genes such as *Egr1* and *cFos* (10,11). Indeed, restricted expression of β -klotho in metabolically potent organs such as liver, adipose tissue, and pancreas (7,12) provides a mechanistic basis for FGF21's tissue-specific influence on glucose and lipid homeostasis, suggesting important roles for β -klotho and FGF21 signaling in these tissues.

Growing evidence points to FGF21 as a potential therapeutic agent for treatment of T2DM, obesity, and their complications since pharmacological doses of FGF21 reduce plasma glucose and triglycerides to near normal levels and improve glucose clearance and insulin sensitivity in both *ob/ob* and *db/db* mice; transgenic mice overexpressing FGF21 exhibit similar effects and are resistant to diet-induced weight gain and fat accumulation (13,14). Furthermore, treatment of nonhuman primates with pharmacologic doses of FGF21 leads to improvements in lipoprotein profiles and levels of circulating cardiovascular risk markers (15). In high-fat diet–induced obese mice, FGF21 treatment reverses hepatic steatosis (16,17), and consistent with its actions on lipid oxidation in liver and lipolysis in white adipose tissue, mice lacking FGF21 develop mild obesity and have increased hepatic fat content when fed a ketogenic diet (18). Notably, FGF21 has also been reported to improve pancreatic β -cell function and preserve islet and β -cell mass (19); most prior studies of this factor have focused on the benefits of treatment of T2DM and obesity with pharmacologic doses of FGF21; however, as a metabolic modulator, the actions of FGF21 in target tissues under normal and diabetic conditions, and in the pathogenesis of T2DM, are not fully understood. Clinical studies have shown that circulating FGF21 levels correlate with abnormalities of glucose metabolism and with insulin resistance (20–22). FGF21 expression in the liver and white adipose tissue is increased in diabetic rodents (23), but these increases occur in the context of impaired glucose tolerance and increased hepatic lipid content, suggesting that the ability of endogenous FGF21 to exert beneficial effects on glucose homeostasis and lipid oxidation is impaired in the diabetic state (i.e., T2DM may be a state of FGF21 resistance) (10).

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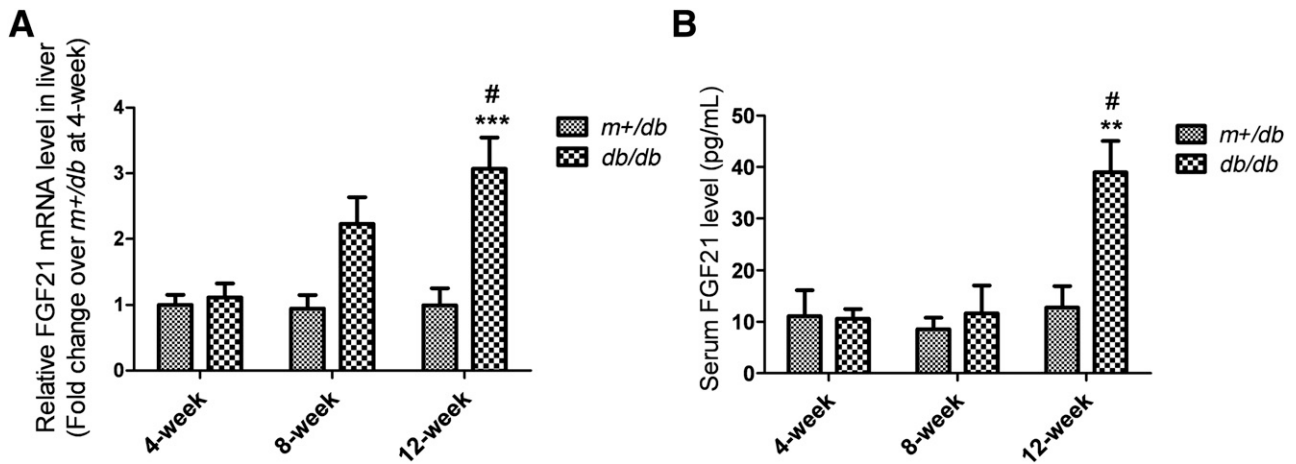


FIG. 1. Hepatic FGF21 mRNA and circulating levels of FGF21 are increased in *db/db* mice by 12 weeks of age. *A*: mRNA expression as quantified by standard quantitative RT-PCR. *B*: Circulating FGF21 levels as determined by ELISA of serum from a terminal bleed. Data are means \pm SEs. ^{*}*P* < 0.01; ^{***}*P* < 0.001 vs. age-matched *m+/db* group; [#]*P* < 0.05 vs. 4-week *db/db* group (*n* = 5 to 6).

Given that pancreatic islet dysfunction is the central factor determining the progression of T2DM and that the pancreatic islet is an FGF21 target, we hypothesized that FGF21 action is altered in pancreatic islets under diabetic as compared with normal conditions. To test this hypothesis, the action of FGF21 on pancreatic islets throughout progression to T2DM in diabetic *db/db* and lean mice was examined. We examined the direct effects of glucose on

FGF21 actions in islets, including involvement of peroxisome proliferator-activated receptor γ (PPAR γ).

RESEARCH DESIGN AND METHODS

Animal models. Male genetically diabetic C57BL/KSJ *db/db* mice, their age-matched, nondiabetic C57BL/KSJ *m+/db* littermates, and C57BL/KSJ mice were obtained from the Laboratory Animal Services Center of the Chinese University of Hong Kong. The experimental procedures were approved by the

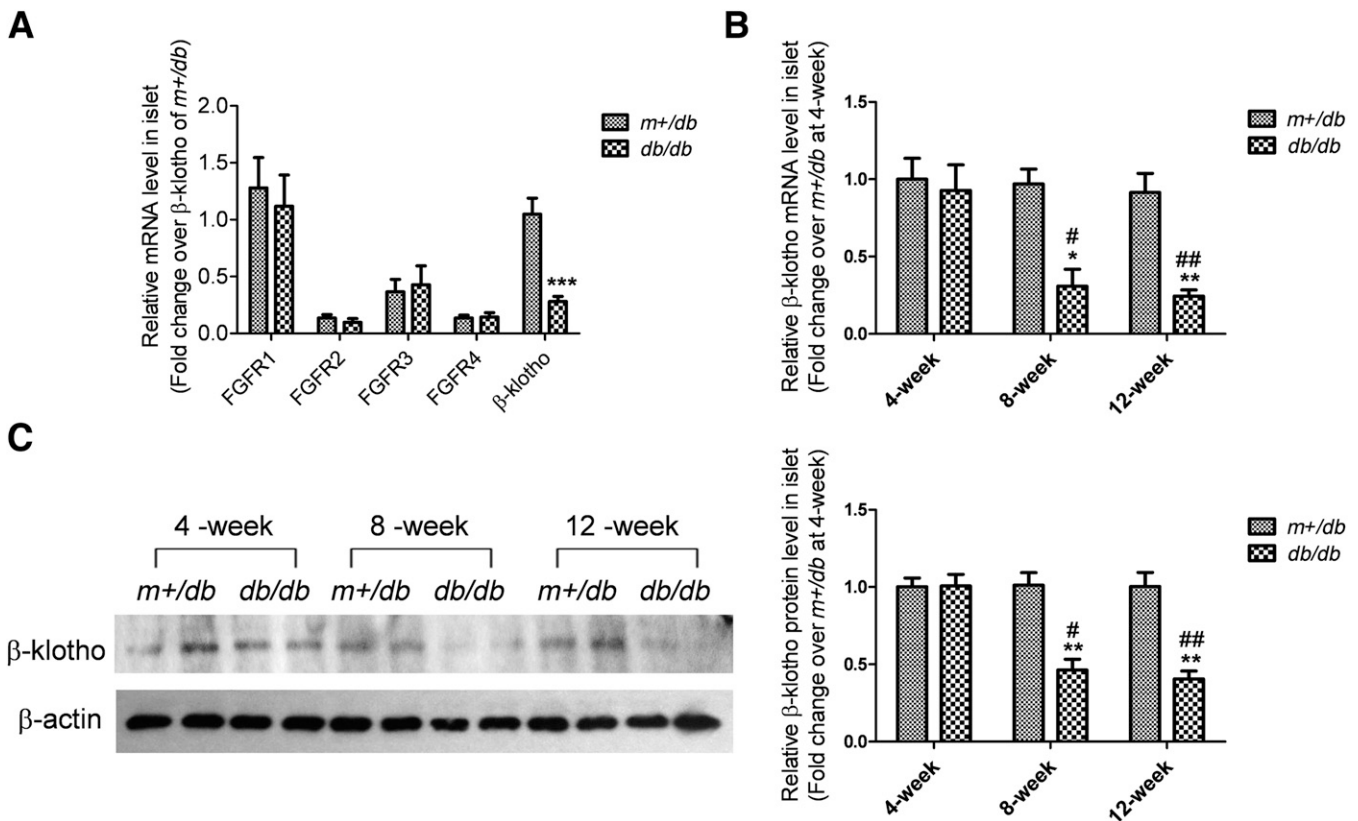


FIG. 2. β -Klotho is downregulated in islets of adult *db/db* mice. mRNA levels of FGFRs and β -klotho (*A*) were determined in 12-week-old *m+/db* and *db/db* mice. mRNA (*B*) and protein (*C*) levels of β -klotho in islets were analyzed in 4-, 8-, and 12-week-old mice. For the Western blot, β -actin was used as a loading control. Densitometry is shown and was calculated as β -klotho/ β -actin. Data are means \pm SEs. ^{*}*P* < 0.05; ^{**}*P* < 0.01; ^{***}*P* < 0.001 vs. age-matched *m+/db* group; [#]*P* < 0.05; ^{##}*P* < 0.01 vs. 4-week *db/db* group (*n* = 4 to 5).

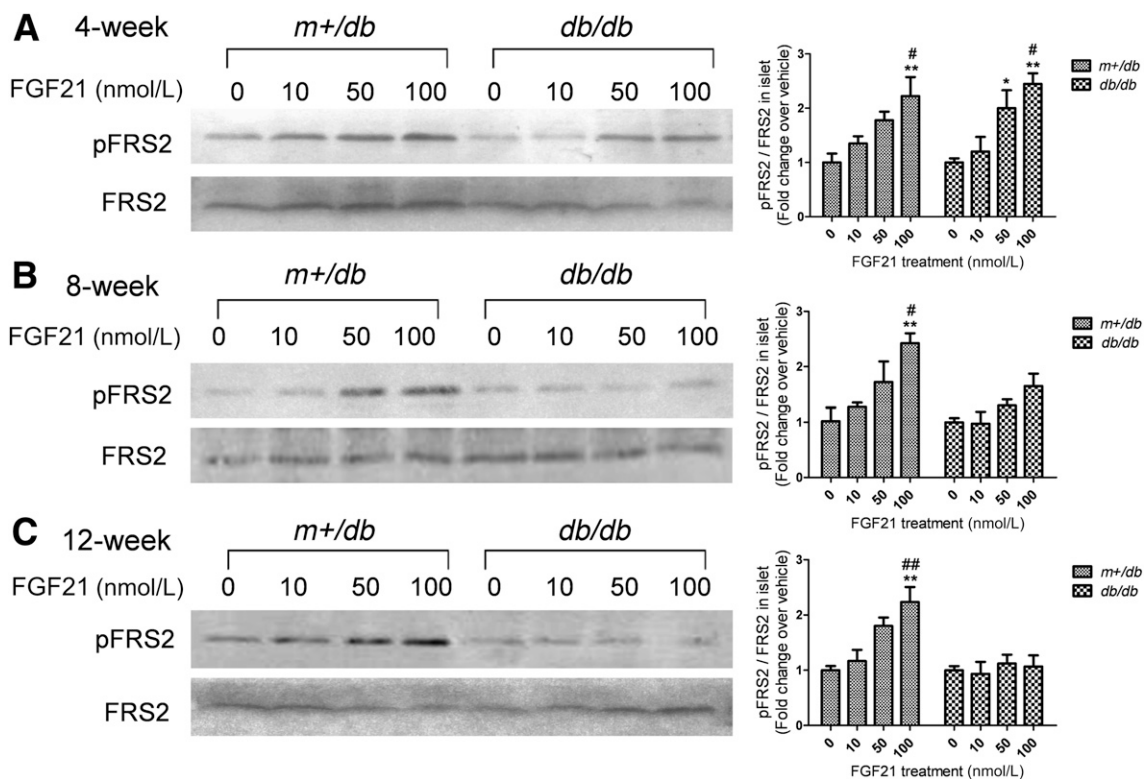


FIG. 3. FGF21-induced FRS2 phosphorylation is attenuated in islets from adult *db/db* mice relative to *m+/db* mice. Islets were isolated from the mice at 4 (A), 8 (B), and 12 weeks (C) of age and treated with FGF21 (at the indicated concentrations) for 10 min. Phosphorylated (pFRS2) and total FRS2 expression were quantitated in Western blots. Densitometry is shown and calculated as pFRS2/total FRS2. Data are means \pm SEs. * $P < 0.05$; ** $P < 0.01$ vs. 0 nmol/L group; # $P < 0.05$; ## $P < 0.01$ vs. 10 nmol/L group ($n = 4$).

Animal Experimentation Ethics Committee of the Chinese University of Hong Kong (reference number 10/059/GRF-4).

Pancreatic islet isolation, primary culture, and treatments. Intact pancreatic islets were isolated from mice as previously described (24). Islets were cultured overnight in RPMI 1640 medium (Invitrogen, Carlsbad, CA) supplemented with 10% (volume for volume) FBS (Gibco Laboratories, Grand Island, NY), 1% (volume for volume) penicillin, and streptomycin (Invitrogen). Isolated islets were treated with 5.6 or 28 mmol/L D-glucose (Sigma-Aldrich, St. Louis, MO), 20 μ mol/L rosiglitazone, or 20 μ mol/L GW9662 (Sigma-Aldrich) for the indicated periods of time.

Analysis of FGF21 signaling. For analyses of acute FGF21-induced signaling events in the pancreatic islet, isolated islets were exposed to endotoxin-free tagless recombinant FGF21 (Antibody and Immunoassay Services, University of Hong Kong) (25); briefly, isolated islets were treated with FGF21 (0–100 nmol/L) for 10 min or 1 h and then subjected to quantitative protein or mRNA analysis, respectively.

Western blotting. Total protein per standardized number of islets was extracted using the CytoBuster Protein Extraction Reagent (Novagen, Madison, WI). Proteins were separated by SDS-PAGE, transferred to nitrocellulose membrane (Bio-Rad, Munich, Germany), and probed with antibodies against the following proteins: β -klotho, β -actin, total FRS2, total PPAR γ (Santa Cruz Biotechnology Inc., Santa Cruz, CA), and phospho-FRS2 (Y196; Cell Signaling Technology, Danvers, MA). The Western blot bands were quantitated with ImageJ software (National Institutes of Health).

Quantitative RT-PCR. Total RNA from islets or flash-frozen liver tissue samples was extracted using TRIzol reagent (Invitrogen) and subjected to reverse transcription using the iScript Select cDNA Synthesis Kit (Bio-Rad). Relative gene expression was quantified by real-time PCR using iQ SYBR Green Supermix (Bio-Rad). The reactions were performed using an i-Cycler Thermal Cycler (version 3.1; Bio-Rad). Relative gene expression was analyzed using the $2^{-\Delta\Delta Ct}$ method (26) and normalized relative to glyceraldehyde 3-phosphate dehydrogenase. The sequences of the primers used are listed in Supplementary Table 1.

Plasma FGF21 concentrations. Blood samples were collected on ice and spun at 4°C. Plasma FGF21 concentrations were determined by a specific mouse ELISA kit (Antibody and Immunoassay Services, University of Hong Kong), as previously described (27).

Statistical analysis. Data are displayed as means \pm SEs. Comparisons between groups were analyzed by two-tailed Student *t* test, or one-way ANOVA, followed by Tukey post hoc test, in which $P < 0.05$ was considered statistically significant.

RESULTS

Hepatic FGF21 mRNA and circulating levels of FGF21 are elevated in diabetic *db/db* mice in an age-dependent manner. Hepatic FGF21 mRNA levels in *db/db* diabetic mice and *m+/db* lean mice were similar at 4 weeks, but were approximately twofold and threefold greater in *db/db* mice than *m+/db* mice at 8 and 12 weeks, respectively (Fig. 1A). Similar results were obtained for circulating FGF21 levels; the levels did not differ significantly between *db/db* and *m+/db* mice at 4 and 8 weeks, but were elevated approximately threefold in *db/db* mice relative to *m+/db* mice at 12 weeks, thereby demonstrating an age-dependent change (Fig. 1B; 4 weeks: *m+/db*, 11.10 ± 5.04 pg/mL and *db/db*, 10.62 ± 1.87 pg/mL; 8 weeks: *m+/db*, 8.58 ± 2.22 pg/mL and *db/db*, 11.65 ± 5.41 pg/mL; 12 weeks: *m+/db*, 12.76 ± 4.53 pg/mL and *db/db*, 39.00 ± 6.08 pg/mL).

β -Klotho is downregulated in pancreatic islets from *db/db* mice in an age-dependent manner. To study the action of FGF21 in islets under diabetic conditions, the expression of FGFRs and the cofactor β -klotho in islets of *db/db* and *m+/db* mice was examined. In 12-week-old mice, there was no apparent effect of diabetes on islet FGFRs expression since the mRNA levels of all FGFRs were unchanged in *db/db* mouse islets relative to levels in *m+/db* mouse islets. However, mRNA levels of the FGF21 cofactor β -klotho were reduced by 72% in *db/db*

mouse islets compared with levels observed in *m+/db* mouse islets (Fig. 2A).

A progressive reduction in β -klotho mRNA levels was seen with increasing age (Fig. 2B). At 4 weeks of age, β -klotho mRNA levels were similar in *db/db* islets versus *m+/db* islets. However, β -klotho mRNA levels were 69 and 72% lower in *db/db* islets versus *m+/db* islets at 8 and 12 weeks of age, respectively. There was a corresponding decrease in β -klotho protein levels in *db/db* mouse islets that became apparent at 8 and 12 weeks of age (Fig. 2C).

FGF21-induced phosphorylation of FRS2 is attenuated in islets from adult *db/db* mice. To determine FGF21 signaling in islets, FRS2 phosphorylation was used as a reporter. In isolated islets treated with FGF21 (0, 10, 50, or 100 nmol/L) for 10 min, FGF21 dose-dependently induced FRS2 phosphorylation in islets from both 4-week-old lean and diabetic mice (Fig. 3A). In contrast, in 8-week-old *db/db* mice, FGF21-induced phosphorylation was decreased with profound impairment noted in islets isolated from 12-week-old *db/db* mice (Fig. 3B and C).

FGF21-induced expression of immediate early genes is impaired in islets from adult *db/db* mice. To confirm FGF21 resistance, FGF21-induced immediate early genes

expression was used as a secondary readout for FGF21 action in islets. As shown in Fig. 4A and B, both *Egr1* and *cFos* mRNA expression was induced in islets from 4-week-old *m+/db* and *db/db* mice after stimulation with FGF21 (0, 50, or 100 nmol/L) for 1 h. However, FGF21 induction of these immediate early genes was attenuated in *db/db* mice, relative to *m+/db* mice, at 8 weeks of age (Fig. 4C and D) and profoundly impaired in islets from 12-week-old *db/db* mice (Fig. 4E and F). Thus, the aforementioned reduced induction of FRS2 phosphorylation in *db/db* islets was accompanied by attenuated induction of expression of immediate early genes in diabetic mouse islets in an age-dependent manner.

High glucose treatment reduces the expression of β -klotho and attenuates FGF21 signaling in isolated islets. To investigate the effects of isolated hyperglycemia on FGF21 action, islets isolated from normal C57BL/KSJ mice were treated with 28 mmol/L glucose and FGF21 signaling was examined. As shown in Fig. 5A, a time-course analysis showed that high glucose (28 mmol/L) reduced β -klotho protein expression with maximal inhibition occurring after 72 h of glucose treatment (55% reduction), while the FGFRs mRNA expression remained unchanged

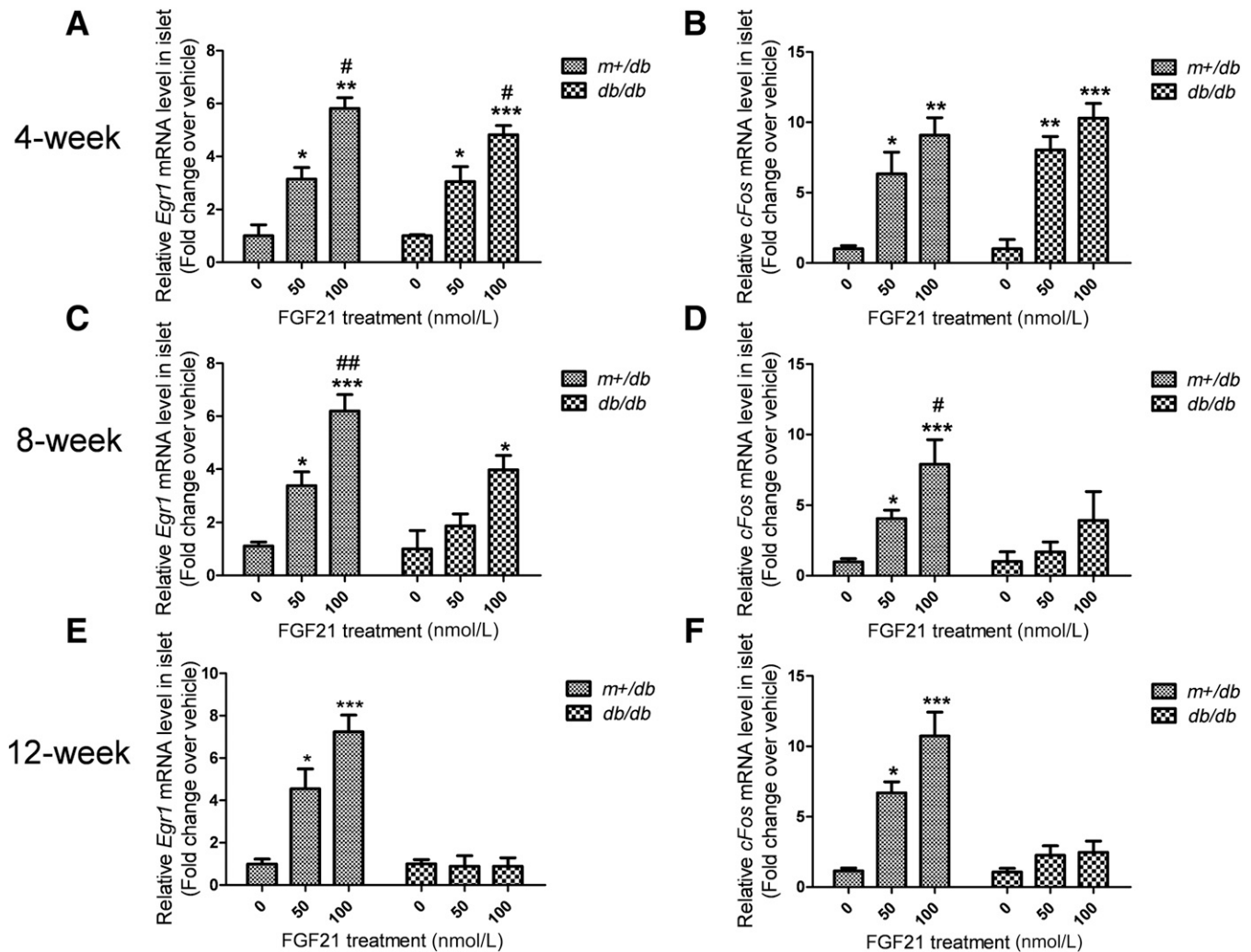


FIG. 4. FGF21-induced expression of immediate early genes is attenuated in islets of adult *db/db* mice relative to *m+/db* mice. Islets were isolated from the mice at 4 (A and B), 8 (C and D), and 12 weeks (E and F) of age and treated with FGF21 (at the indicated concentrations) for 1 h. *Egr1* (A, C, and E) and *cFos* (B, D, and F) mRNA levels were analyzed. Data are means \pm SEs. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ vs. 0 nmol/L group; # $P < 0.05$; ## $P < 0.01$ vs. 50 nmol/L group ($n = 5$ to 6).

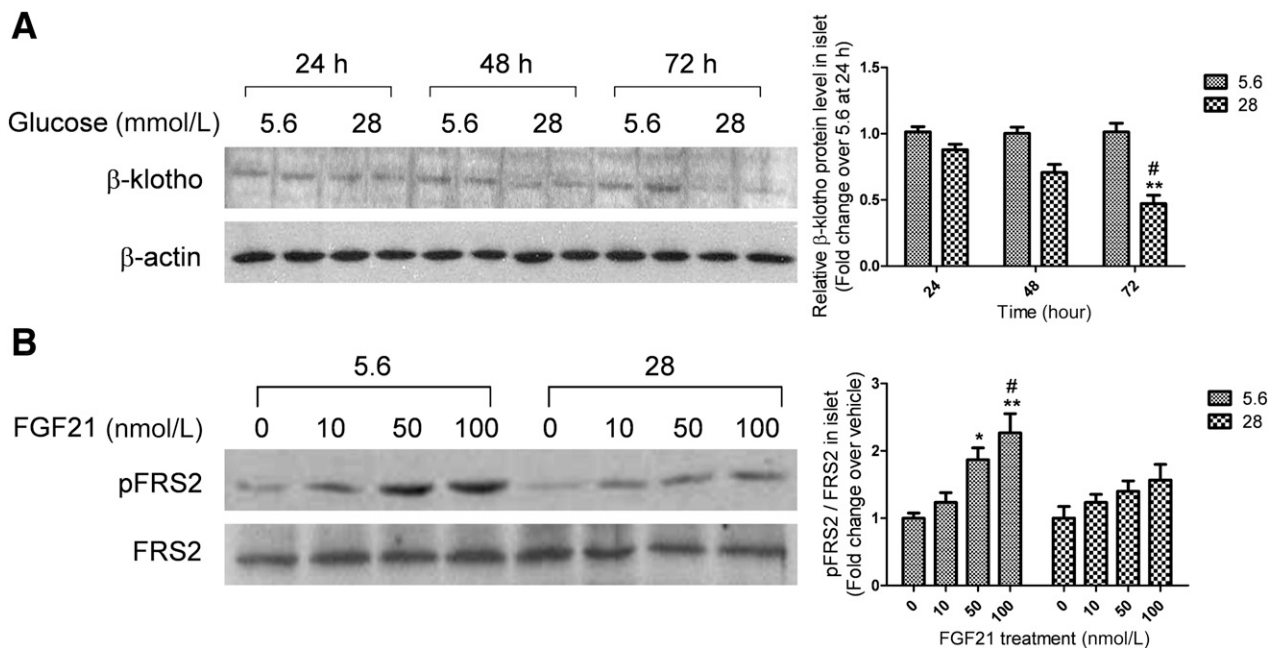


FIG. 5. High glucose reduces β -klotho expression and impairs FGF21-induced FRS2 phosphorylation in islets. **A:** Islets were isolated from lean mice and treated with 5.6 or 28 mmol/L glucose for 24, 48, or 72 h. $**P < 0.01$ vs. time-matched 5.6 mmol/L group; $\#P < 0.05$ vs. 24-h 28 mmol/L group ($n = 4$). **B:** Islets were isolated from lean mice and pretreated with 5.6 mmol/L or 28 mmol/L glucose for 72 h, then were treated with FGF21 (at the indicated concentrations) for 10 min. $*P < 0.05$; $**P < 0.01$ vs. 0 nmol/L group; $\#P < 0.05$ vs. 10 nmol/L group ($n = 4$). Data are means \pm SEs. pFRS2, phosphorylated FRS2.

(Supplementary Fig. 1). Exposure to 22.4 mmol/L L-glucose together with 5.6 mmol/L D-glucose for 72 h (to control for potential nonspecific effects of high sugar osmolarity) did not affect β -klotho expression. Palmitic acid (400 μ mol/L) also did not exert any effect (Supplementary Fig. 2A and B). To determine whether downregulation of β -klotho by high glucose was associated with alterations in FGF21 action, we evaluated the ability of FGF21 to induce FRS2 phosphorylation in islets after glucose treatment. Islets that were pretreated with high glucose for 72 h displayed markedly weakened FGF21-induced FRS2 phosphorylation compared with those pretreated with normal glucose (5.6 mmol/L) (Fig. 5B).

PPAR γ activation prevents downregulation of β -klotho and rescues impairment of FGF21 signaling in high glucose-treated islets or *db/db* mouse islets. Since elevated glucose reduced β -klotho expression and FRS2 phosphorylation in pancreatic islets, we tested whether the PPAR γ ligand rosiglitazone, an agent that protects islets against glucotoxicity, might prevent β -klotho repression. As shown in Fig. 6A, for islets treated with 28 mmol/L glucose for 72 h, cotreatment with rosiglitazone restored β -klotho protein expression to levels not significantly different from levels observed in islets maintained in normal conditions. Overnight pretreatment with the PPAR γ antagonist GW9662 blocked the effect of rosiglitazone.

The alterations of β -klotho expression by rosiglitazone and GW9662 were accompanied by corresponding alterations in FGF21-induced FRS2 phosphorylation. FRS2 phosphorylation impaired by high glucose was restored by rosiglitazone, and this restoration could then be counteracted by GW9662 (Fig. 6B). Similarly, rosiglitazone rescued β -klotho repression and impaired FRS2 phosphorylation in *db/db* mouse islets, while GW9662 again cancelled the effects of rosiglitazone (Fig. 6C and D).

PPAR γ expression is reduced in high glucose-treated islets and *db/db* mouse islets, but is improved by rosiglitazone. To elucidate the interactions between glucose and PPAR γ in pancreatic islets, PPAR γ expression was evaluated in high glucose-treated islets and *db/db* mouse islets. As shown in Fig. 7A, 72-h high glucose treatment decreased PPAR γ expression. Rosiglitazone inhibited such change, while GW9662 cancelled rosiglitazone's effect. Relative to islets from *m+/db* mice, islets from 12-week-old *db/db* mice showed a significant decrease in PPAR γ level. Again, rosiglitazone reversed such an effect, and the rosiglitazone's effect was blocked by GW9662 (Fig. 7B). A progressive age-related decrease in PPAR γ expression was observed in *db/db* mouse islets at 8 and 12 weeks of age, while no significant difference was detected at 4 weeks versus *m+/db* mice (Fig. 7C). Consistent with the aforementioned alterations in β -klotho expression, age-related changes in PPAR γ expression were observed in high glucose-treated islets and *db/db* mouse islets.

DISCUSSION

The present data show for the first time that high glucose, a pivotal component of the type 2 diabetic milieu, induces FGF21 resistance in pancreatic islets, likely through a reduction in PPAR γ expression. The concept of FGF21 resistance originated from studies showing that serum concentrations of FGF21 were increased in *db/db* mice and human patients with T2DM (23,28). Clinical studies have revealed that serum FGF21 levels correlate with severity of glucose intolerance and insulin resistance (20,21). In this study, we confirmed that increased hepatic expression and serum levels of FGF21 were associated with the diabetic state and showed that these increases were age-dependent and, as with progressive

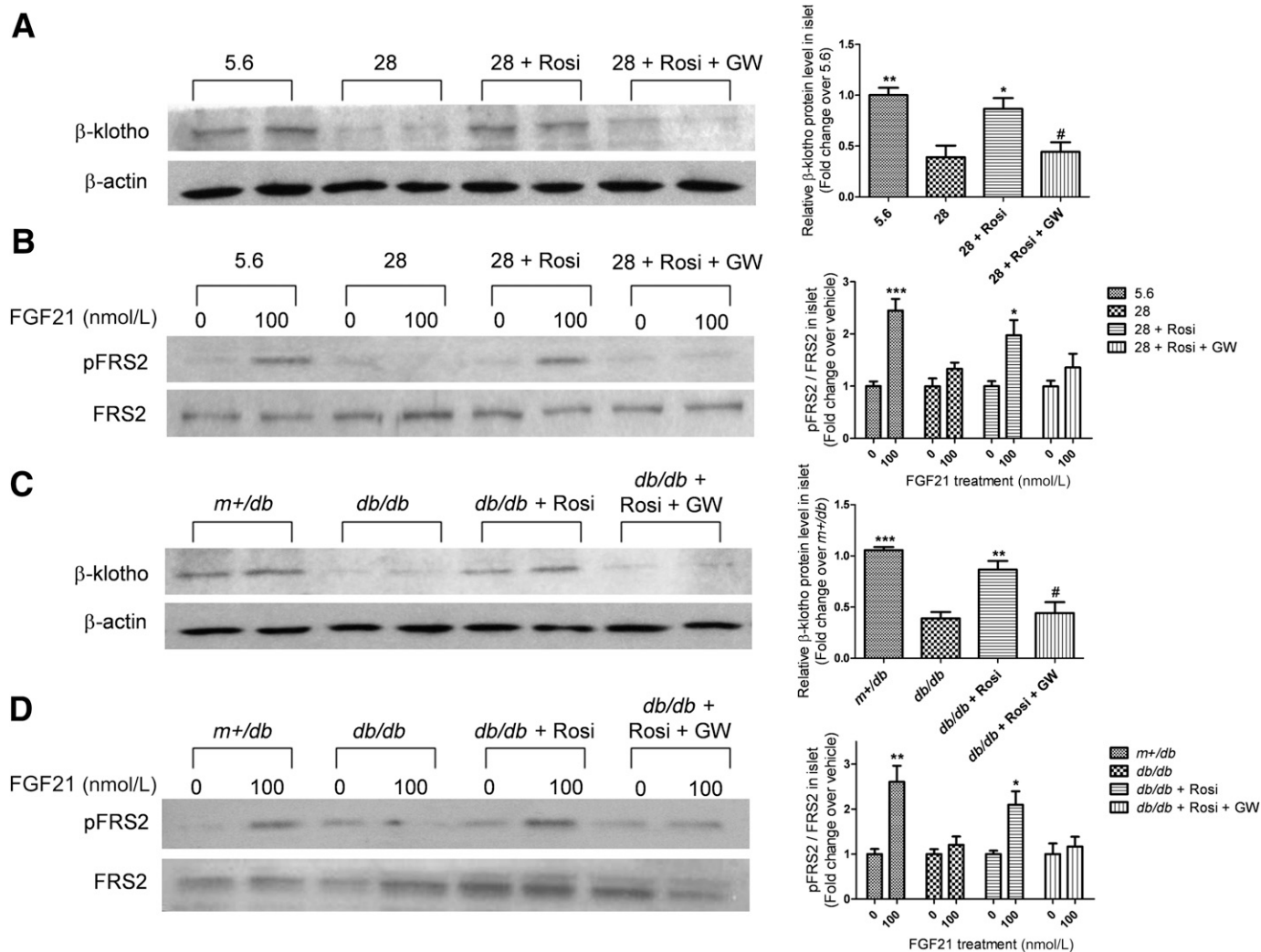


FIG. 6. Rosiglitazone reverses the downregulation of β -klotho and impairment of FGF21-induced FRS2 phosphorylation in islets subjected to high glucose treatment and in adult *db/db* mouse islets, and these effects are blocked by the PPAR γ antagonist GW9662. **A:** Islets were isolated from lean mice and treated with 5.6 or 28 mmol/L glucose, 20 μ mol/L rosiglitazone (Rosi), with or without 20 μ mol/L GW9662 (GW) for 72 h. * P < 0.05; ** P < 0.01 vs. 28 mmol/L group; # P < 0.05 vs. 28 mmol/L + Rosi group (n = 4). **B:** Pretreated islets were exposed to 0 or 100 nmol/L FGF21 for 10 min for signaling analyses. * P < 0.05; *** P < 0.001 vs. 0 nmol/L group (n = 4). **C:** Isolated islets of adult *m+/db* and *db/db* mice were treated with 20 μ mol/L Rosi, with or without 20 μ mol/L GW, for 72 h. ** P < 0.01; *** P < 0.001 vs. *db/db* group; # P < 0.05 vs. *db/db* + Rosi group (n = 4). **D:** Pretreated islets were exposed to 0 or 100 nmol/L FGF21 for 10 min for signaling analyses. * P < 0.05; ** P < 0.01 vs. 0 nmol/L group (n = 4). Data are means \pm SEs. pFRS2, phosphorylated FRS2.

hyperglycemia, appeared only in adult *db/db* mice with overt T2DM.

Paradoxically, despite high endogenous levels of FGF21 in diabetic adult *db/db* mice, pharmacological doses of exogenous FGF21 have been shown to improve metabolic parameters (13). The fact that high endogenous FGF21 levels fail to produce beneficial effects while high pharmacological doses induce the expected results suggests a state of FGF21 resistance existing in overt T2DM. We postulated that FGF21 actions in pancreatic islets might be abnormal in T2DM (19). Our results indicated that, under diabetic conditions, pancreatic islets were FGF21-resistant, largely due to downregulation of β -klotho, an essential cofactor for FGF21 (5,7). That was associated with a reduction in the capacity of FGF21 to induce signaling in *db/db* mouse islets, as shown by marked reductions in the induction of FRS2 phosphorylation, the essential step linking FGFRs to the ERK1/2 signaling pathway (9); and the reduced expression of immediate early genes (*Egr1*

and *cFos*) regulated by the ERK1/2 pathway (10,11). These effects were not apparent until the *db/db* mice reached adulthood (after 8 weeks of age), a finding matching previous work showing that mice lacking β -klotho are unresponsive to FGF21 stimulation (29).

The natural history of *db/db* mice follows a distinct pattern: they have normal glycemia at 4 weeks of age due to compensatory increases in circulating insulin; hyperglycemia develops once insulin secretion can no longer compensate for the increased insulin resistance, from ~7 weeks of age, and blood glucose continues to increase with age (30). We found that the patterns of β -klotho expression and FGF21's actions in *db/db* mouse islets followed these changes during the progression toward overt diabetes, suggesting that glucose itself is a potential mediator of islet FGF21 resistance. Our ex vivo experiments further demonstrated that prolonged high glucose exposure can dramatically reduce β -klotho expression and impair FGF21 signaling in pancreatic islets. High glucose

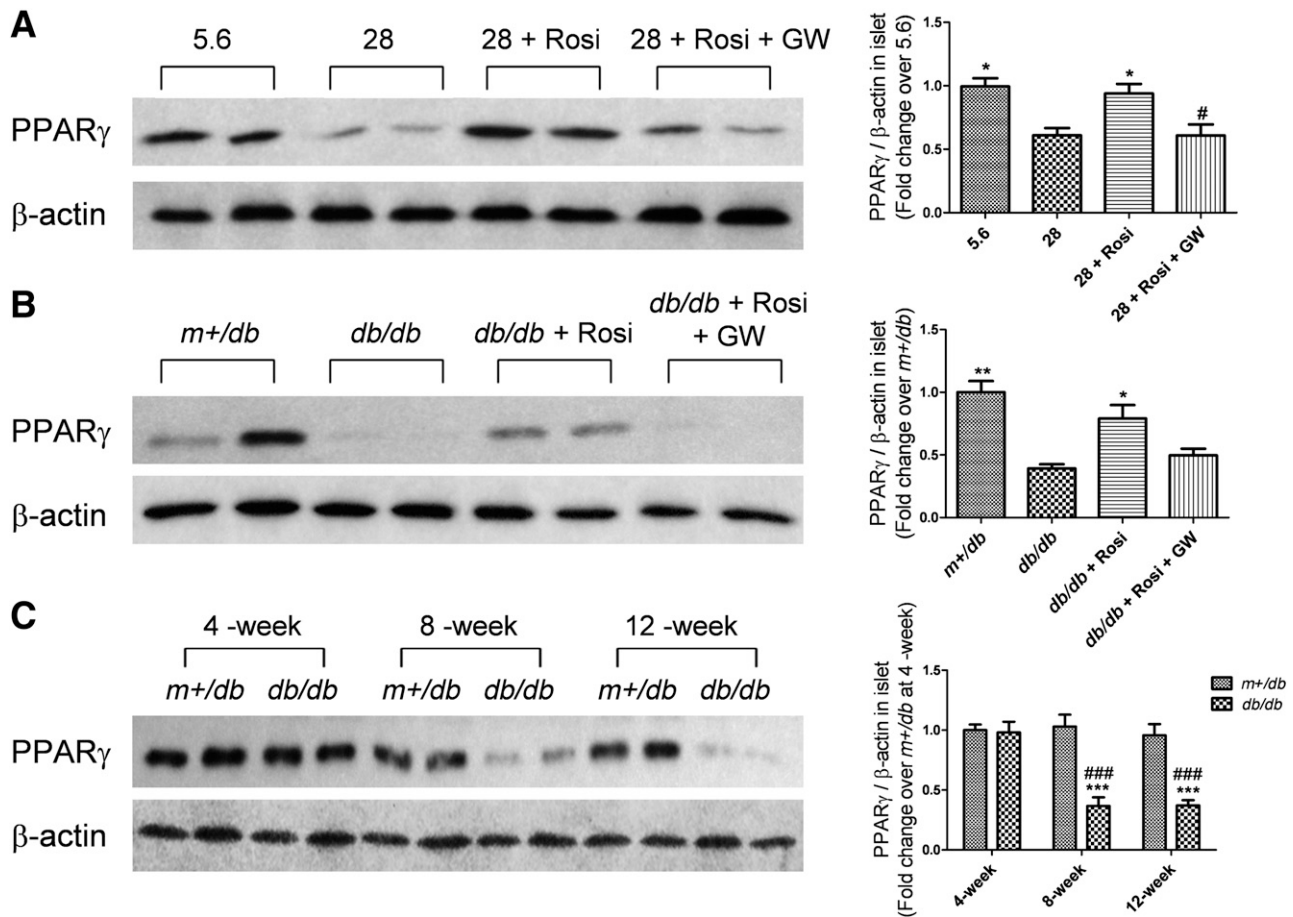


FIG. 7. PPAR γ expression is reduced in high glucose-treated islets and adult *db/db* mouse islets, which is reversed by rosiglitazone. **A:** Isolated islets from lean mice were treated with 5.6 or 28 mmol/L glucose, 20 μ mol/L rosiglitazone (Rosi), with or without 20 μ mol/L GW9662 (GW), for 72 h. * P < 0.05 vs. 28 mmol/L group; # P < 0.05 vs. 28 mmol/L + Rosi group (n = 6). **B:** Isolated islets from 12-week-old *m+/db* and *db/db* mice were treated with 20 μ mol/L Rosi with or without 20 μ mol/L GW for 72 h. * P < 0.05; ** P < 0.01 vs. *db/db* group (n = 6). **C:** Islet PPAR γ expression was examined in *m+/db* and *db/db* mice at 4, 8, and 12 weeks of age. *** P < 0.001 vs. age-matched *m+/db* group; ### P < 0.001 vs. 4-week *db/db* group (n = 4). β -Actin was used as a loading control. Densitometry is shown and calculated as PPAR γ / β -actin. Data are means \pm SEs.

concentrations in culture media mimicked diabetic hyperglycemia, supporting the possibility that β -klotho repression and blunted FGF21 activity in diabetic mouse islets were, at least in part, due to hyperglycemia. Furthermore, in both high glucose-treated and *db/db* mouse islets, β -klotho, but not the FGFRs, was downregulated, and, since β -klotho has tissue-restricted expression as opposed to the widespread localization of FGFRs (31), this suggests different roles for β -klotho and FGF21 signaling in modulating glucose-induced toxicity in islets in T2DM.

We hypothesized that antihyperglycemic drugs (e.g., thiazolidinediones) exert some of their beneficial effects through improvement in FGF21 responsiveness in islets. The thiazolidinediones rosiglitazone and pioglitazone are strong synthetic agonists of PPAR γ and have potent antidiabetic effects (32,33). PPAR γ is expressed in pancreatic islets (34) and PPAR-responsive elements have been identified in the promoters of genes involved in islet function, such as *Glut2* and *Pdx1* (35–37). Moreover, PPAR γ activation has a number of direct pro-survival and profunction effects on pancreatic islet cells (38,39). In our study, we found that rosiglitazone reversed the downregulation of β -klotho in both *db/db* mouse islets and high glucose-treated islets, leading to restoration of FGF21 signaling. GW9662, a PPAR γ antagonist (40), completely blocked the effects

of rosiglitazone, implying that direct binding of the ligand was required for PPAR γ 's actions. These findings are consistent with prior reports showing that rosiglitazone promoted β -klotho expression and enhanced FGF21 actions on adipocytes (41,42). Our data illustrate that β -klotho expression and FGF21 activity in pancreatic islets can be regulated by PPAR γ modulation. Therefore, we suggest that glucose may regulate islet FGF21 actions through modulation of PPAR γ expression and/or activity, a signaling that can be interrupted by rosiglitazone.

PPAR γ expression may be altered by environmental changes or stress. Chronic high glucose exposure can decrease PPAR γ mRNA levels in mouse islets (43). Consistently, in both high glucose-treated islets and *db/db* mouse islets, PPAR γ expression was found to be reduced that could be reversed with rosiglitazone. The timeline of these effects is worthwhile noting; the age-associated development of the diabetic phenotype: *db/db* mice developing high blood glucose as they reach adulthood was associated with reductions in PPAR γ and β -klotho expression. These data suggest that hyperglycemia reduces PPAR γ expression in *db/db* mouse islets. The fact that rosiglitazone-induced PPAR γ activation rescues β -klotho repression makes it likely that repression of β -klotho by high glucose is the result of glucose-mediated downregulation of PPAR γ

and provides clues as to why rosiglitazone-mediated PPAR γ activation favors FGF21 effects (42). PPAR γ has been characterized as a master regulator of genes transcription; in islets, pharmacologic PPAR γ activation regulates various genes for which products mediate key aspects of β -cell function. Transcription of klotho, the homolog of β -klotho, was also shown to be directly regulated by PPAR γ (44,45), suggesting that the whole klotho family would be regulated by PPAR γ . However, whether PPAR γ regulates β -klotho expression by direct activation of gene transcription or through modulation of other signaling pathways is still unclear, and further investigation is needed to delineate PPAR γ and β -klotho interactions.

Chronic hyperglycemia contributes to the progressive loss of islet function and survival through mechanisms involving activation of oxidative stress and/or fatty acid toxicity (46). In this study, we present a novel pathway for glucotoxicity in islets through hyperglycemia-induced FGF21 resistance, probably through downregulation of PPAR γ .

In conclusion, our results highlight the involvement of PPAR γ in the negative regulation of islet β -klotho expression and FGF21 action by high glucose. Thus, we suggest that FGF21 could prove to have therapeutic value, especially in combination with an antihyperglycemic agent that itself promotes FGF21 actions in T2DM.

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No potential conflicts of interest relevant to this article were reported.

W.Y.S. designed and performed experiments, analyzed and interpreted data, and drafted the manuscript. Q.C. and L.C. performed experiments and reviewed the manuscript. C.E.-M. reviewed the manuscript. A.X. and K.S.L.L. reviewed and edited the manuscript. P.S.L. designed the experiments, analyzed and interpreted data, and edited and revised the manuscript. P.S.L. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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REFERENCES

- Kahn SE. The importance of the beta-cell in the pathogenesis of type 2 diabetes mellitus. *Am J Med* 2000;108(Suppl. 6a):2S–8S
- Bell GI, Polonsky KS. Diabetes mellitus and genetically programmed defects in beta-cell function. *Nature* 2001;414:788–791
- Del Prato S, Marchetti P, Bonadonna RC. Phasic insulin release and metabolic regulation in type 2 diabetes. *Diabetes* 2002;51(Suppl. 1):S109–S116
- Gerich JE. The genetic basis of type 2 diabetes mellitus: impaired insulin secretion versus impaired insulin sensitivity. *Endocr Rev* 1998;19:491–503
- Suzuki M, Uehara Y, Motomura-Matsuzaka K, et al. betaKlotho is required for fibroblast growth factor (FGF) 21 signaling through FGF receptor (FGFR) 1c and FGFR3c. *Mol Endocrinol* 2008;22:1006–1014
- Kharitonov A, Dunbar JD, Bina HA, et al. FGF-21/FGF-21 receptor interaction and activation is determined by betaKlotho. *J Cell Physiol* 2008; 215:1–7
- Ogawa Y, Kurosu H, Yamamoto M, et al. BetaKlotho is required for metabolic activity of fibroblast growth factor 21. *Proc Natl Acad Sci U S A* 2007;104:7432–7437
- Yie J, Hecht R, Patel J, et al. FGF21 N- and C-termini play different roles in receptor interaction and activation. *FEBS Lett* 2009;583:19–24
- Kouhara H, Hadari YR, Spivak-Kroizman T, et al. A lipid-anchored Grb2-binding protein that links FGF-receptor activation to the Ras/MAPK signaling pathway. *Cell* 1997;89:693–702
- Fisher FM, Chui PC, Antonellis PJ, et al. Obesity is a fibroblast growth factor 21 (FGF21)-resistant state. *Diabetes* 2010;59:2781–2789
- Chai J, Tarnawski AS. Serum response factor: discovery, biochemistry, biological roles and implications for tissue injury healing. *J Physiol Pharmacol* 2002;53:147–157
- Ito S, Kinoshita S, Shiraiishi N, et al. Molecular cloning and expression analyses of mouse betaklotho, which encodes a novel Klotho family protein. *Mech Dev* 2000;98:115–119
- Kharitonov A, Shiyanova TL, Koester A, et al. FGF-21 as a novel metabolic regulator. *J Clin Invest* 2005;115:1627–1635
- Kharitonov A, Shanafelt AB. Fibroblast growth factor-21 as a therapeutic agent for metabolic diseases. *BioDrugs* 2008;22:37–44
- Kharitonov A, Wroblewski VJ, Koester A, et al. The metabolic state of diabetic monkeys is regulated by fibroblast growth factor-21. *Endocrinology* 2007;148:774–781
- Coskun T, Bina HA, Schneider MA, et al. Fibroblast growth factor 21 corrects obesity in mice. *Endocrinology* 2008;149:6018–6027
- Xu J, Lloyd DJ, Hale C, et al. Fibroblast growth factor 21 reverses hepatic steatosis, increases energy expenditure, and improves insulin sensitivity in diet-induced obese mice. *Diabetes* 2009;58:250–259
- Badman MK, Koester A, Flier JS, Kharitonov A, Maratos-Flier E. Fibroblast growth factor 21-deficient mice demonstrate impaired adaptation to ketosis. *Endocrinology* 2009;150:4931–4940
- Wente W, Efanov AM, Brenner M, et al. Fibroblast growth factor-21 improves pancreatic beta-cell function and survival by activation of extracellular signal-regulated kinase 1/2 and Akt signaling pathways. *Diabetes* 2006;55:2470–2478
- Chavez AO, Molina-Carrion M, Abdul-Ghani MA, Folli F, DeFronzo RA, Tripathy D. Circulating fibroblast growth factor-21 is elevated in impaired glucose tolerance and type 2 diabetes and correlates with muscle and hepatic insulin resistance. *Diabetes Care* 2009;32:1542–1546
- Semba RD, Sun K, Egan JM, Crasto C, Carlson OD, Ferrucci L. Relationship of serum fibroblast growth factor 21 with abnormal glucose metabolism and insulin resistance: the Baltimore Longitudinal Study of Aging. *J Clin Endocrinol Metab* 2012;97:1375–1382
- Hojman P, Pedersen M, Nielsen AR, et al. Fibroblast growth factor-21 is induced in human skeletal muscles by hyperinsulinemia. *Diabetes* 2009;58: 2797–2801
- Zhang X, Yeung DC, Karpisek M, et al. Serum FGF21 levels are increased in obesity and are independently associated with the metabolic syndrome in humans. *Diabetes* 2008;57:1246–1253
- Chu KY, Cheng Q, Chen C, et al. Angiotensin II exerts glucose-dependent effects on Kv currents in mouse pancreatic beta-cells via angiotensin II type 2 receptors. *Am J Physiol Cell Physiol* 2010;298: C313–C323
- Ge X, Chen C, Hui X, Wang Y, Lam KS, Xu A. Fibroblast growth factor 21 induces glucose transporter-1 expression through activation of the serum response factor/Ets-like protein-1 in adipocytes. *J Biol Chem* 2011;286: 34533–34541
- Chu KY, Lau T, Carlsson PO, Leung PS. Angiotensin II type 1 receptor blockade improves beta-cell function and glucose tolerance in a mouse model of type 2 diabetes. *Diabetes* 2006;55:367–374
- Chen W, Hoo RL, Konishi M, et al. Growth hormone induces hepatic production of fibroblast growth factor 21 through a mechanism dependent on lipolysis in adipocytes. *J Biol Chem* 2011;286:34559–34566
- Mraz M, Bartlova M, Lacinova Z, et al. Serum concentrations and tissue expression of a novel endocrine regulator fibroblast growth factor-21 in patients with type 2 diabetes and obesity. *Clin Endocrinol (Oxf)* 2009;71: 369–375
- Ding X, Boney-Montoya J, Owen BM, et al. β Klotho is required for fibroblast growth factor 21 effects on growth and metabolism. *Cell Metab* 2012; 16:387–393
- Wyse BM, Dulin WE. The influence of age and dietary conditions on diabetes in the db mouse. *Diabetologia* 1970;6:268–273
- Kharitonov A, Larsen P. FGF21 reloaded: challenges of a rapidly growing field. *Trends Endocrinol Metab* 2011;22:81–86
- Balfour JA, Plosker GL. Rosiglitazone. *Drugs* 1999;57:921–930; discussion 931–932

33. Campbell IW. Long-term glycaemic control with pioglitazone in patients with type 2 diabetes. *Int J Clin Pract* 2004;58:192–200
34. Dubois M, Pattou F, Kerr-Conte J, et al. Expression of peroxisome proliferator-activated receptor gamma (PPARgamma) in normal human pancreatic islet cells. *Diabetologia* 2000;43:1165–1169
35. Gupta D, Jetton TL, Mortensen RM, Duan SZ, Peshavaria M, Leahy JL. In vivo and in vitro studies of a functional peroxisome proliferator-activated receptor gamma response element in the mouse pdx-1 promoter. *J Biol Chem* 2008;283:32462–32470
36. Im SS, Kim JW, Kim TH, et al. Identification and characterization of peroxisome proliferator response element in the mouse GLUT2 promoter. *Exp Mol Med* 2005;37:101–110
37. Kim HI, Ahn YH. Role of peroxisome proliferator-activated receptor-gamma in the glucose-sensing apparatus of liver and beta-cells. *Diabetes* 2004;53(Suppl. 1):S60–S65
38. Gupta D, Kono T, Evans-Molina C. The role of peroxisome proliferator-activated receptor γ in pancreatic β cell function and survival: therapeutic implications for the treatment of type 2 diabetes mellitus. *Diabetes Obes Metab* 2010;12:1036–1047
39. Campbell IW, Mariz S. Beta-cell preservation with thiazolidinediones. *Diabetes Res Clin Pract* 2007;76:163–176
40. Leesnitzer LM, Parks DJ, Bledsoe RK, et al. Functional consequences of cysteine modification in the ligand binding sites of peroxisome proliferator activated receptors by GW9662. *Biochemistry* 2002;41:6640–6650
41. Díaz-Delfín J, Hondares E, Iglesias R, Giral M, Caelles C, Villarroya F. TNF- α represses β -Klotho expression and impairs FGF21 action in adipose cells: involvement of JNK1 in the FGF21 pathway. *Endocrinology* 2012;153:4238–4245
42. Moyers JS, Shiyanova TL, Mehrbod F, et al. Molecular determinants of FGF-21 activity-synergy and cross-talk with PPARgamma signaling. *J Cell Physiol* 2007;210:1–6
43. Chuang JC, Cha JY, Garmey JC, Mirmira RG, Repa JJ. Research resource: nuclear hormone receptor expression in the endocrine pancreas. *Mol Endocrinol* 2008;22:2353–2363
44. Zhang H, Li Y, Fan Y, et al. Klotho is a target gene of PPAR-gamma. *Kidney Int* 2008;74:732–739
45. Yang HC, Deleuze S, Zuo Y, Potthoff SA, Ma LJ, Fogo AB. The PPARgamma agonist pioglitazone ameliorates aging-related progressive renal injury. *J Am Soc Nephrol* 2009;20:2380–2388
46. Del Prato S. Role of glucotoxicity and lipotoxicity in the pathophysiology of Type 2 diabetes mellitus and emerging treatment strategies. *Diabet Med* 2009;26:1185–1192