Fasting exacerbates hepatic growth differentiation factor 15 to promote fatty acid β-oxidation and ketogenesis via activating XBP1 signaling in liver

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ABSTRACT

Liver coordinates a series of metabolic adaptations to maintain systemic energy balance and provide adequate nutrients for critical organs, tissues and cells [1]. These adaptive responses in liver are wellorchestrated including supplying glucose to the circulation initially from hepatic glycogen followed by gluconeogenesis produced by other noncarbohydrate materials. In sustained fasting state, ketones are synthesized by liver and provide an alternative energy source instead of glucose for highly oxidative tissues [2]. Hepatic fatty acid β-oxidation is considered to be critical for these processes for providing ATP and NADP to facilitate gluconeogenesis and acetyl-CoA for ketogenesis [3]. Accumulative studies reveal plenty of molecular taking part in regulating hepatic β-oxidation during fasting or other physiological states, including peroxisome proliferator-activated receptor alpha (Ppara) [4], fibroblast growth factor 21 (Fgf21) [5]. It is still valuable to discover novel mediator(s) to further understand the regulation of hepatic fatty acid β-oxidation and ketogenesis.

Growth differentiation factor 15 (GDF15), also known as MIC-1, is a member of the transforming growth factor-β (TGF-β)/bone morphogenetic protein (BMP) super family and a secreted protein circulating in plasma as a 25-kDa homodimer [6,7]. GDF15 is found to be highly expressed in the placenta and prostate and also in heart, pancreas, liver, kidney and colon [8–10]. Notably, Circulating GDF15 are increased in obese and type 2 diabetic patients and display high correlation with body mass index (BMI), body fat, glucose, and C-reactive proteins, indicating its crucial functions in the pathogenesis of metabolic diseases [11]. GDF15 is wildly accepted as a biomarker and higher levels are closely associated with increased risk of cardiovascular disease [12,13]. GDF15 is implicated in governing systemic energy balance and protecting against obesity through enhancing thermogenesis and oxidative metabolism [14,15]. However, physiological roles of GDF15 in hepatic lipid homeostasis and underlying molecular mechanism(s) are still obscure.

Accumulation of unfolded or misfolded proteins in lumen of endoplasmic reticulum causes ER stress and subsequently activates the cellular unfolded protein response (UPR) [16,17]. Mammalian UPR pathways are composed of three branches which are initiated by ER membrane-located proteins, including activating transcription factor 6 (ATF6), inositol requiring enzyme 1 (IRE1), and double-stranded RNA-activated protein kinase-like ER kinase (PERK). Among the UPR pathways, IRE1α-XBP1 branch is the most conserved one, indicating its

1. Introduction

During starvation, liver coordinates a series of metabolic adaptations to maintain systemic energy balance and provide adequate nutrients for critical organs, tissues and cells [1]. These adaptive responses in liver are well orchestrated including supplying glucose to the circulation initially from hepatic glycogen followed by gluconeogenesis produced by other noncarbohydrate materials. In sustained fasting state, ketones are synthesized by liver and provide an alternative energy source instead of glucose for highly oxidative tissues [2]. Hepatic fatty acid β-oxidation is considered to be critical for these processes for providing ATP and NADP to facilitate gluconeogenesis and acetyl-CoA for ketogenesis [3]. Accumulative studies reveal plenty of molecular taking part in regulating hepatic β-oxidation during fasting or other physiological states, including peroxisome proliferator-activated receptor alpha (Ppara) [4], fibroblast growth factor 21 (Fgf21) [5]. It is still valuable to discover novel mediator(s) to further understand the regulation of hepatic fatty acid β-oxidation and ketogenesis.

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Abbreviations: GDF15, growth differentiation factor 15; UPR, unfolded protein response; IRE1α, inositol requiring enzyme 1 alpha; XBP1, x-box binding protein 1; STZ, streptozotocin; ChIP, chromatin immunoprecipitation; ERSE, ER stress-response element; Ppara, peroxisome proliferator-activated receptor alpha; Accl, acyl-Coenzyme A dehydrogenase; Accl, acyl-Coenzyme A oxidase 1; Cpt1a, carnitine palmitoyltransferase 1a; Hmgcs2, 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2; KD, ketogenic diet; NCD, normal chow diet; Hsl, hormone sensitive lipase; Atgl, adipose triglyceride lipase; NAFLD, nonalcoholic fatty liver disease

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IRE1α catalyzes the non-conventional splicing of the mRNA encoding X-box-binding protein 1 (XBPI) by removing 26nt intron, and thereby products an active spliced form (XBPIs) to initiate a key UPR program [19]. Growing studies in animal models have revealed the crucial role of IRE1α or XBPIs in both glucose and lipid metabolism [20–26]. Although several studies implicate IRE1α-XBP1s in lipid homeostasis of liver in fasted studies [20,21], it still remains largely elusive how IRE1α-XBPIs branch acts in regulating hepatic lipid metabolism under physiological conditions. Here we present that hepatic GDF15 which is controlled by IRE1α-XBPIs branch could be upregulated and promote fatty acids β-oxidation and ketogenesis of liver during fasting.

2. Materials and methods

2.1. Animals

C57BL/6J wild type mice were obtained from Shanghai Laboratory Animal Co. Ltd. (SILAC) and housed in laboratory cages at a temperature of 23 ± 3 °C with a humidity of 35 ± 5% under 12-h dark/light cycle (lights on at 7:00 a.m.).

For fed and fasting experiments, fasted mice were kept from food (regular chow diet) for 24 h and sacrificed within 2 h after lights on; mice fed ad libitum were free to access to food and sacrificed at the same time of the day as fasted mice. For diet-induced ketogenesis studies, mice were fed with a ketogenic diet (containing 17.6% protein, 0.2% carbohydrate and 57.2% fat, w/w, Research Diets Inc., #D12369B) for 3 days and sacrificed within 2 h after lights on.

For diabetic ketoacidosis, mice were firstly administered with adenosine of ad-NC and ad-siGp15 as indicated, respectively. Two weeks later, these mice were injected intraperitoneally (i.p.) with streptozotocin (STZ, Sigma-Aldrich, #S0130) at a dose of 250 mg per kg body weight as previously described [21] and sacrificed within 2 h after lights on. All experiments in this study were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee of Fudan University.

2.2. Serum and liver measurements

Hepatic levels of TGs, Cholesterol and FFAs were respectively determined using the triglyceride determination kit (Sigma, triglyceride reagent #T2449 and free glycerol reagent #T2448), Cholesterol assay (reagent #T2449 and free glycerol reagent #F6428), Cholesterol assay (Sigma, triglyceride determination kit, #MGD150). For measurement of hepatic TG, FFA and Cholesterol, ~30 mg of liver tissue was homogenized in 0.5 ml PBS. After sufficient mixing of liver homogenate with 1.6 ml of CHCl3-CH3OH (2:1, v/v), the suspension was centrifuged at 3,000 rpm for 10 min at room temperature and then the lower organic phase was transferred for air-dried overnight. The residual liquid was re-suspended in 400 μl of ethanol with 1% Triton X-100. TG contents were determined using the triglyceride determination kit (Sigma, triglyceride reagent #T2449 and free glycerol reagent #F6428). Cholesterol contents were determined using the Cholesterol Assay Kit (Sigma, Cholesterol Quantiitation Kit #MK0433-1KT). For determining hepatic levels of GDF15, liver tissues were firstly homogenized in the homogenization buffer (0.01 N HCl, 1 mM EDTA, 4 mM Na2S2O3), and then centrifuged at 13,000 rpm for 15 min at 4 °C to remove the cellular debris. The homogenates were collected and stored at ~80 °C prior to measurement by ELISA. For determining secreted GDF15, cell culture medium (3 ml) was collected as indication. Then the supernatant was centrifuged at 200 μl by the using of an Amicon Ultra-4 centrifugal filter unit (Millipore). GDF15 was measured using the ELISA kit (R&D System, #MGD150) according to the manufacturer's instructions.

2.3. Isolation of primary hepatocytes of mice

Primary hepatocytes were isolated from male mice at 8–12 weeks of age as previously described [21]. Briefly, collagenase perfusion was performed with 50 ml of perfusion buffer through the portal vein of mice after anesthetizing. Livers were aseptically removed and minced in a sterile 10-cm cell culture dish with ice-cold perfusion buffer without collagenase. Then, hepatocytes were filtrated through a 70 μm cell strainer (BD Falcon) into a new tube followed with centrifugation at 50 g for 2 min at 4 °C. Cells were then washed with cold heparate wash medium (Invitrogen) three times and re-suspended in 15 ml of cold HepatoZyme-SFM medium (Invitrogen) supplemented with 2 mM-l-glutamine, 20 units/ml penicillin, and 20 μg/ml streptomycin. After trypan blue staining for determination of viability, cells were plated at 6 × 10⁵ cells/well in 6-well culture dishes or at 3 × 10⁵ cells/well in 12-well dishes pre-coated with collagen. Cells were cultured for at least 8 h before further use. Cells were subsequently treated with the desired reagents prior to further analysis.

2.4. Quantitative real-time RT-PCR analysis

Total RNAs were isolated from cells or liver tissues by TRizol reagent (Invitrogen), and cDNA was generated by M-MLV reverse transcriptase with random hexamer primers (Invitrogen). Quantitative Real-time PCR (qRT-PCR) was performed with an ABI Prism 7500 sequence detection system, using the SYBR Green PCR Master Mix (Applied Biosystems). Ct values were normalized to mouse Rpl18 and then the lower organic phase was transferred for air-dried overnight. The methods followed the ΔΔCt method. The sequences of primers mainly used in this study were as following:

- Rpl18: sense 5′-ATGATGTGGATCTCAGAACAG-3′, antisense 5′-CTGGCGCTTGGCAAAATT-3′;
- Gdf15: sense 5′-CTGGAATCTGCTGAACAGC-3′, antisense 5′-GTCGAGGAGATCTCTTCGAG-3′;
- Pparα: sense 5′-AGAGGCCCATCTGCTCTCTC-3′, antisense 5′-ACTG GTAGTCTGCAAACAACAAA-3′;
- Fasn: sense 5′-CTGCTGAGGGGCTCTACCAAG-3′, antisense 5′-CTGC GCCCTACACTGTTCC-3′;
- Acac: sense 5′-TCTTTTCTCTCGAGCAGTACA-3′, antisense 5′-GAC CTCTCTACTCTACCTCCAG-3′;
- Acalx: sense 5′-TAACTTCCCTCCTACGAAAGC-3′, antisense 5′-GAC TCTCACACACCATCTGTC-3′;
- Cpt1a: sense 5′-TCTCCGGCCGTGACCATAGAAG-3′, antisense 5′-CACC ATGTAATGACCATCT-3′;
- Hmgcs2: sense 5′-CTCCGGCAGTGACCATAGAAG-3′, antisense 5′-CAC TACAGTATGACCATTCT-3′;
- Pparγ2: sense 5′-TCGCGTGTCAGCTCCTATG-3′, antisense 5′-GAG AGGTCCACAGACTGCATT-3′;
- Hsl: sense 5′-GATTTACCGCAGCAGACAGT-3′, antisense 5′-ACC TGCAAAACACATAGACGC-3′;
- Atg1: sense 5′-CTGGAATCTCAGACCAATACATCT-3′, antisense 5′-CACC ATGTAATGACCATCT-3′;
- Xbp1s: sense 5′-CTGAGTGGCAAATCATGAG-3′, antisense 5′-GTC CATGGAAATGTCTTTGG-3′;
- Bip: sense 5′-ATCTGGGAGGACACACTATATTCT-3′, antisense 5′-ATCG CCAATCAGAGGTCCTCC-3′;
- Chap: sense 5′-CTTGGAGAGGGCTGATGAGGAT-3′, antisense 5′-GCC ATTGAGTTGAG-3′;
- Erdj4: sense 5′-ATAAACGCTTGGTACGTTGAGA-3′, antisense 5′-GCC ATTGAGGAGTTGAG-3′;
- Fasn: sense 5′-GGCTTCTATGATTACCACGAC-3′, antisense 5′-CCAG TTGGTTCTTCCGGAGA-3′;
- Scdl: sense 5′-AGATCTCCAGGTTTTCGACACAC-3′, antisense 5′-GAGGATGTCGTTCTTCCAGTTG-3′;
- Pgcl1α: sense 5′-TACAGTCTTATACGACGTCCT-3′, antisense 5′-CCAA TCAATTCCACGACAAGA-3′;
- Accl: sense 5′-GATTGACACCATCCGTGGGC-3′, antisense 5′-CACA
ATTATGAATGGGATGTC-3',
Cd36: sense 5'-GAAACCTGCTTTCAAAAAACTGG-3', antisense 5'-TGCTGTCTCAGGCAGCACTGCA-3';
ApoB: sense 5'-GCTAATCTCATGTTACGTA-3', antisense 5'-AGGAGCTGACATTGACTTG-3';
Srebplc: sense 5'-GAGCCACCTGGATCAGC-3', antisense 5'-GGCGGCAAGATGACC-3';
Ppar: sense 5'-TGCTGATGACCTGCTATG-3', antisense 5'-GAGAAGCTAGTGG-3';
ApoDII: sense 5'-TACAGGGCACATTGAGA-3', antisense 5'-CAGGGATCTGAACTGTCC-3'.

2.5. Luciferase reporter assays

The luciferase (Luc) reporter plasmids for mouse GDF15 promoter spanning the region from −1998 to +3 were constructed in pGL3 (Promega) utilizing a PCR-based cloning strategy. For luciferase activity assays, HEK293T cells were co-transfected with the Gdf15 promoter-Luc and β-galactosidase plasmids before treatment with thapsigargin (Tg, 1 μM) or tunicamycin (Tm, 5 μg/ml) for 6 h, or co-transfected with the Luc reporter plasmid, pCMV-CHOP and β-galactosidase plasmids. Luciferase activity was measured by the using of Dual-Luciferase Reporter Assay System (Promega) following the manufacturer’s instructions, and β-galactosidase activity was used for normalization.

2.6. Chromatin immunoprecipitation (ChIP)

ChIP assays were performed with the Agarose ChIP Kit (Pierce, Thermo Fisher) according to the manufacturer’s instructions. In brief, cells or liver tissues were subjected to cross-linking with 1% formaldehyde before the preparation of nuclear extracts. Chromatin-CHOP complexes were immunoprecipitated with normal rabbit IgG or anti-CHOP antibodies (Cell Signaling Technology) by incubation at 4 °C overnight on a rocking platform, and then incubated with the beads from the ChIP kit at 4 °C for 2 h with gentle rocking. After washing sufficiently with the washing buffer, the complexes were eluted with the elution buffer from the beads and were subjected to further PCR analysis.

2.7. Histology analysis

Liver were harvested from indicated mice after perfusion (4% paraformaldehyde) and fixed in 4% paraformaldehyde for at least 72 h and then embedded in paraffin before cut into 5 μm sections for being stained with hematoxylin and eosin (H&E).

For Oil-Red O staining, frozen liver sections (10 μm) were rinsed with 60% isopropanol and then applied to stain with freshly prepared Oil-red O solution (Sigma) for 10 min. The sections were then rinsed with 60% isopropanol and then applied to stain with freshly prepared Oil-red O solution (Sigma) for 10 min. The sections were then rinsed with 60% isopropanol and then applied to stain with freshly prepared Oil-red O solution (Sigma) for 10 min. The sections were then rinsed with 60% isopropanol and then applied to stain with freshly prepared Oil-red O solution (Sigma) for 10 min. The sections were then rinsed with 60% isopropanol and then applied to stain with freshly prepared Oil-red O solution (Sigma) for 10 min. The sections were then rinsed with 60% isopropanol and then applied to stain with freshly prepared Oil-red O solution (Sigma) for 10 min. 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mouse Gdf15. A putative ER stress-response element (ERSE) CCATT…N (n)…CCAGC, was identified in the promoter sequence of mouse (Fig. 2E), which was a potential XBP1s-binding core site as previously reported [27,28]. Thus, luciferase reporter plasmids were constructed to evaluate the importance of this core site, which contain mouse Gdf15 promoter of full length (WT) or “CCATT” deletion (ΔCCATT). In the results of luciferase assays performed in HEK293T cells, the transcriptional activities of Gdf15 promoter were dramatically enhanced when XBP1s were overexpressed but were almost abolished when “CCATT” core sequence was deleted (Fig. 2F). Next, we conducted chromatin immunoprecipitation (ChIP) assays to determine whether XBP1s could bind directly to Gdf15 promoter. Interestingly, exogenous Flag-tagged XBP1s protein were co-immunoprecipitated with DNA of Gdf15 promoter as shown of the result (n)

3.3. Abrogation of hepatic GDF15 impairs fatty acid β-oxidation and ketogenesis in fasted mice

During extended periods of food deprivation, the liver is known to undergo metabolic reprogramming to oxidize fatty acids and produce ketones for the brain and other tissues as the primary energy source [21]. To investigate whether fasting-induced hepatic GDF15 is critical in regulating fatty acid β-oxidation and ketone production of liver, we applied adenovirus expressing siRNA specially targeted at mouse Gdf15 mRNA (ad-siGdf15) to inhibit GDF15 expression in liver rather than other major tissues or organs (Fig. 3G, Fig. S1C), and eventually resulted in dramatic reduction of serum GDF15 contents (Fig. 3H), which further confirmed that fasting-upregulated GDF15 in circulation are mainly produced by liver. Ad-siGdf15-injected mice exhibited massive accumulation of lipids (Figs. 3A and 2B) and markedly increased hepatic triglyceride (TG) contents (Fig. 3C) in the livers of 24-h fasting, impressively, decreased hepatic GDF15 resulted in significant reduction of fasting-upregulated serum β-hydroxybutyrate, indicating possible defects in ketogenic output of liver (Fig. 3D). Moreover, relative to mice of ad-NC, liver extracts of fasted ad-siGdf15-administered mice displayed dramatically decreased β-oxidation activity (Fig. 3E). Fasted animals administered with ad-siGdf15 also have a severe reduction in their ability to produce ketones (β-hydroxybutyrate) when treated octanoate, a ketogenic substrate with high mitochondria permeability (Fig. 3F) [29]. Further studies also demonstrate that loss of hepatic GDF15 resulted in marked decreases of mRNA levels of key genes involved in hepatic fatty acid β-oxidation and ketogenesis, including Ppara, Peroxisome proliferator-activated receptor γ coactivator 1α (Pgc1α), Acox1, Carnitine palmitoyltransferase 1a (Cpt1a) and 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (Hmgs2) (Fig. 3G), but showed no impacts on expression levels of other genes involved in liver metabolism, such as lipogenesis, fatty acid uptake and vLDL secretion (Fig. 3H). Interestingly, ad-siGdf15-treated mice displayed reduced fasting-induced free fat acids (FFA) in circulation (Fig. 3J) and downregulated expression levels of lipolysis-associated genes (Hormone sensitive lipase (Hsl) and Adipose triglyceride lipase (Atgl)) in gWAT (Fig. 3K), indicating that impaired hepatic GDF15 attenuate lipolysis in white adipose tissue. These data implicate that hepatic GDF15 is of great importance in fasting-induced fatty acid β-oxidation and ketogenesis.

3.4. Abrogation of hepatic GDF15 reduces diet-induced ketogenesis and alleviates ketoacidosis of diabetic mice

To determine the role of GDF15 in ketogenesis of liver, we fed the mice with a ketogenic diet (KD) to enforce hepatic ketones production in liver. Relative to feeding a normal chow diet (NCD), KD consumption for 3 days efficiently caused excess lipid accumulation in liver (Fig. 4A-C) and extremely upregulated serum content of β-hydroxybutyrate (Fig. 4D). After ad-siGdf15-mediated ablation of hepatic GDF15 (Fig. 4E), KD-feeding mice displayed much more TGs content in the liver but dramatic reduction of serum ketones (Fig. 4A-D). Moreover, decreased hepatic GDF15 also repressed expression levels of genes involved in fatty acid β-oxidation and ketogenesis in livers, including
Subsequently, we applied streptozotocin (STZ)-induced type 1 diabetic mice as another kind of ketotic model [30] to further investigate physiological roles of hepatic GDF15 in liver ketogenesis. First of all, at 3 days after STZ injection, mice with non-fasting blood glucose levels higher than 300 mg/dl and almost none circulating insulin were selected for further analysis (Fig. 5A and B). In consisting with previous study [21], STZ treatment dramatically enlarged Xbp1 mRNA splicing levels in liver (Fig. 5C). Importantly, hepatic Gdf15 mRNA abundance also showed greatly upregulated after STZ treatment and was significantly suppressed when ad-siGdf15 were injected (Fig. 5C). Indeed, levels of serum β-hydroxybutyrate were greatly elevated in STZ-treated mice but significantly repressed after the loss of hepatic GDF15, indicating impairment of ketone production in Gdf15-abrogated liver (Fig. 5D). Consistently, reduced hepatic GDF15 repressed expression levels of genes involved in fatty acid β-oxidation and ketogenesis in livers of STZ-treated mice, including Ppara, Acadl, Acox1, Cpt1a and Hmgcs2 (Fig. 5E). To investigate whether insulin could correct changes of hepatic Gdf15 expression, we treated the STZ-induced diabetic mice with insulin. Interestingly, insulin treatment efficiently repressed the upregulated Xbp1 splicing and Gdf15 mRNA levels in livers of STZ-treated mice (Fig. S4A-B), and then reduced the elevated β-hydroxybutyrate in blood (Fig. S4C).

To explore whether GDF15 plays a role in the development of obesity-induced fatty liver, we analyzed GDF15 expression in the livers of leptin receptor-deficient mice (db/db mice). Interestingly, relative to control littermates (WT), db/db mice displayed dramatic increases of hepatic Gdf15 mRNA abundance and protein levels in circulation and liver (Fig. S5A-C). To further explore physiological functions of GDF15 in NAFLD, db/db mice were injected with adenovirus expressing GFP (Ad-GFP) or XBP1s (Ad-XBP1s).
(ad-GFP) as control or GDF15 (ad-GDF15) to enforce GDF15 levels in livers (Fig. 6A–B) and circulation (Fig. 6C). In comparison to control mice, with similar body weight and food intake (Fig. S6A–B), ectopic GDF15 resulted in significantly reduced liver weight (Fig. 6D), hepatic contents of TG (Fig. 6E), impaired hepatosteatosis (Fig. 6F–G), improved NAFLD (Fig. 6H–I) and liver damage (Fig. 6J) in obese mice. Furthermore, GDF15-expressed obese mice exhibited dramatically improved glucose tolerance (Fig. 6K), reduced insulin resistance (Fig. 6L) and hyperinsulinemia (Fig. 6M). As expected, ectopic expression of hepatic GDF15 heightened serum contents of ketones, indicating

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**Fig. 3. Abrogation of hepatic GDF15 attenuates fasting-induced fatty acid β-oxidation and ketogenesis.** Male C57BL/6 J mice were administered adenovirus expressing negative control siRNA (Ad-NC) or siGdf15 targeted at mouse Gdf15 mRNA (Ad-siGdf15) at the age of 10 weeks and then subjected for 24-h fasting two weeks later (n = 10 for groups of ad-NC and ad-siGdf15) or fed ad libitum (n = 6 for groups of ad-NC and ad-siGdf15). A-B, Representative images of liver sections stained with H&E (A) and Oil-red O (B). Scale bar is 100 µm. C, Liver contents of triglycerides (TG). D, Serum contents of β-hydroxybutyrate. E, Determination of fatty acid β-oxidation by the using of fresh liver homogenates. F, Serum β-hydroxybutyrate were determined at indicated time points of the fasted mice which were i.p. injected with sodium octanoate. G-H, qRT-PCR analysis of the mRNA abundance of the indicated genes in liver. RPL18 was used as the internal control. I, ELISA assays determined serum contents of GDF15. J, Serum contents of free fat acids (FFA). K, qRT-PCR analysis of the mRNA abundance of Hsl and Atgl in gonadal white adipose tissue (gWAT). All data are shown as mean ± s.e.m. *, p < .05; **, p < .01 or ***, p < .001 by unpaired two-tailed Student’s t-test or two-way ANOVA.
β-ketoacidosis, which results in diabetic ketoacidosis (DKA), a life-threatening complication of Type 1 diabetes. Conversely, GDF15 knockdown in liver could enhance hepatic β-oxidation and ketogenesis, which may help the liver produce ketones for metabolic adaptation during fasting or diabetes. In our study, GDF15 knockdown in liver enhanced β-oxidation and ketogenesis, suggesting the critical roles of hepatic GDF15 in metabolic adaptation of liver to fasting or ketogenic diet feeding. 

4. Discussion

Growing studies reveal that elevated GDF15 in blood display a close association with type 2 diabetes, cardiovascular diseases and liver diseases [31–33]. GDF15 is considered as a crucial hormone in regulating lipid and carbohydrate metabolism, and is even to be used as a biomarker for diabetes and cardiovascular diseases [6,14,15]. It still remains elusive the physiological roles of GDF15 in metabolic adaptations and nonalcoholic fatty liver disease (NAFLD) development of liver, however. In our present study, Gdf15 mRNA (Ad-siGdf15) expression was reduced in liver, which might diminish the activities of hepatic fatty acid β-oxidation and ketone production via repressing key oxidation-associated genes expression (such as Ppara, Pgc1a Cpt1a, Acox1 and Hmgcs2) in the livers of ketotic mice (Fig. 3), as well as the mice with STZ-induced type 1 diabetes [14]. Importantly, GDF15 secreted by hepatocytes acts as an important hepatokine in regulating lipid homeostasis of liver [3,34]. In our study, hepatocyte-expressed GDF15 is indeed needed for activating fatty acid β-oxidation in liver when faced to 24-h fasting (Fig. 3). Notably, deleting carnitine palmitoyltransferase 2 (Cpt2) in liver could lead to enhanced hepatic Gdf15 expression and serum GDF15 contents, and even higher when the mice were subjected to fasting, suggesting the critical roles of hepatic GDF15 in fatty acid β-oxidation and metabolic adaptations of liver in facing to starvation [3]. Inhibition of GDF15 expression in liver could sufficiently reduce the activities of fatty acid β-oxidation and ketone production via repressing key oxidation-associated genes expression (such as Ppara, Pgc1a Cpt1a, Acox1 and Hmgcs2) in the livers of fasted mice (Fig. 3), as well as the mice with STZ-induced type 1 diabetes (Fig. 4). As ketoacidosis is one of the most dangerous diabetes complications, inhibition of hepatic GDF15 might be a promising method to control circulating ketone levels in diabetic patients.

In our current study, decreased hepatic GDF15 resulted in reduced oxidative metabolism-associated genes but didn’t affect the expression of Fgf21 in liver. Although PPARα is considered as the main mediator in regulating hepatic Fgf21 expression, the loss of PPARα didn’t cause significant impairment of hepatic Fgf21 expression in mice subjected to 24-h fast [35], indicating that some other regulators might activate Fgf21 expression as a compensation of PPARα loss during fasting, i.e. XBP1s [28]. More XBP1s are generated by heightened ER stress in GDF15-deficient liver with higher TG accumulation (data not shown) and subsequently promote Fgf21 expression, which might diminish the effects of PPARα reduction. Indeed, the underlying molecular metabolism(s) of GDF15 in regulating hepatic fatty acid β-oxidation are still to be elucidated.

Fig. 4. Ablation of hepatic GDF15 reduces diet-induced ketogenesis. Male C57BL/6J mice were administered adenovirus expressing negative control siRNA (Ad-NC) or siGdf15 targeted at mouse Gdf15 mRNA (Ad-siGdf15) at the age of 10 weeks and then fed with ketogenic diet (KD, n = 8 for groups of ad-NC and ad-siGdf15) or normal chow diet (NCD) (n = 6 for groups of ad-NC and ad-siGdf15) for 3 days. A-B, Representative images of liver sections stained with H&E (A) and Oil-red O (B). Scale bar is 100 µm. C, Liver contents of TG. D, Serum -hydroxybutyrate. E, qRT-PCR analysis of the mRNA abundance of the indicated genes in liver. Rps18 was used as the internal control. All data are shown as mean ± s.e.m., *, p < .05 or **, p < .01 by two-way ANOVA.
needed to be elucidated to help people understand the interrelationships of GDF15 and FGF21 in regulating the complicated processes of liver lipid metabolism.

Circulating GDF15 levels are positively correlated with body weight in human and elevated GDF15 in tissues and blood has been revealed in obese mice [11,14,36,37]. Recently, 3 independent studies demonstrate that GDF15 could bind with high affinity to GDNF family receptor a-like (GFRAL) in neurons to promote weight loss in mice and nonhuman primates, enabling a more comprehensive assessment of GDF15 as a potential pharmacotherapy for the treatment of obesity [38–40]. In our present study, ectopic expression of GDF15 in liver of obese mice could conversely augment lipid catabolism in liver and eventually result in alleviated NAFLD development and improved insulin resistance (Fig. 5), which are in consistent with the metabolic beneficial effects of upregulated GDF15 in Cpt2-deficient mice [34]. Interestingly, we didn’t find overexpressed hepatic Gdf15 affect body weight and calorie uptake of obese mice even circulating GDF15 levels were much higher in ad-GDF15-injected mice (Fig. 6C, Fig. S6A-B). These may be due to the in vivo experiments we did in the db/db mice were all completed within 4 weeks after the adenovirus injection.

ER stress is thought to underlie metabolic dysfunctions [41,42], and the UPR pathways are implicated in many metabolic processes [21,26,43]. Previous study has well revealed the crucial role of IRE1α-XBP1s branch of the UPR pathway in orchestrating fatty acid β-oxidation and ketogenesis through activating Ppara transcription upon fasting and ketogenic diet feeding [21]. Moreover, accumulative evidence demonstrates that IRE1α-XBP1 pathway controls takes part in the regulation of various metabolic processes, such as protein disulphide isomerase (PDI) [44], fatty acid synthase (FAS) or UDP-galactose-4-epimerase [20], or through non-transcriptional actions such as promoting degradation of the forkhead box O1 transcription factor (FoxO1) [45]. We found that fasting-induced increased activation of IRE1α-XBP1s could drive the transcription of Gdf15 in liver (Fig. 2). Chung et al. reported that CHOP could activate GDF15 expression in skeletal muscle [14]. As fasting and ketogenic diet show no effects on hepatic Chop expression (Fig. S2), we can exclude the possibility of CHOP-regulating Gdf15 expression in fasted liver, indicating various molecular mechanisms in regulating GDF15 expression in different tissues. Despite of these, increased activation of the UPR pathways in fatty liver also implicates that elevated GDF15 levels of obese mice might be mostly contributed by upregulated XBP1s in liver. To investigate whether classical ER stress could exacerbate Gdf15 expression in liver, we treat the mice with Tm to induce ER stress in the liver [25]. Interestingly, we found that the Tm treatment could also activate hepatic Gdf15 expression and serum levels of GDF15, which further indicates the important role of the UPR pathways in Gdf15 expression in different tissues. Our results are supported by an independent research work from another lab, which was recently published [46]. Thus, the UPR pathways are critical in controlling hepatic Gdf15 expression to regulate lipid metabolism of liver. Further studies are needed to elucidate underlying mechanisms of UPR pathways and GDF15 in future.

In summary, our present study reveals a key role of hepatocyte-derived GDF15 in hepatic lipid homeostasis. These findings will be applicable in the clinical treatment and drug discovery for diabetes and NAFLD.
Fig. 6. Elevated hepatic GDF15 reduces obesity-induced hepatosteatosis via enhancing hepatic lipid catabolism. db/db mice at 10 weeks of age were administered with adenovirus expressing GFP (Ad) or GDF15 (Ad-GDF15). A, qRT-PCR analysis of mRNA abundance of Gdf15 in liver. B-C, GDF15 contents in liver (C) and serum (B). D, Liver weight. E, Hepatic TG. F-H, Representative images of liver sections stained with H&E (F), Oil-red O (G) and Masson’s trichrome (H). Scale bar is 100 µm. I, NAFLD activity. J, Levels of ALT and AST. K, Glucose tolerance tests (GTT). L, Insulin tolerance tests (ITT). M-N, Serum levels of insulin (M) and β-hydroxybutyrate (N). O-P, qRT-PCR analysis of mRNA abundance of indicated genes in liver (O) and Hsl and Atgl in gWAT (P). For A, O and P, Rps18 was used as the internal control. All data are shown as mean ± s.e.m.. *, p < .05; **, p < .01 or ***, p < .001 by unpaired two-tailed Student’s t-test or two-way ANOVA.
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Data availability statement

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Authors’ contributions

M. Zhang and Y. Tang conceived and designed the studies. M. Zhang, W. Sun and J. Qian conducted most of the experiments and analyzed the data. M. Zhang and Y. Tang wrote the manuscript.

Competing interests

The authors declare no competing financial interests.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.redox.2018.01.013.

References

[1] G.F. Cahill Jr., Fuel metabolism in starvation, Annu. Rev. Nutr. 26 (2006) 1–22.
[2] D.E. Owen, A.P. Morgan, H.G. Kemp, J.M. Sullivan, M.G. Herrera, G.F. Cahill Jr., Brain metabolism during fasting. J. Clin. Investig. 46 (10) (1968) 1589–1595.
[3] J. Lee, J. Choi, S. Scalfi, M.J. Wolfgang, Hepatic fatty acid oxidation represses systemic catabolism during starvation. Cell Rep. 16 (1) (2016) 201–212.
[4] S. Kirsten, J. Seydoux, J.M. Peters, F.J. Gonzalez, B. Desvergne, W. Wahli, Peroxisome proliferator-activated receptor alpha mediates the adaptive response to fasting. J. Clin. Investig. 103 (11) (1999) 1489–1498.
[5] M.K. Badman, P. Piston, A.R. Kennedy, G. Kourous, J.S. Flier, E. Manzoni-Flier, Hepatic fibroblast growth factor 21 is regulated by paaparla and is a key mediator of hepatic lipid metabolism in ketotic states. Cell Metab. 5 (6) (2007) 426–437.
[6] R. Adela, S.K. Banerjee, Gdf-15 as a target and biomarker for diabetes and cardiovascular diseases: a translational perspective. J. Diabetes Res. 2015 (2015) 490842.
[7] H. Johnson, S. Lin, T. Kuffner, D.A. Brown, V.W. Tsai, A.R. Baukün, et al., Tumor-induced anorexia and weight loss are mediated by the tgf-beta superfamily cytokine mic-1. Nat. Med. 13 (11) (2007) 1323–1340.
[8] M. Yokoyama-Kobayashi, M. Saeki, S. Sekine, S. Kato, Human cdna encoding a novel tgf-beta superfamily protein highly expressed in placenta. J. Biochem. 122 (3) (1997) 622–626.
[9] J. Koepmann, P. Buckhaults, D.A. Brown, M.L. Zahurak, N. Sato, N. Fukushima, et al., Serum macropage inhibitory cytokine-1 as a marker of pancreatic and other peripancreatic cancers. Cancer Res. 60 (10) (2000) 2384–2392.
[10] T. Kempf, R. Horn-Wichmann, G. Brabant, T. Peter, T. Allhoff, G. Klein, et al., Circulating concentrations of growth-differentiation factor 15 in apparently healthy elderly individuals and patients with chronic heart failure as assessed by a new immunoradiometric sandwich assay. Clin. Chem. 53 (2) (2007) 284–291.
[11] Q. Ding, T. Mraček, P. González-Muniesa, K. Kos, J. Wilding, P. Traynor, et al., Identification of macropage inhibitory cytokine-1 in adipose tissue and its secretion as an adipokine by human adipocytes. Endocrinology 150 (4) (2009) 1688–1696.
[12] D.A. Brown, S.N. Breit, J. Buring, W.D. Fairchild, A.R. Baukün, T. Liu, et al., Concentration in plasma of macropage inhibitory cytokine-1 and risk of cardiovascular events in women: a nested-case control study. Lancet 359 (9324) (2002) 622–623.
[13] T. Kempf, E. Bjorklund, S. Olofsson, B. Lindahl, T. Allhoff, T. Peter, et al., Growth-differentiation factor 15 improves risk stratification in st-elevation myocardial infarction. Eur. Heart J. 28 (23) (2007) 2858–2865.
[14] H.K. Chung, D. Ryu, K.S. Kim, J.Y. Chang, Y.K. Kim, H.S. Yi, et al., Growth differentiation factor 15 is a myokinin governing systemic energy homeostasis. J. Cell Biol. 216 (1) (2017) 149–165.
[15] K. Chrysovergis, X. Wang, J. Kosak, S.H. Lee, J.S. Kim, J.F. Foley, et al., Nag-1/gdf15 prevents obesity by increasing thermogenesis, lipolysis and oxidative metabolism. Int. J. Obes. (Lon). 38 (12) (2014) 1555–1564.
[16] R. Dong, P. Walter, S. Sengupta, T.R. Petersson, M. Laplante, S.H. Back, et al., Pka phosphorylation of the ligand, Nat. Med. 13 (11) (2007) 1333–1340.
[17] V.W. Tsai, L. Macia, C. Feinle-Bisset, R. Manandhar, A. Asprou, A. Raben, et al., Sensitivity levels of human gdf15 vary in a diurnal pattern, do not display a profile suggestive of a satiety factor and are related to bmi, PLoS One 10 (7) (2015) e0133362.
[18] F. Verdegher, M.S. Soustek, M. Hatting, S.M. Blatter, D. McDonald, J.J. Barrow, et al., Brown adipose yil defeiciency activates expression of secreted proteins linked to energy expenditure and prevents diet-induced obesity, Mol. Cell. Biol. 36 (1) (2016) 184–196.
[19] S.E. Mullican, A. Lin-Schmidt, C.N. Chiu, A.J. Chavez, L.J. Furman, A.A. Armstrong, et al., Gfral is the receptor for gdf15 and the ligand promotes weight loss in mice and nonhuman primates. Nat. Med. (2017).
[20] L. Yang, C.C. Chang, Z. Sun, D. Madsen, H. Zhu, S.B. Padjaker, et al., Gfral is the receptor for gdf15 and it is required for the anti-obesity effects of the ligand. Nat. Med. (2017).
[21] P.J. Emmerson, F. Wang, Y. Du, Q. Liu, R.T. Pickard, M.D. Gonzalez-Crespo, et al., The metabolic effects of gdf15 are mediated by the orphan receptor gfral. Nat. Med. (2017).
[22] G.S. Hotamisligil, Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. Cell 140 (6) (2010) 900–917.
[23] H. Mahli, R.J. Kaufman, Endoplasmic reticulum stress in liver disease. J. Hepatol. 54 (4) (2011) 795–809.
[24] S. Pu, S.M. Watkins, G.S. Hotamisligil, The role of endoplasmic reticulum in hepatic lipid homeostasis and stress signaling. Cell Metab. 15 (5) (2012) 623–634.
[25] S. Wang, Z. Chen, V. Lam, J. Han, J. Hasler, B.N. Finck, et al., irf1a is regulated by ageing. Nature 469 (7327) (2010) 1100–1104.
[26] J.P. Blackshear, K.G. Alberti, Experimental diabetic ketoacidosis. Sequential changes in metabolic intermediates in blood, liver, cerebrospinal fluid and brain after acute insulin deprivation in the streptozotocin-diabetic rat. J. Biochem. 138 (1) (1974) 107–117.
[27] H. Zhang, W. Zhang, X. Tu, Y. Niu, X. Li, L. Qin, et al., Elevated serum growth differentiation factor 21 levels are associated with thyroid nodules in type 2 diabetes aged over 60 years, Oncotarget 8 (25) (2017) 41797–41806.
[28] M.Y. Shin, J.M. Kim, E.Y. Kang, M.K. Kim, H.K. Juong, J.H. Lee, et al., Association between growth differentiation factor 15 (gdf15) and cardiovascular risk in patients with newly diagnosed type 2 diabetes mellitus. J. Korean Med. Sci. 31 (9) (2016) 1413–gdf1418.
[29] E.S. Lee, S.H. Kim, H.J. Kim, H.K. Kim, B.S. Lee, J.B. Ku, Growth differentiation factor 15 predicts chronic liver disease severity. Gut Liver 11 (2) (2017) 276.
[30] J. Lee, C. Choi, E.S. Selen Alpergin, L. Zhao, T. Hartung, S. Scalfi, et al., Loss of hepatic mitochondrial long-chain fatty acid oxidation confers resistance to diet-induced obesity and glucose intolerance. Cell Rep. 20 (3) (2015) 655–667.
[31] S. Jiang, C. Yan, Q.C. Fang, M.L. Shao, Y.L. Zhang, Y. Liu, et al., Fibroblast growth factor-21 is regulated by the irf1alpha-xbp1 branch of the unfolded protein response and counteracts endoplasmic reticulum stress-induced hepatic steatosis. J. Biol. Chem. 289 (43) (2014) 29751–29765.
[32] S. Sengupta, T.R. Petersson, M. Laplante, S.H. Back, D.M. Sabatini, Mtorc1 controls fasting-induced ketogenesis and its modulation by ageing. Nature 468 (7327) (2011) 1100–1104.
[33] E.M. Greenberg, Z. Feng, Y. Zeng, S. Jin, F. Li, J. Fang, et al., Gdf15 expression is increased in diet-induced obesity in the liver. J. Clin. Investig. 103 (4) (1999) 1498–1505.
[34] S. Jiang, M. Kuroki, T. Tanaka, A. Matsumoto, K. Hata, H. Kobayashi, et al., A novel human serum protein, c51, acts as a satiety factor and is related to bmi, PLoS One 10 (7) (2015) e0133362.