Induction of fibroblast growth factor 21 does not require activation of the hepatic X-box binding protein 1 in mice

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ABSTRACT

Objective: Fibroblast growth factor 21 (FGF21), a key regulator of the metabolic response to fasting, is highly induced by endoplasmic reticulum (ER) stress. The X-box binding protein 1 (Xbp1) is one of several ER stress proteins that has been shown to directly activate the FGF21 promoter. We aimed to determine whether hepatic Xbp1 is required for induction of hepatic FGF21 in vivo.

Methods: Mice bearing a hepatocyte-specific deletion of Xbp1 (Xbp1Flox/Flox) were subjected to fasting, pharmacologic ER stress, or a ketogenic diet, all potent stimuli of Fgf21 expression.

Results: Hepatocyte-specific Xbp1 knockout mice demonstrated normal induction of FGF21 in response to fasting or pharmacologic ER stress and enhanced induction of FGF21 in response to a ketogenic diet. Consistent with preserved induction of FGF21, Xbp1Flox/Flox mice exhibited normal induction of FGF21 target genes and normal ketogenesis in response to fasting or a ketogenic diet.

Conclusion: Hepatic Xbp1 is not required for induction of FGF21 under physiologic or pathophysiologic conditions in vivo.

Keywords Unfolded protein response; Endoplasmic reticulum stress; Fasting; Fatty acid oxidation; Ketogenic diet

1. INTRODUCTION

Fasting induces well-characterized changes in hepatic lipid and glucose metabolism that are critical to maintaining energy balance within an organism. Fibroblast growth factor 21 (FGF21), a key regulator of the metabolic response to fasting, is highly induced by endoplasmic reticulum (ER) stress. The X-box binding protein 1 (Xbp1) is one of several ER stress proteins that has been shown to directly activate the FGF21 promoter. Among other cellular stressors, induces hepatic FGF21 expression, the mechanism remains controversial. ER stress response elements have been identified in the Fgf21 promoter that are capable of binding several ER stress-induced transcription factors [25,26]. The PERK-eIF2α-ATF4 branch of the UPR in particular has clearly been shown to mediate ER stress-induced FGF21 expression in vitro and in vivo [25,28]; however, the physiologic role of other ER stress-induced transcription factors in the regulation of FGF21 is less well understood.

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Abbreviations: FGF21, fibroblast growth factor 21; XBP1, X-box binding protein 1; IRE1, inositol requiring enzyme 1; UPR, unfolded protein response; ER, endoplasmic reticulum; PPARα, proliferator-activator receptor alpha; Pgc1α, PPAR-gamma cofactor 1α; O2t1α, carnitine palmityl acyl-CoA transferase 1; Ac, acyl-CoA-oxidase; KD, ketogenic diet; shRNA, short hairpin RNA; CHOP, C/EBP homologous protein; ATF4, activating transcription factor 4; eIF2α, eukaryotic translation initiation factor 2-α; Sreb1c, sterol regulatory element binding protein 1c; Fas, fatty acid synthase; Dgat2, diacylglycerol acyltransferase 2; Scd-1, stearoyl-CoA desaturase 1

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The IRE1α-XBP1 branch of the UPR has been strongly implicated in regulating hepatic lipid and glucose metabolism, making this particular branch of the UPR an intriguing candidate for mediating the effect of ER stress on FGF21 expression [29–31]. XBP1 has been shown to bind to the promoter of FGF21 in vitro, and there has been recent interest in and controversy over the role of XBP1 in the regulation of FGF21 [25–27]. In vitro experiments have shown that overexpression of XBP1s in primary hepatocytes induces FGF21 expression whereas knockdown of XBP1 blunts FGF21 induction [27]. Furthermore, it has previously been shown that mice bearing a liver-specific deletion of IRE1a, the primary activator of Xbp1, show impaired activation of Fgf21 when challenged with pharmacologic ER stress [27]. Despite this compelling data, the role of hepatic Xbp1 in the regulation of hepatic Fgf21 under physiologic or pathophysiologic conditions remains unclear. To directly determine whether hepatic Xbp1 is required for induction of hepatic Fgf21 in vivo, we subjected mice bearing a hepatocyte-specific deletion of Xbp1 to fasting, a ketogenic diet, or pharmacologic ER stress, all potent stimuli of Fgf21 expression.

2. EXPERIMENTAL PROCEDURES

2.1. Animals and treatments
CS7BL/6 -Xbp1fl/fl mice with loxP sites flanking exon 2 of the Xbp1 gene were kindly provided by Dr. Laurie H. Gilmer. The generation of the Xbp1fl/fl strain has been previously reported [29]. Xbp1fl/fl mice were bred with CS7BL/6 -Albumin-Cre mice (Jackson Laboratory, ME) that express Cre-recombinase in albumin-producing hepatocytes as previously described [32]. Xbp1fl/fl mice expressing Cre recombinase were confirmed to be liver-specific Xbp1-knockout mice (Xbp1fl/fl) by western blot for XBP1 and real-time PCR using primers targeting a deleted region of the transcript in exon 2. Littermate Xbp1fl/fl mice negative for expression of Cre recombinase were used as control mice. To determine the effect of hepatic Xbp1 deletion on fasting-induced FGF21 induction, female Xbp1fl/flKDO mice and Xbp1fl/fl controls (6 weeks of age) were fasted overnight (18 h, from 4pm to 10am). To study the effects of a ketogenic diet in mice lacking hepatic Xbp1, female Xbp1fl/KDO mice and Xbp1fl/fl controls (6 weeks of age) were fed a high-fat, ketogenic diet (KD) for 2 weeks. Given the potential confounding variables of sex and age on FGF21 expression, we also fed aged (20 week old), male Xbp1fl/KDO mice and Xbp1fl/fl controls a KD for 2 weeks. The KD is composed of 84% fat, 16% protein, 0% carbohydrate (TestDiet, St. Louis, MO). Food intake was measured in KD-fed mice and found to be similar among Xbp1fl/KDO mice and Xbp1fl/fl controls. To determine the effect of pharmacologic ER stress on the regulation of FGF21, female Xbp1fl/KDO mice and Xbp1fl/fl controls (6 weeks of age) were treated with a single intraperitoneal injection of tunicamycin (0.5 mg/kg) or vehicle (20% DMSO/PBS) and were sacrificed 6 h post-injection. All mice underwent 14/10-hour light/dark cycling before and during the treatment protocol and were given free access to water during dietary manipulation. Mice were sacrificed by CO2 inhalation followed by cardiac puncture. The cardiac blood was immediately centrifuged to collect the plasma. The livers were rapidly excised, flushed with ice-cold saline, and sectioned. An aliquot of liver was fixed in formalin and the remaining liver was snap-frozen in liquid nitrogen. All animal protocols were approved by the Northwestern University Institutional Animal Care and Use Committee (IACUC).

2.2. Liver and plasma chemistries
Plasma FGF21 levels were measured using an FGF21 ELISA assay kit (RND Systems, Minneapolis, MN). Plasma β-hydroxybutyrate levels were measured using a biochemical assay (Stanbio Laboratory, Boerne, TX). Glucose was measured on whole blood obtained by tail bleed using a One Touch Ultra glucometer (Lifescan, Milpitas, CA). Plasma insulin was measured using an Insulin ELISA assay kit (Thermo Scientific, Frederick, MD). The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as (fasting glucose (in mg/dL) X fasting insulin (mU/L))/405. Plasma FGF21, β-hydroxybutyrate, and insulin were measured on plasma collected from cardiac blood at the termination of the experiment. Liver samples were homogenized in Dulbecco’s phosphate buffered saline for hepatic lipid analysis (100 mg liver tissue/1 mL). Triglyceride levels were measured in liver homogenate using an Infinity spectrophotometric assay per the manufacturer’s protocol (Thermo Electron Corporation, Melbourne, Australia).

2.3. Cell culture experiments
Human hepatoma HuH7 cells transfected with short hairpin RNA (shRNA) targeting XBP1 (HuH7shXBP1) or control shRNA (HuH7shCON) were generated and characterized as previously described [33] (kindly provided by Dr. Richard Green, Northwestern University, Chicago, IL). HuH7shCON and HuH7shXBP1 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum, L-glutamine, and penicillin-streptomycin at 37 °C with 5% CO2. To induce ER stress, cells were grown to 80% confluence in 6-well plates and treated with tunicamycin (5 μg/mL) or vehicle (DMSO/saline) in serum-free media for 6 h. RNA isolation was performed using TRIzol Reagent (Ambion Life Technologies, Carlsbad, CA) per protocol.

2.4. Analysis of gene and protein expression
Total RNA from frozen liver or cultured cells was isolated using TRIzol reagent, and real-time quantitative PCR was performed as previously described [34,35]. Total protein was isolated from frozen liver samples, and western blotting was performed as previously described [34,35]. Protein detection was performed using polyclonal rabbit antibodies to IRE1α, CHOP, total and phosphorylated eIF2α, and GAPDH (Cell Signaling Technology, Danvers, MA). Bound antibody was detected using goat anti-rabbit polyclonal HRP antibody (Cell Signaling Technology) and developed using ECL Western Blotting Substrate (Cell Signaling Technology). Representative western blots of pooled samples are shown.

2.5. Statistical analysis
Data are presented as mean ± standard deviation (SD). Comparisons between groups were performed using Student’s t-test analysis.

3. RESULTS

3.1. Hepatic Xbp1 is not required for induction of hepatic Fgf21 in response to fasting
We first examined the effect of hepatic Xbp1-deletion on induction of Fgf21 in response to fasting, a potent stimulus of hepatic Fgf21 expression. As expected, fasting markedly increased the hepatic expression of Fgf21 in Xbp1fl/fl control mice (Figure 1A). Paralleling the induction of hepatic Fgf21 expression, the plasma concentration of FGF21 was also markedly increased by fasting (Figure 1B). Surprisingly, Xbp1fl/KDO mice showed an equivalent increase in hepatic Fgf21 expression and plasma FGF21 concentration relative to Xbp1fl/fl mice in response to fasting (Figure 1A,B). These findings indicate that hepatic Xbp1 does not mediate fasting-related hepatic Fgf21 induction.

Having shown that Xbp1fl/fl mice demonstrate normal induction of hepatic Fgf21 in response to fasting, we next examined whether fasted Xbp1fl/KDO mice show normal induction of FGF21-mediated downstream signaling. FGF21 has been shown to induce hepatic Pgc1α expression;
Figure 1: Hepatic Xbp1 is not required for fasting-induced activation of FGF21. A) Hepatic Fgf21 expression, B) plasma FGF21 concentration, C) hepatic Pgc1α expression, D) hepatic Cpt1α expression, E) hepatic Aco expression, F) plasma β-hydroxybutyrate concentration (mM), G) fasting whole blood glucose (mg/dL), H) fasting plasma insulin concentration (mU/L), and I) calculated homeostatic model assessment of insulin resistance (HOMA-IR) in Xbp1LKO or Xbp1fl/fl mice in the fed or fasted state. mRNA expression shown as mean (n = 7–9) ± SD. *p < 0.05.
however, whether the metabolic effects of FGF21 are mediated by Pgc1α is controversial [5,6,36]. Paralleling the induction of Fgf21 among Xbp1fl/fl mice, the hepatic expression of Pgc1α was appropriately induced by fasting in Xbp1fl/fl mice (Figure 1C). FGF21 has also been shown to regulate other key genes controlling fatty acid β-oxidation including carnitine palmitoyl acyl-CoA transferase 1 (Cpt1α) and acyl-CoA-oxidase (Aco) [1,6]. Xbp1fl/fl mice showed equivalent induction of hepatic Cpt1α and Aco expression relative to Xbp1fl/fl mice in response to fasting (Figure 1D,E). Consistent with normal induction of fatty acid oxidation genes, Xbp1fl/fl and Xbp1fl/fl control mice showed an equivalent increase in plasma β-hydroxybutyrate in response to fasting (Figure 1F). These data suggest that hepatic Xbp1 is not a major regulator of fatty acid β-oxidation in response to fasting. Complementary to its role in regulating fasting-induced fatty acid oxidation, FGF21 has also been shown to be a critical regulator of glucose metabolism [19,37–41]. Consistent with the known function of hepatic Xbp1 in regulating glucose metabolism [42–45], we found that Xbp1fl/fl mice demonstrated increased fasting blood glucose, a trend toward increased fasting plasma insulin, and an increased HOMA-IR relative to control mice (Figure 1G,H,I). Given our finding of normal FGF21 production (Figure 1A,B) in the setting of impaired glucose metabolism, we conclude that hepatic Xbp1 regulates glucose metabolism independently of FGF21.

### 3.2. Hepatic Xbp1 is not required for induction of hepatic Fgf21 in response to ER stress

It is now well-established that ER stress induces Fgf21 in the liver [25–27]. Having shown that hepatic Xbp1 is not required for activation of hepatic Fgf21 in response to fasting, we next determined whether hepatic Xbp1 mediates ER stress-related Fgf21 induction. Xbp1fl/fl and Xbp1fl/fl mice were treated with tunicamycin for 6 h to pharmacologically induce ER stress. As expected, Xbp1fl/fl mice showed induction of spliced Xbp1 mRNA and markedly increased hepatic expression of hepatic Fgf21 in response to ER stress (Figure 2A,B). Xbp1fl/fl mice failed to induce Xbp1 splicing yet demonstrated normal induction of hepatic Fgf21 in response to ER stress.

We next determined the effect of ER stress on Fgf21 expression in a human cell line with a stable knockdown of XBP1 (Huh7ΔXBP1). Huh7ΔXBP1 knockdown and Huh7ΔΔCON control cells were treated with tunicamycin for 6 h. Huh7ΔΔCON control cells showed induction of Xbp1 splicing and induction of Fgf21 expression in response to ER stress (Figure 2C,D). As expected, Huh7ΔXBP1 knockdown cells showed 80% suppression of Xbp1 mRNA at baseline and no induction of Xbp1 splicing in response to ER stress. Despite the failure to induce Xbp1 splicing, Huh7ΔXBP1 cells showed equal induction of Fgf21 expression compared to control cells in response to ER stress. These data indicate that XBP1 does not mediate ER stress-induced FGF21 activation.

### 3.3. Deletion of hepatic Xbp1 enhances ketogenic diet-induced hepatic Fgf21 expression

Feeding mice a high-fat, low-carbohydrate, ketogenic diet (KD) increases circulating FGF21 levels and induces fatty acid β-oxidation pathways [1,7]. We next examined the effect of a KD on hepatic Fgf21 expression in Xbp1fl/fl and Xbp1fl/fl mice. As expected, the KD increased hepatic expression of Fgf21 and increased plasma levels of FGF21 among Xbp1fl/fl control mice (Figure 3A,B). Interestingly, Xbp1fl/fl mice fed the KD showed enhanced hepatic expression and plasma level of FGF21 relative to KD-fed Xbp1fl/fl mice. Given this unexpected finding and the potential influence of age and sex on FGF21 levels, we repeated the experiment in older, male mice. Paralleling our findings in female mice, we found that male Xbp1fl/fl mice demonstrated increased hepatic expression of Fgf21 and higher plasma levels of FGF21 compared to male Xbp1fl/fl mice when fed a KD (Figure 3A,B). Similar to our observations in the fasted state, Xbp1fl/fl and Xbp1fl/fl mice showed equal induction of fatty acid oxidation genes and an equivalent increase in plasma β-hydroxybutyrate in response to a KD (Figure 3C–E). Hepatic Fgf21 is transcriptionally activated by several UPR signaling proteins other than Xbp1, most notably, ATF4 and CHOP [25,26]. We considered whether the enhanced hepatic Fgf21 expression observed in Xbp1fl/fl mice fed a KD may be due to compensatory hyperactivation of non-Xbp1-dependent elements of the UPR. Feeding a KD was
associated with activation of the UPR in the livers of Xbp1^{fl/fl} control mice as evidenced by an increase in mRNA level of spliced Xbp1, ATF4, and Chop, as well as increased protein levels of phosphorylated IRE1, phosphorylated eIF2, and CHOP (Figure 4). As we and others have previously shown [29,32], Xbp1^{fl/KO} mice showed a near-absence of hepatic spliced Xbp1 mRNA and compensatory hyperactivation of IRE1 at baseline (Figure 4A,B). In response to a KD, Xbp1^{fl/KO} mice showed enhanced activation of hepatic IRE1 relative to Xbp1^{fl/fl} mice. Xbp1^{fl/KO} showed a similar degree of activation of the eIF2α-ATF4 signaling cascade as evidenced by equal hepatic levels of phosphorylated eIF2α protein and ATF4 mRNA (Figure 4A,C). On the contrary, Xbp1^{LKO} mice showed markedly enhanced induction of hepatic CHOP at both the mRNA and protein level relative to Xbp1^{fl/fl} mice when fed a KD (Figure 4A,D). Consistent with enhanced induction of CHOP, KD-fed Xbp1^{LKO} mice showed enhanced hepatic expression of death receptor 5 (Dr5), a pro-apoptotic transcriptional target of CHOP (Figure 4E).

It has been shown that mice lacking hepatic Xbp1 are protected from lipogenic diet-induced hepatic steatosis associated with suppressed expression of lipogenesis genes [29,33]. Xbp1^{fl/fl} mice fed a KD for 2 weeks showed a four-fold increase in hepatic triglyceride content associated with scant hepatic steatosis on H&E staining of liver sections (Figure 4F,G). There was no overt histologic evidence of liver injury or fibrosis at this early time point. Consistent with the known effect of hepatic Xbp1-deletion on hepatic lipid accumulation, we found that Xbp1^{fl/KO} mice fed a KD showed reduced hepatic steatosis (Figure 4F), modestly attenuated hepatic triglyceride accumulation (Figure 4G), and enhanced suppression of hepatic lipogenesis genes (Figure 4H) relative to Xbp1^{fl/fl} mice.

4. DISCUSSION

FGF21 is a key regulator of the metabolic response to fasting and has been shown to be highly induced by ER stress [25,26]. UPR response elements have been identified within the Fgf21 promoter, and several UPR signaling elements have been implicated in the regulation of Fgf21 including ATF4, CHOP, and XBP1 [25–27]. While the PERK-eIF2α-ATF4 branch of the UPR has been strongly implicated in the regulation of ER stress-induced FGF21 expression, the physiologic role of XBP1 in FGF21 regulation is controversial [25,27,28,46]. We find that mice lacking hepatic Xbp1 show normal induction of hepatic Fgf21 in response to fasting, pharmacologic ER stress, or a ketogenic diet. Furthermore, we find that a human cell line bearing a stable knockdown of XBP1 also demonstrates normal induction of FGF21 in response to ER stress. Although Xbp1 is capable of binding to UPR response elements within the Fgf21 promoter, our data demonstrate that Xbp1 is not required for induction of Fgf21 under physiologic or pathophysiologic conditions.

It has been shown previously that mice bearing a liver-specific deletion of Ire1α show impaired activation of hepatic Fgf21 when challenged with pharmacologic ER stress [27]. It has been speculated that the effect of Ire1α-deletion on hepatic Fgf21 is a consequence of impaired Xbp1 splicing in this model. Challenging hepatocyte-specific Xbp1 knockout mice with stimuli that induce hepatic Fgf21 is the most direct and definitive way to determine whether Xbp1 is required for Fgf21 activation in vivo. Our observation that mice lacking hepatic Xbp1 demonstrate normal induction of Fgf21 in response to fasting, ER stress, and a ketogenic diet clearly demonstrates that hepatic Xbp1 is not necessary for
Figure 4: Deletion of hepatic Xbp1 enhances ketogenic diet-induced UPR activation. A) Hepatic protein levels of total and phosphorylated IRE1α, total and phosphorylated eIF2α, CHOP, and GAPDH, hepatic mRNA expression of B) Xbp1s, C) Atf4, D) Chop, and E) Dr5, F) representative Oil Red O stained liver sections, G) quantification of hepatic triglyceride content (mg triglyceride per gram liver), and H) relative hepatic mRNA expression of lipogenesis genes in Xbp1LKO or Xbp1fl/fl mice treated with a control or high-fat ketogenic diet (KD) for 2 weeks. Representative western blot is of pooled samples (n = 6–7). mRNA expression shown as mean (n = 6–7) ± SD. *p < 0.05.
induction of Fgf21 in vivo. Furthermore, these data suggest that the effect of Ire1α-deletion on Fgf21 expression is not a function of Xbp1.

In addition to demonstrating that hepatic Xbp1 does not regulate hepatic Fgf21 expression in vivo, we also show that Xbp1 does not regulate ER stress-induced Fgf21 expression in vitro. Although consensus exists that XBP1 is capable of binding to the FGF21 pro-
in vitro

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REFERENCES

[1] Badman, M.K., Pissios, P., Kennedy, A.R., Koukos, G., Flier, J.S., Maratos-Flier, E., 2007. Hepatic fibroblast growth factor 21 is regulated by PPARalpha and is a key mediator of hepatic lipid metabolism in ketotic states. Cell Metabolism 5:426–437.

[2] Inagaki, T., Dutchak, P., Zhao, G., Ding, X., Gautron, L., Parameswaran, V., et al., 2007. Endocrine regulation of the fasting response by PPARalpha-mediated induction of fibroblast growth factor 21. Cell Metabolism 5:415–425.

[3] Lundasen, T., Hunt, M.C., Nilsson, L.M., Sanyal, S., Angelin, B., Alexson, S.E., et al., 2007. PPARalpha is a key regulator of hepatic Fgf21. Biochemical and Biophysical Research 360:437–440.

[4] Galimain, C., Lundasen, T., Kharitonenkov, A., Bina, H.A., Eriksson, M., Hafstrom, I., et al., 2006. Increased liver fibroblast growth factor 21 (FGF21) mRNA expression through ChREBP activation in rat hepatocytes. FEBS Letters 583:2882–2886.

[5] Fisher, F.M., Estall, J.L., Adams, A.C., Antonellis, P.J., Bina, H.A., Flier, J.S., et al., 2011. Integrated regulation of hepatic metabolism by fibroblast growth factor 21 (FGF21) in vivo. Endocrinology 152:2996–3004.

[6] Kennedy, A.R., Pissios, P., Otu, H., Roberson, R., Xue, B., Asakura, K., et al., 2007. A high-fat, ketogenic diet induces a unique metabolic state in mice. American Journal of Physiology. Endocrinology and Metabolism 292:E1724–E1739.

[7] Sanchez, J., Palou, A., Pico, C., 2009. Response to carbohydrate and fat refeeding in the expression of genes involved in nutrient partitioning and metabolism: striking effects on fibroblast growth factor-21 induction. Endocrinology 150:5341–5350.

[8] Iizuka, K., Takeda, J., Horikawa, Y., 2009. Glucose induces FGF21 mRNA expression through ChREBP activation in rat hepatocytes. FEBS Letters 583:2882–2886.

[9] Fisher, F.M., Chui, P.C., Nasser, I.A., Popov, Y., Cuniff, J.C., Lundasen, T., et al., 2014. Fibroblast growth factor 21 limits lipotoxicity by promoting hepatic


carcinology 150:5341


carcinology 150:5341


carcinology 150:5341


carcinology 150:5341
fatty acid activation in mice on methionine and choline-deficient diets. Gastroenterology 147:1073–1083 e1078.

[11] Tanaka, N., Takahashi, S., Zhang, Y., Krausz, K.W., Smith, P.B., Patterson, A.D., et al., 2015. Role of fibroblast growth factor 21 in the early stage of NASH induced by methionine- and choline-deficient diet. Biochimica et Biophysica Acta 1852:1242–1252.

[12] Fisher, F.M., Chui, P.C., Antonellis, P.J., Bina, H.A., Kharitonkoven, A., Flier, J.S., et al., 2010. Obesity is a fibroblast growth factor 21 (FGF21)-resistant state. Diabetes 59:2781–2789.

[13] Dushay, J., Chui, P.C., Gopalakrishnan, G.S., Varela-Rey, M., Crawley, M., Fisher, F.M., et al., 2010. Increased fibroblast growth factor 21 in obesity and nonalcoholic fatty liver disease. Gastroenterology 139:456–463.

[14] Chen, W.W., Li, L., Yang, G.Y., Li, K., Qi, X.Y., Zhu, W., et al., 2008. Circulating FGF-21 levels in normal subjects and in newly diagnose patients with Type 2 diabetes mellitus. Experimental and Clinical Endocrinology & Diabetes 116: 65–68.

[15] Zhang, X., Yeung, D.C., Karpisek, M., Stejskal, D., Zhou, Z.G., Liu, F., et al., 2008. Serum FGF21 levels are increased in obesity and are independently associated with the metabolic syndrome in humans. Diabetes 57:1246–1253.

[16] Chavez, A.O., Molina-Carrion, M., Abdul-Ghani, M.A., Folli, F., Defronzo, R.A., Tripathy, D., 2009. 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 32:1542–1546.

[17] Yilmaz, Y., Eren, F., Yonal, O., Kaptan, B., Celikel, C.A., et al., 2010. Increased serum FGF21 levels in patients with nonalcoholic fatty liver disease. European Journal of Clinical Investigation 40:887–892.

[18] Coskun, T., Bina, H.A., McNally, M.A., Barnard, J.D., Hu, C.C., et al., 2011. p38 MAPK-mediated regulation of Xbp1s is crucial for glucose homeostasis. Nature Medicine 17:1251–1267.

[19] Kharitonkov, A., Shiyanova, T.L., Koester, A., Ford, A.M., Micanovic, R., Kharitonkov, S.A., et al., 2015. Fibroblast growth factor-21 is elevated in the early stages of hepatic steatosis. Biochimica et Biophysica Acta 1852:1242–1252.

[20] Lee, J., Sun, C., Zhou, Y., Lee, J., Gokalp, D., Herrema, H., et al., 2011. p38 alpha-mediated c-Jun N-terminal kinase activation from hepatic insulin resistance increases hepatic FGF-21 mRNA decay lowers plasma lipids in mice. Cell Metabolism 16:478–486.

[21] Soberg, S., Sandholt, C.H., Jespersen, N.Z., Toft, U., Madsen, A.L., von Holstein-Rathlou, S., et al., 2017. FGF21 is a sugar-induced hormone associated with sweet intake and preference in humans. Cell Metabolism 25:1045–1059.

[22] Dusey, J., Chui, P.C., Gopalakrishnan, G.S., Varela-Rey, M., Crawley, M., Fisher, F.M., et al., 2010. Increased fibroblast growth factor 21 in obesity and nonalcoholic fatty liver disease. Gastroenterology 139:456–463.

[23] Liang, Q., Zhong, L., Zhang, J., Wang, Y., Bornstein, S.R., Triggle, C.R., et al., 2012. p38 MAPK-mediated regulation of Xbp1s is crucial for glucose homeostasis. Nature Medicine 17:1251–1267.

[24] Winnay, J.N., Boucher, J., Mori, M.A., Ueki, K., Kahn, C.R., 2010. A regulatory subunit of phosphoinositide 3-kinase increases the nuclear accumulation of Xbp1s in response to hepatic ischemia-reperfusion injury. American Journal of Physiology Endocrinology and Metabolism 299:E1046–E1055.

[25] Lee, J., Sun, C., Zhou, Y., Lee, J., Gokalp, D., Herrema, H., et al., 2011. p38 alpha-mediated c-Jun N-terminal kinase activation from hepatic insulin resistance increases hepatic FGF-21 mRNA decay lowers plasma lipids in mice. Cell Metabolism 16:478–486.

[26] Soberg, S., Sandholt, C.H., Jespersen, N.Z., Toft, U., Madsen, A.L., von Holstein-Rathlou, S., et al., 2017. FGF21 is a sugar-induced hormone associated with sweet intake and preference in humans. Cell Metabolism 25:1045–1059.

[27] Lee, J., Sun, C., Zhou, Y., Lee, J., Gokalp, D., Herrema, H., et al., 2011. p38 alpha-mediated c-Jun N-terminal kinase activation from hepatic insulin resistance increases hepatic FGF-21 mRNA decay lowers plasma lipids in mice. Cell Metabolism 16:478–486.

[28] Dusey, J., Chui, P.C., Gopalakrishnan, G.S., Varela-Rey, M., Crawley, M., Fisher, F.M., et al., 2010. Increased fibroblast growth factor 21 in obesity and nonalcoholic fatty liver disease. Gastroenterology 139:456–463.

[29] Liang, Q., Zhong, L., Zhang, J., Wang, Y., Bornstein, S.R., Triggle, C.R., et al., 2012. p38 MAPK-mediated regulation of Xbp1s is crucial for glucose homeostasis. Nature Medicine 17:1251–1267.

[30] Winnay, J.N., Boucher, J., Mori, M.A., Ueki, K., Kahn, C.R., 2010. A regulatory subunit of phosphoinositide 3-kinase increases the nuclear accumulation of Xbp1s in response to hepatic ischemia-reperfusion injury. American Journal of Physiology Endocrinology and Metabolism 299:E1046–E1055.

[31] Lee, J., Sun, C., Zhou, Y., Lee, J., Gokalp, D., Herrema, H., et al., 2011. p38 MAPK-mediated regulation of Xbp1s is crucial for glucose homeostasis. Nature Medicine 17:1251–1267.

[32] Dusey, J., Chui, P.C., Gopalakrishnan, G.S., Varela-Rey, M., Crawley, M., Fisher, F.M., et al., 2010. Increased fibroblast growth factor 21 in obesity and nonalcoholic fatty liver disease. Gastroenterology 139:456–463.

[33] Liang, Q., Zhong, L., Zhang, J., Wang, Y., Bornstein, S.R., Triggle, C.R., et al., 2012. p38 MAPK-mediated regulation of Xbp1s is crucial for glucose homeostasis. Nature Medicine 17:1251–1267.

[34] Winnay, J.N., Boucher, J., Mori, M.A., Ueki, K., Kahn, C.R., 2010. A regulatory subunit of phosphoinositide 3-kinase increases the nuclear accumulation of Xbp1s in response to hepatic ischemia-reperfusion injury. American Journal of Physiology Endocrinology and Metabolism 299:E1046–E1055.

[35] Lee, J., Sun, C., Zhou, Y., Lee, J., Gokalp, D., Herrema, H., et al., 2011. p38 MAPK-mediated regulation of Xbp1s is crucial for glucose homeostasis. Nature Medicine 17:1251–1267.

[36] Dusey, J., Chui, P.C., Gopalakrishnan, G.S., Varela-Rey, M., Crawley, M., Fisher, F.M., et al., 2010. Increased fibroblast growth factor 21 in obesity and nonalcoholic fatty liver disease. Gastroenterology 139:456–463.
Brief Communication

resistance in conditional X-box-binding protein-1 (XBP1) knock-out mice. The
Journal of Biological Chemistry 287:2558—2567.

[46] Shao, M., Shan, B., Liu, Y., Deng, Y., Yan, C., Wu, Y., et al., 2014. Hepatic
IRE1alpha regulates fasting-induced metabolic adaptive programs through the
XBP1s-PPARalpha axis signalling. Nature Communications 5:3528.

[47] Laeger, T., Henagan, T.M., Albarado, D.C., Redman, L.M., Bray, G.A.,
Noland, R.C., et al., 2014. FGF21 is an endocrine signal of protein restriction.
Journal of Clinical Investigation 124:3913—3922.

[48] Tong, X., Muchnik, M., Chen, Z., Patel, M., Wu, N., Joshi, S., et al., 2010.
Transcriptional repressor E4-binding protein 4 (E4BP4) regulates metabolic
hormone fibroblast growth factor 21 (FGF21) during circadian cycles and
feeding. The Journal of Biological Chemistry 285:36401—36409.

[49] Oishi, K., Uchida, D., Ishida, N., 2008. Circadian expression of FGF21 is induced
by PPARalpha activation in the mouse liver. FEBS Letters 582:3639—3642.