The role of fibroblast growth factor 21 in the pathogenesis of non-alcoholic fatty liver disease and implications for therapy

Jia Liu, Yuan Xu, Yanjin Hu, Guang Wang

Department of Endocrinology, Beijing Chao-yang Hospital, Capital Medical University, No. 8, Gongti South Road, Chaoyang District, Beijing 100020, China

ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) includes a cluster of liver disorders ranging from simple fatty liver to non-alcoholic steatohepatitis (NASH) and cirrhosis. Due to its liver and vascular complications, NAFLD has become a public health problem with high morbidity and mortality. The pathogenesis of NAFLD is considered a “multi-hit hypothesis” that involves lipotoxicity, oxidative stress, endoplasmic reticulum stress, a chronic inflammatory state and mitochondrial dysfunction. Fibroblast growth factor 21 (FGF21) is a member of the fibroblast growth factor family with multiple metabolic functions. FGF21 directly regulates lipid metabolism and reduces hepatic lipid accumulation in an insulin-independent manner. Several studies have shown that FGF21 can ameliorate the “multi-hits” in the pathogenesis of NAFLD. The administration of FGF21 reverses hepatic steatosis, counteracts obesity and alleviates insulin resistance in rodents and nonhuman primates. Using several strategies, we show that the reversal of simple fatty liver and NASH is mediated by activation of the FGF21 signaling pathway. In this review, we describe the molecular mechanisms involved in the onset and/or progression of NAFLD, and review the current literature to highlight the therapeutic procedures associated with the FGF21 signaling pathway for simple fatty liver and NASH, which are the two most important types of NAFLD.

© 2015 Elsevier Inc. All rights reserved.

Abbreviations: NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; FGF21, fibroblast growth factor 21; SREBP-1c, sterol regulatory element-binding protein-1c; ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; SCD1, stearoyl-CoA desaturase 1; ChREBP, carbohydrate response element binding protein; PKC, protein kinase C; ERK, extracellular regulated protein kinases; ROS, reactive oxygen species; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; CRP, C-reactive protein; JNK, c-jun N-terminal kinase; iNOS, inhibitor of nuclear factor-κB; NF-κB, nuclear factor-κB; CHOP, CCAAT enhancer binding protein homologous protein; FGFR, fibroblast growth factor receptor; PPARα, peroxisome proliferator activated receptor α; AMPK, AMP-activated protein kinase; SIRT1, sirtuin 1; PPARγ, peroxisome proliferators-activated receptor γ; UCP-1, uncoupling protein 1; UCP-2, uncoupling protein 2; P38, phosphatidylinositol 3-kinase; ATF4, activating transcription factor 4; OLETF, Otsuka Long Evans Tokushima Fatty; GLP-1, glucagon-like peptide-1; MCAD, medium-chain acyl-CoA dehydrogenase.

* Disclosure statement: The authors have nothing to disclose.

* Corresponding author at: Department of Endocrinology; Beijing Chao-yang Hospital, Capital Medical University, No. 8, Gongti South Road, Chaoyang District, Beijing 100020, China. Tel./fax: +86 10 85231710.

E-mail address: drwg6688@126.com (G. Wang).

http://dx.doi.org/10.1016/j.metabol.2014.11.009

0026-0495/© 2015 Elsevier Inc. All rights reserved.
1. Introduction

Non-alcoholic fatty liver disease (NAFLD) includes a cluster of liver disorders ranging from simple fatty liver to non-alcoholic steatohepatitis (NASH) to cirrhosis [1]. A sedentary lifestyle along with excessive energy intake contributes to obesity, insulin resistance and NAFLD [1]. NAFLD is a public health problem with high morbidity and mortality due to its liver and vascular complications [1]. The current therapies are limited to reducing weight and improving insulin sensitivity with drugs or lifestyle interventions, such as dietary changes and physical activity, which have uncertain therapeutic effects [1].

Fibroblast growth factor 21 (FGF21) is a member of the fibroblast growth factor family with multiple metabolic functions [2]. The administration of FGF21 reverses hepatic steatosis, counteracts obesity, and alleviates insulin resistance and dyslipidemia in both rodents and nonhuman primates. These findings support its development as a novel therapy for the treatment of NAFLD and other metabolic disorders [3–9]. In this review article, we describe the molecular mechanisms involved in the onset and/or progression of NAFLD. Furthermore, we review the current literature to highlight the therapeutic procedures associated with the FGF21 signaling pathway for simple fatty liver and NASH, which are the two most important types of NAFLD.

2. The Pathogenesis of NAFLD

The pathogenesis of NAFLD has been modified to the “multi-hit hypothesis” from the “two-hit hypothesis” [10]. The “first-hit” is known as hepatic lipid accumulation/steatosis, which is the initial histological characteristic of NAFLD [11]. Insulin resistance causes an imbalance of fatty acid metabolism of hepatocytes and further contributes to hepatic steatosis [10]. Under physiological conditions, insulin suppresses the lipolysis of white adipose tissue and hepatic gluconeogenesis and also promotes hepatic lipogenesis by stimulating the transcription of sterol regulatory element-binding protein-1c (SREBP-1c). SREBP-1c is a master regulator of lipogenesis that regulates the transcription of acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS) and stearoyl-CoA desaturase 1 (SCD1) [12,13]. However, in some insulin-resistant states, including obesity, type 2 diabetes and metabolic syndrome, lipolysis and hepatic gluconeogenesis are not inhibited by insulin. However, insulin retains the ability to effectively promote hepatic lipogenesis [14]. Elevated lipolysis of white adipose tissue increases plasma free fatty acids and enhances gluconeogenesis. These changes result in hyperglycemia and further increase de novo lipogenesis by stimulating carbohydrate response element binding protein (ChREBP) [14]. In NAFLD patients, approximately 60% of hepatic lipid accumulation is derived from the re-esterification of plasma free fatty acids. 26% occurs by de novo lipogenesis and 14% is derived from dietary fatty acids [15]. Additional lipids are deposited in the liver and simple fatty liver histologically manifests as steatosis with more than 5% hepatic lipid accumulation [11].

The exposure and overload of fatty acid harm hepatocytes by intracellular accumulation of lipid intermediates, such as diglycerides and ceramides, which is defined as lipotoxicity [16]. The lipids and intermediates induce endoplasmic reticulum stress and mitochondrial dysfunction directly or via activation of Toll-like receptors 2 and 4 [16]. Ceramides activate protein kinase C (PKC), inhibit Akt and increase protein phosphatase 2A levels. The increase in protein phosphatase 2A levels contributes to mitochondrial dysfunction and endoplasmic reticulum stress [17]. In addition, the accumulation of lipids and intermediates activates p38 mitogen-activated protein kinase, extracellular regulated protein kinases (ERK) and c-Jun. The activation of these pathways further exacerbates insulin resistance [18]. Elevated fatty acid β-oxidation increases reactive oxygen species (ROS) and activates oxidative stress [16]. The hepatic lipid accumulation and intracellular stresses activate the transcription and release of pro-inflammatory factors, such as interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and C-reactive protein (CRP) [19,20]. A sedentary lifestyle in conjunction excessive energy intake promotes obesity and dysfunction of white adipose tissue. White adipose tissue secretes more TNF-α and IL-6 and reduces the secretion of adiponectin [20]. The elevated circulating levels of pro-inflammatory cytokines and reduced anti-inflammatory factors cause a chronic low-grade inflammatory state that is recognized as an important pathogenic mechanism of NAFLD [20,21].

These “hits” further deteriorate insulin resistance and initiate inflammatory signaling pathways and apoptosis cascades [10]. TNF-α activates c-Jun N-terminal kinase (JNK), PKC and inhibitor of nuclear factor-κB (IκB), which inhibits insulin signal and triggers inflammatory pathways [20]. TNF-α also activates de novo ceramide synthesis by stimulating sphingomyelinase [22]. IL-6 stimulates Janus kinase activation and induces the phosphorylation of signal transducers and activators of transcription to increase the transcription of the suppressor of cytokine signaling and promote insulin resistance [23]. An increase of ROS levels and oxidative stress triggers the ROS–PKC–nuclear factor-κB (NF-κB) pathway and further activates inflammatory signaling pathways that stimulate the infiltration of inflammatory cells [10,20]. Endoplasmic reticulum stress also stimulates cell apoptosis through both CCAAT enhancer binding protein homologous protein (CHOP)-induced and JNK-mediated mitochondria-dependent apoptosis pathways [24,25].

Hepatocytes with excess lipid accumulation are susceptible to subsequent “multi-hits”, including oxidative stress, endoplasmic reticulum stress, a chronic inflammatory state and mitochondrial dysfunction. These changes lead to the infiltration of inflammatory cells, activation of inflammatory signaling pathways and hepatocytes apoptosis. Additionally, these changes promote the progression from simple fatty liver to NASH [10] (Fig. 1). Previous studies have shown more than 25% of NASH patients will develop cirrhosis within 10 years, and a few patients will develop end-stage liver disease and hepatocellular carcinoma [26]. Thus, NASH is a turning point from benign to irreversible lesions including cirrhosis and hepatocellular carcinoma [26].
3. FGF21 and NAFLD

FGF21 is predominantly released from hepatocytes and to a lesser extent from other tissues, including white adipose tissue, skeletal muscle and pancreatic β-cells [2]. FGF21 binds to β-klotho and fibroblast growth factor receptor (FGFR) and induces the dimerization and autophosphorylation of FGFR. Activated FGFR then initiates its biological functions [2]. Recent studies have shown that FGF21 is a metabolic regulator of energy homeostasis, glucose-lipid metabolism and insulin sensitivity [2]. FGF21 reverses hepatic steatosis and prevents diet-induced obesity in both rodents and nonhuman primates [3–9]. There are several underlying mechanisms responsible for the above-mentioned beneficial effect of FGF21 (Fig. 1).

FGF21 directly regulates lipid metabolism and reduces hepatic lipid accumulation in an insulin-independent manner [12,27,28]. Additionally, FGF21 inhibits the lipolysis of white adipose tissue and further decreases circulating free fatty acids levels [29]. The over-expression of FGF21 reverses the up-regulated expression of SREBP-1c and FAS, the two key enzymes for lipid synthesis, in fatty acids-treated human liver-derived HepG2 cells [12]. In addition to suppressing lipid synthesis by lowering the hepatic influx of fatty acids and reducing the expression of lipid synthesis genes, FGF21 also promotes fatty acid β-oxidation mediated by the peroxisome proliferator activated receptor α (PPARα)-FGF21 transcriptional regulatory axis [28,30,31]. Moreover, FGF21 activates AMP-activated protein kinase (AMPK) and sirtuin 1 (SIRT1). The activation of these proteins up-regulates gene expression required for mitochondrial biogenesis and fatty acids β-oxidation [32]. A recent study showed that FGF21 also had the ability to ameliorate endoplasmic reticulum stress-mediated apoptosis in ApoE-/- mice [33]. FGF21 phosphorylates Akt and ameliorates insulin resistance in peripheral tissues [5]. The elevated insulin sensitivity decreased ChREBP-mediated de novo lipogenesis by promoting glucose utilization in the liver and skeletal muscle and inhibiting hepatic gluconeogenesis [14,17]. Moreover, the reduced insulin resistance also decreased the accumulation of lipid and its intermediates and lowered PKC activation in liver and skeletal muscle. Collectively, the result is the inhibition of inflammatory signaling pathways and apoptosis [24].

White adipose tissue highly expresses FGR1 and β-klotho, and is considered to be the predominant site of FGF21 action [34,35]. FGF21 stimulated glucose uptake by adipocytes is mediated through the activation of ERK1/ERK2 and the induction of glucose transporter-1 expression in an insulin-independent manner [27]. FGF21 inhibits lipolysis stimulated by growth hormone and catecholamine in adipocytes [29]. In white adipocytes, FGF21 also enhances mitochondrial oxidative capacity and potentiates peroxisome proliferator activated receptor γ (PPARγ) activity [36]. FGF21 is expressed in human brown adipose tissue and has a significant positive correlation with uncoupling protein 1 (UCP-1) expression [37]. FGF21 signaling elicits thermogenic activation and stimulates browning of white adipose tissue by inducing the expression of UCP-1 and ACC2, and then leading to an increase of energy expenditure [38]. FGF21 has been identified as a key modulator for adiponectin secretion in white adipose tissue [3]. FGF21 also counteracts the negative effects of TNF-α on adiponectin secretion [39]. Adiponectin alleviates steatosis, suppresses the NF-κB signaling pathway and improves insulin sensitivity by promoting fatty acid β-oxidation, reducing de novo lipogenesis and inhibiting the expression of pro-inflammatory cytokines [40]. Moreover, adiponectin also stimulates deacetylation of ceramides and decreases lipotoxicity [39]. Adiponectin has been reported to ameliorate hepatic mitochondrial dysfunction by stimulating uncoupling protein 2 (UCP-2) expression, which is a mitochondrial inner membrane transporter [41].

FGF21 administration significantly decreases the lipid content and improves the insulin sensitivity of skeletal muscle [4]. In human skeletal muscle myotubes, FGF21 prevents palmitate-induced insulin resistance by inhibiting the activation of stress-related kinases including JNK, NF-κB and PKC [42]. FGF21 has been proven as a myokine expressed by growth hormone and catecholamine in adipocytes [29]. In white adipocytes, FGF21 also enhances mitochondrial oxidative capacity and potentiates peroxisome proliferator activated receptor γ (PPARγ) activity [36]. FGF21 is expressed in human brown adipose tissue and has a significant positive correlation with uncoupling protein 1 (UCP-1) expression [37]. FGF21 signaling elicits thermogenic activation and stimulates browning of white adipose tissue by inducing the expression of UCP-1 and ACC2, and then leading to an increase of energy expenditure [38]. FGF21 has been identified as a key modulator for adiponectin secretion in white adipose tissue [3]. FGF21 also counteracts the negative effects of TNF-α on adiponectin secretion [39]. Adiponectin alleviates steatosis, suppresses the NF-κB signaling pathway and improves insulin sensitivity by promoting fatty acid β-oxidation, reducing de novo lipogenesis and inhibiting the expression of pro-inflammatory cytokines [40]. Moreover, adiponectin also stimulates deacetylation of ceramides and decreases lipotoxicity [39]. Adiponectin has been reported to ameliorate hepatic mitochondrial dysfunction by stimulating uncoupling protein 2 (UCP-2) expression, which is a mitochondrial inner membrane transporter [41].

FGF21 administration significantly decreases the lipid content and improves the insulin sensitivity of skeletal muscle [4]. In human skeletal muscle myotubes, FGF21 prevents palmitate-induced insulin resistance by inhibiting the activation of stress-related kinases including JNK, NF-κB and PKC [42]. FGF21 has been proven as a myokine expressed by growth hormone and catecholamine in adipocytes [29]. In white adipocytes, FGF21 also enhances mitochondrial oxidative capacity and potentiates peroxisome proliferator activated receptor γ (PPARγ) activity [36]. FGF21 is expressed in human brown adipose tissue and has a significant positive correlation with uncoupling protein 1 (UCP-1) expression [37]. FGF21 signaling elicits thermogenic activation and stimulates browning of white adipose tissue by inducing the expression of UCP-1 and ACC2, and then leading to an increase of energy expenditure [38]. FGF21 has been identified as a key modulator for adiponectin secretion in white adipose tissue [3]. FGF21 also counteracts the negative effects of TNF-α on adiponectin secretion [39]. Adiponectin alleviates steatosis, suppresses the NF-κB signaling pathway and improves insulin sensitivity by promoting fatty acid β-oxidation, reducing de novo lipogenesis and inhibiting the expression of pro-inflammatory cytokines [40]. Moreover, adiponectin also stimulates deacetylation of ceramides and decreases lipotoxicity [39]. Adiponectin has been reported to ameliorate hepatic mitochondrial dysfunction by stimulating uncoupling protein 2 (UCP-2) expression, which is a mitochondrial inner membrane transporter [41].

FGF21 administration significantly decreases the lipid content and improves the insulin sensitivity of skeletal muscle [4]. In human skeletal muscle myotubes, FGF21 prevents palmitate-induced insulin resistance by inhibiting the activation of stress-related kinases including JNK, NF-κB and PKC [42]. FGF21 has been proven as a myokine expressed by growth hormone and catecholamine in adipocytes [29]. In white adipocytes, FGF21 also enhances mitochondrial oxidative capacity and potentiates peroxisome proliferator activated receptor γ (PPARγ) activity [36]. FGF21 is expressed in human brown adipose tissue and has a significant positive correlation with uncoupling protein 1 (UCP-1) expression [37]. FGF21 signaling elicits thermogenic activation and stimulates browning of white adipose tissue by inducing the expression of UCP-1 and ACC2, and then leading to an increase of energy expenditure [38]. FGF21 has been identified as a key modulator for adiponectin secretion in white adipose tissue [3]. FGF21 also counteracts the negative effects of TNF-α on adiponectin secretion [39]. Adiponectin alleviates steatosis, suppresses the NF-κB signaling pathway and improves insulin sensitivity by promoting fatty acid β-oxidation, reducing de novo lipogenesis and inhibiting the expression of pro-inflammatory cytokines [40]. Moreover, adiponectin also stimulates deacetylation of ceramides and decreases lipotoxicity [39]. Adiponectin has been reported to ameliorate hepatic mitochondrial dysfunction by stimulating uncoupling protein 2 (UCP-2) expression, which is a mitochondrial inner membrane transporter [41].
4. **FGF21 Resistance**

There are several stimulators that regulate the expression and release of FGF21. Acute, hour-long stimulation of overnutrition manifests as an inhibitory effect on FGF21 release [48]. However, a 3-day overfeeding significantly raises circulating FGF21 in healthy humans [49]. An increased fatty acid influx has been shown to induce hepatic FGF21 production via activating PPARα [50], and glucose activates FGF21 mRNA expression in a ChREBP-dependent way in rat hepatocytes [12]. Both oxidative stress and endoplasmic reticulum stress are related to increased expression of FGF21 [51,52]. A recent study demonstrated that FGF21 is the target gene of activating transcription factor 4 (ATF4) and CHOP [53]. Endoplasmic reticulum stress induced by triglycerides can directly regulate transcription factor 4 and CHOP. Moreover, intraperitoneal injection of the endoplasmic reticulum stressor – tunicamycin – can stimulate hepatic FGF21 expression and increase circulating FGF21 levels [52]. In vivo, insulin increases FGF21 levels in patients with insulin resistance during a euglycemic clamp test [50]. The circulating FGF21 levels are positively correlated with plasma insulin levels in patients with impaired glucose tolerance and type 2 diabetes [54]. Moreover, mitochondrial disorders in patients with NAFLD/NASH are related to elevated FGF21 expression [41].

Circulating FGF21 levels are significantly elevated in patients with simple fatty liver and NASH. The plasma levels and hepatic mRNA expression of FGF21 increase with the degree of steatosis [Table 1] [55-64]. There were similar results found in animal studies [65,66]. The induction of ERK1/2 phosphorylation by FGF21 is impaired in diet-induced obese mice [67]. Furthermore, the ability of FGF21 to promote adiponectin production is decreased significantly in mice with obesity and type 2 diabetes. The ability of FGF21 to decrease plasma levels of fatty acids, triglycerides and glucose is also restricted in diet-induced obese mice [6,68], and more notably, a higher dose of intravenous FGF21 still reverses hepatic steatosis, improves insulin sensitivity and decreases fasting glucose in NASH animal models [6,7,9]. These findings might suggest an FGF21-resistant state in animal models and patients with NAFLD/NASH. The increased FGF21 levels may be a protective response against disorders of glucose-lipid metabolism. In animal models of NAFLD, the expressions of FGF21 and β-klotho in white adipose tissue are markedly lower. Furthermore, the expressions of FGF2R, FGR substrates 2 and β-klotho in liver are also reduced [67,69]. The activation of inflammatory signaling pathways represses β-klotho expression by the JNK1 pathway in adipose tissue [68]. Additionally, in vitro studies have shown high glucose directly inhibits the expression of β-klotho [70]. Thus, the mechanisms responsible for FGF21 resistance may involve the inhibition of

<table>
<thead>
<tr>
<th>Year</th>
<th>Country (ethnicity)</th>
<th>Study design (diagnostic method)</th>
<th>Major findings</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
<td>China (Asian)</td>
<td>253 subjects (hepatic ultrasonography)</td>
<td>NAFLD patients showed significantly higher serum FGF21 than those without NAFLD.</td>
<td>[55]</td>
</tr>
<tr>
<td>2012</td>
<td>Turkey (European)</td>
<td>136 subjects NASH (n = 60)/healthy controls (n = 76) (biopsy)</td>
<td>NASH patients have higher serum FGF21 than those without NAFLD.</td>
<td>[56]</td>
</tr>
<tr>
<td>2012</td>
<td>China Hong Kong (Asian)</td>
<td>220 subjects NAFLD (n = 146)/healthy controls (n = 74) (biopsy)</td>
<td>NASH patients have higher serum FGF21 than controls.</td>
<td>[57]</td>
</tr>
<tr>
<td>2011</td>
<td>China (Asian)</td>
<td>138 subjects with ultrasonography diagnosed fatty liver (hepatic fat contents were measured by proton magnetic resonance spectroscopy)</td>
<td>Serum FGF21 levels were positively correlated with hepatic fat content especially in patients with mild/moderate hepatic steatosis.</td>
<td>[58]</td>
</tr>
<tr>
<td>2011</td>
<td>USA (Caucasian)</td>
<td>74 subjects NASH (n = 36)/healthy controls (n = 38) (biopsy)</td>
<td>Fasting plasma FGF21 levels were significantly higher in patients with NASH.</td>
<td>[59]</td>
</tr>
<tr>
<td>2011</td>
<td>China (Asian)</td>
<td>99 subjects patients with newly diagnosed type 2 diabetes (n = 69)/healthy controls (n = 30) (hepatic ultrasonography)</td>
<td>Serum FGF21 in newly diagnosed type 2 diabetic patients with NAFLD group was significantly increased.</td>
<td>[60]</td>
</tr>
<tr>
<td>2010</td>
<td>Turkey (European)</td>
<td>159 subjects NAFLD (n = 82)/healthy controls (n = 77) (biopsy)</td>
<td>NAFLD patients have significantly higher serum FGF21 levels than healthy controls; serum FGF21 levels were an independent predictor of hepatic steatosis scores in NAFLD patients.</td>
<td>[61]</td>
</tr>
<tr>
<td>2010</td>
<td>China (Asian)</td>
<td>348 subjects NAFLD (n = 224)/control subjects (n = 124) (hepatic ultrasonography); 17 subjects with different degrees of steatosis (biopsy)</td>
<td>Serum FGF21 levels of NAFLD patients were significantly higher than control subjects; in human liver tissues, FGF21 mRNA expression increased with the degree of steatosis.</td>
<td>[62]</td>
</tr>
<tr>
<td>2010</td>
<td>Spain (Caucasian)</td>
<td>30 subjects overweight or obese nondiabetic women (n = 10)/healthy, lean subjects (n = 20) (biopsy)</td>
<td>Serum FGF21 of NASH patients was increased.</td>
<td>[63]</td>
</tr>
<tr>
<td>Longitudinal studies</td>
<td></td>
<td></td>
<td>Baseline FGF21 level was an independent predictor of NAFLD.</td>
<td>[64]</td>
</tr>
</tbody>
</table>
FGF21 downstream signaling pathways induced by multiple pathologic conditions.

5. FGF21 and the Therapy of NAFLD

As a novel metabolic regulator, FGF21 has beneficial effects on simple fatty liver and NASH, which are the two most important types of NAFLD [6,7,9]. Several strategies have shown the ability to reverse simple fatty liver and NASH is potentially mediated by activation of the FGF21 signaling pathway (Tables 2 and 3) (Fig. 2).

Lifestyle modifications, including a calorie-restricted diet and regular physical exercise, are widely believed as the first-line treatment of simple fatty liver and NASH [1]. Previous population studies have shown that both exercise and caloric restriction significantly decrease liver enzyme levels and reverse hepatic steatosis [81]. Uebanso et al. demonstrated that a hypocaloric high-protein diet promotes hepatic lipolysis and lipid utilization and further leads to a significant improvement of simple fatty liver through a FGF21-dependent pathway [78]. Physical activity for two weeks has been shown to significantly increase circulating FGF21 levels in sedentary young healthy women [82]. However, both exercise and caloric restriction prevent obesity and NAFLD development of Otsuka Long Evans Tokushima Fatty (OLETF) rats by reducing circulating FGF21 levels and hepatic FGF21 mRNA expression [69]. The interesting finding is the up-regulation of hepatic FGF21 downstream effectors including FGRF2, FGF21 receptor substrate 2 and β-klotho in this model [69]. These data might indicate chronic exercise and caloric restriction alleviate FGF21 resistance. Therefore, it is likely that the acute effect of lifestyle modification is due to the stimulation of FGF21 expression, whereas chronic actions are attributable to improving hepatic FGF21 resistance. Another study showed that moderate weight loss by exercise and caloric restriction did not cause the changes of FGF21 levels in humans [73]. This controversy might be related to different duration of treatment, excise intensity and the degree of body weight loss.

In addition to the improvement of lipid profiles, PPARα agonists significantly reverse hepatic steatosis, necro-inflammation and collagen deposition in animal models with simple fatty liver and NASH [83,84]. The FGF21 gene has a PPARα response element and is considered as an important downstream target factor of PPARα [30]. Increasing evidence suggests that fenofibrate, by activating PPARα, shows the therapeutic benefits on NAFLD by increasing fatty acid β-oxidation, improving lipid profiles, decreasing hepatic insulin resistance and inhibiting the expression of inflammatory mediators [83]. However, the above-mentioned effects of fenofibrate are impaired in the FGF21 knockout mice [30]. Therefore, these data suggest the effects of PPARα agonists are mediated by activating the FGF21 signaling pathway.

Metformin treatment alleviates hepatic inflammation, steatosis and fibrosis in simple fatty liver and NASH patients

<table>
<thead>
<tr>
<th>Year</th>
<th>Country (ethnicity)</th>
<th>Study design diagnostic method</th>
<th>Interventions (duration)</th>
<th>Major findings</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>Germany (Caucasian)</td>
<td>Obese children (n = 60)/normal-weight children (n = 40)</td>
<td>Exercise, behavior, and nutrition therapy (12 months)</td>
<td>Compared with the normal-weight children, obese children had significantly increased FGF21 levels; a decrease of BMI was associated with a significant reduction of FGF21 levels.</td>
<td>[71]</td>
</tr>
<tr>
<td>2011</td>
<td>China (Asian)</td>
<td>Patients with newly diagnosed type 2 diabetes (n = 68)/controls (n = 52)</td>
<td>30 patients with newly diagnosed type 2 diabetes with FBG ≥14.0 mmol/L were treated with CSII (2 weeks)</td>
<td>Plasma FGF21 levels were higher in the newly diagnosed type 2 diabetes mellitus group than controls; fasting plasma FGF21 levels were significantly decreased after CSII treatment.</td>
<td>[72]</td>
</tr>
<tr>
<td>2011 USA (Caucasian)</td>
<td>Type 2 diabetic patients (n = 24) on diet and/or metformin (magnetic resonance spectroscopy)</td>
<td>Pioglitazone (45 mg/d) or pioglitazone (45 mg/d) + exenatide (10 μg twice daily) (12 months)</td>
<td>Pioglitazone treatment reduced hepatic fat, but plasma FGF21 levels did not change; combined treatment with pioglitazone and exenatide decreased FGF21 levels and hepatic fat.</td>
<td>[66]</td>
<td></td>
</tr>
<tr>
<td>2011 Germany (Caucasian)</td>
<td>Obese subjects (n = 30)</td>
<td>A weight reduction program (6 months)</td>
<td>Type 2 diabetic patients with poor glycemic control were added rosiglitazone (4 mg/day) (12 weeks)</td>
<td>Moderate weight loss did not induce changes of FGF21 levels in humans. Plasma FGF21 levels were higher in type 2 diabetic patients than in the controls; rosiglitazone treatment significantly decreased plasma FGF21 levels.</td>
<td>[73]</td>
</tr>
<tr>
<td>2009 China (Asian)</td>
<td>Patients with newly diagnosed type 2 diabetes (n = 30)/type 2 diabetic patients with poor glycemic control after the treatment with metformin (n = 34)/healthy controls (n = 30)</td>
<td></td>
<td></td>
<td>Both 3 weeks of VLC and 3 months of fenofibrate treatment significantly increased FGF21 levels.</td>
<td>[74]</td>
</tr>
<tr>
<td>2009 Czech (European)</td>
<td>Obese patients (n = 26)/type 2 diabetic patients (n = 11)/healthy controls (n = 32)</td>
<td>Very low calorie diet (3 weeks) or fenofibrate (200 mg/d) (3 months)</td>
<td>Placebo or fenofibrate (200 mg/d) (3 weeks)</td>
<td>Fenofibrate treatment significantly increased FGF21 levels.</td>
<td>[75]</td>
</tr>
<tr>
<td>2008 Sweden (Caucasian)</td>
<td>Normal-weight, non diabetic patients with primary hypertriglyceridemia (n = 19)</td>
<td></td>
<td></td>
<td></td>
<td>[76]</td>
</tr>
</tbody>
</table>
### Table 3 – Experimental studies investigating implicating FGF21 in the treatment of NAFLD.

<table>
<thead>
<tr>
<th>Year</th>
<th>Study design</th>
<th>Effect and potential mechanism</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2014</td>
<td>Liver-specific SIRT1 knockout (SIRT1 LKO) mice</td>
<td>Hepatic overexpression of FGF21 increased the gene expressions of fatty acid β-oxidation, decreased fasting-induced steatosis, reduced obesity and promoted browning of white adipose tissue.</td>
<td>[77]</td>
</tr>
<tr>
<td>2013</td>
<td>Diet-induced obese mice were infused with FGF21 (1 mg/kg/d) or vehicle (1 week)</td>
<td>In diet-induced obese mice, FGF21 infusion improved insulin responsiveness, decreased hepatocellular and myocellular diacylglycerol content and reduced protein kinase C activation in liver and skeletal.</td>
<td>[4]</td>
</tr>
<tr>
<td>2013</td>
<td>Adiponectin knockout mice were injected intraperitoneally with either rmFGF21 (2 mg/kg/d) or vehicle</td>
<td>The effects of FGF21 on ameliorating insulin resistance of liver and skeletal muscle were abrogated in adiponectin knockout mice, whereas FGF21-mediated activation of ERK1/ERK2 in adipose tissues remained unaffected.</td>
<td>[3]</td>
</tr>
<tr>
<td>2012</td>
<td>Db/db mice were treated with PEGylation of human FGF21 (0.25 mg/kg/d) (12 days)</td>
<td>PEGylation of human FGF21 reduced blood glucose, lipid profiles and hepatic lipid content, and enhanced insulin and glucose-dependent insulin secretion.</td>
<td>[5]</td>
</tr>
<tr>
<td>2009</td>
<td>Diet-induced obese mice were randomly assigned to intraperitoneally injection with FGF21 twice daily (0.1, 1, and 10 mg/kg/d) or vehicle (6 weeks)</td>
<td>FGF21 significantly reversed hepatic steatosis, reduced body weight, blood glucose, insulin and lipid levels, and improved hepatic and peripheral insulin sensitivity; the reduction of hepatic triglyceride levels was associated with FGF21-dependent inhibition of nuclear SREBP-1 expression.</td>
<td>[6]</td>
</tr>
<tr>
<td>2008</td>
<td>Diet-induced obese mice and ob/ob mice were treated with FGF21(0.1, 0.3, and 1 mg/kg/d) or vehicle (2 weeks)</td>
<td>In diet-induced obese and ob/ob mice, administration of FGF21 lowered body weight, increased energy expenditure, reduced hepatic steatosis and ameliorated hyperglycemia.</td>
<td>[7]</td>
</tr>
<tr>
<td>2007</td>
<td>Male diabetic rhesus monkeys were administered FGF21 (1.0 mg/kg/d) (6 weeks)</td>
<td>Exendin-4 could improve fatty liver by increasing SIRT1-mediated FGF21 expression.</td>
<td>[28]</td>
</tr>
<tr>
<td>2011</td>
<td>Diet-induced obese mice were treated with exendin-4 (4 weeks)</td>
<td>Exendin-4 significantly reduced hepatic triglyceride content and FGF21 expression, and enhanced phosphorylation of hepatic AMPK and ACC, but no significant difference in weight and body fat was observed.</td>
<td>[66]</td>
</tr>
<tr>
<td>2012</td>
<td>OLETF rats were grouped into ad libitum fed, sedentary (OLETF-SED), voluntary wheel running exercise (OLETF-EX), or caloric restriction (CR) (OLETF-CR, 70% of SED) (36 weeks)</td>
<td>Both daily EX and CR prevented obesity and NAFLD development, accompanied by significantly lower serum FGF21 and hepatic FGF21 expression; Hepatic FGF21 receptor substrate 2 and β-klotho mRNAs were elevated in the OLETF-EX and OLETF-CR animals compared with the OLETF-SED animals.</td>
<td>[69]</td>
</tr>
<tr>
<td>2009</td>
<td>High-sucrose induced obese rats were assigned randomly to one of two energy restricted diets: a carbohydrate-based control diet (CD) or a high-protein diet (HPD) (4 weeks)</td>
<td>High-sucrose diet caused greater improvement in fatty liver and hypertriglyceridemia than the CD diet; Expression of FGF21 was significantly increased in obese rats fed the HPD.</td>
<td>[78]</td>
</tr>
<tr>
<td>2011</td>
<td>Diet-induced fatty liver mice and db/db mice were given molecular hydrogen-water (12 weeks)</td>
<td>Drinking molecular hydrogen-water significantly enhanced the expression of FGF21, reduced hepatic oxidative stress, and alleviated fatty liver in db/db mice as well as diet-induced fatty liver mice.</td>
<td>[79]</td>
</tr>
<tr>
<td>2007</td>
<td>Wild-type mice, PPARα deficient mice and ob/ob mice were treated with the PPARα agonist Wy-14,643</td>
<td>Fasting or treatment of mice with the PPARα agonist induced FGF21 mRNA expression; in PPARα deficient mice, FGF21 expression was lower than wild-type mice, and fasting or treatment with PPARα agonist did not induce FGF21.</td>
<td>[30]</td>
</tr>
<tr>
<td>2013</td>
<td>C57/B6J mice were randomly divided into three groups: high-fat diet, artemisia santolinaefolia (SANT) and artemisia scoparia (SCO) (4 weeks)</td>
<td>Fewer lipid droplets were observed in the SCO group; SCO significantly increased hepatic insulin sensitivity and AMPK activity, decreased the expressions of lipid synthesis genes. Neither SANT nor SCO significantly altered plasma FGF21 levels.</td>
<td>[80]</td>
</tr>
</tbody>
</table>
These beneficial effects are caused by its ability to decrease hepatic gluconeogenesis, stimulate glucose uptake by skeletal muscle and increase fatty acid β-oxidation of adipose tissue and liver by activating AMPK [85]. Metformin stimulates the FGF21 expression of rat and human hepatocytes in vitro in a dose-dependent manner, and this can be blocked by the AMPK inhibitor Compound C [86]. Therefore, FGF21 may be involved in the AMPK signaling pathway and further contributes to the therapeutic effects of metformin on NAFLD. However, in human clinical trials, metformin is not beneficial for NAFLD patients [87]. This controversial result can be explained because these studies were short-term and have inconsistent outcomes due to the different duration and dose. Further larger randomized controlled trials with sufficient duration and histological endpoints are urgently needed to assess the effectiveness of metformin on NAFLD.

PPARγ agonist, another insulin sensitizer, has two representative medicine—pioglitazone and rosiglitazone. The PPARγ agonists ameliorate insulin resistance, reverse hepatic steatosis and inhibit liver inflammation and ballooning necrosis [36]. In vitro, PPARγ agonists induce FGF21 expression of murine and human adipocytes [88]. Animal studies have shown that FGF21 is an autocrine factor that regulates the PPARγ activity of adipose tissues [36]. In FGF21-/- mice, rosiglitazone fails to exert its beneficial effects [36]. This might suggest that FGF21 is required for the therapeutic effects of PPARγ agonists in NAFLD patients. However, treatment with rosiglitazone alleviates metabolic disorders by reducing circulating FGF21 levels in type 2 diabetic patients [74]. Several in vitro studies have shown that rosiglitazone reverses the inhibition on β-klotho caused by high glucose [70]. It suggests PPARγ agonists alleviate FGF21 resistance, which might contribute to the beneficial effects of PPARγ agonists in reversing the progression of NAFLD.

The glucagon-like peptide-1 (GLP-1) receptor agonist is a novel agent approved for treating type 2 diabetes [89]. Its therapeutic effects on simple fatty liver and NASH have been demonstrated by several human and animal studies [90–92]. Dipeptidyl peptidase IV inhibitors, which enhance endogenous GLP-1 levels by inhibiting degradation of GLP-1, ameliorate hepatocyte ballooning and reduce liver enzyme levels in type 2 diabetic patients with NASH [93]. Liraglutide, a long-acting GLP-1 receptor agonist, significantly increases FGF21 expression in animal models of NAFLD [65]. In high fat diet-induced obese mice, exendin-4 (GLP-1 analog) injections increased hepatic FGF21 expression [28]. Exendin-4 promotes fatty acid β-oxidation by increasing expression of PPARα and medium-chain acyl-CoA dehydrogenase (MCAD). However, the inhibition of FGF21 by siRNA attenuated the effect on PPARα and medium-chain acyl-CoA dehydrogenase (MCAD). Therefore, the beneficial effect of exendin-4 treatment may be due to the activation of the FGF21 signaling pathway. Interestingly, GLP-1 treatment for 16 weeks significantly decreases plasma levels and hepatic mRNA expression of FGF21 [94]. A combination of exenatide and pioglitazone treatment is associated with a significant decrease in plasma FGF21 levels and hepatic fat content [94]. Moreover, exendin-4 administration for 4 weeks significantly reduces hepatic triglyceride content and hepatic FGF21 expression in diet-induced obese mice, despite the lack of any significant change in weight or body mass index [94]. In high-fat diet fed ApoE^-/- mice with adiponectin knockdown, liraglutide upregulated β-klotho expression in adipose tissue, and FGFR1-3 and β-klotho levels in the liver [65]. The intraperitoneal injection of exendin-4 for 10 weeks increases the expression of FGFR in diet-induced obese mice [28].
studies showed that SIRT1 promotes fatty acid from grapes and other plants [95]. Animal studies showed [77,79,80]. Resveratrol, a natural SIRT1 activator, is extracted simple fatty liver and NASH by regulating the FGF21 pathway partly mediated by its direct effect on FGF21 activity. Thus, the therapeutic effects of GLP-1 adiponectin expression suggests the amelioration of FGF21 hepatic FGF21 downstream effectors as well as the raised FGF21-related biological agents.

Moreover, in diet-induced obese mice, exendin-4 treatment increases hepatic AMPK phosphorylation and circulating adiponectin levels, and reduces hepatic expression and plasma levels of FGF21 [66,94]. The elevated expression of hepatic FGF21 downstream effectors as well as the raised ability of FGF21 to promote AMPK phosphorylation and adiponectin expression suggests the amelioration of FGF21 resistance [66,94]. Thus, the therapeutic effects of GLP-1 receptor agonists on simple fatty liver and NASH may be partly mediated by its direct effect on FGF21 activity.

There are several emerging drugs with the ability to treat simple fatty liver and NASH by regulating the FGF21 pathway [77,79,80]. Resveratrol, a natural SIRT1 activator, is extracted from grapes and other plants [95]. Animal studies showed that resveratrol prevents hepatic steatosis and hyperlipidemia in diabetic and diet-induced obese mice [95,96]. In vitro studies showed that SIRT1 promotes fatty acid β-oxidation by stimulating FGF21 expression [77]. The FGF21 signaling pathway may be involved in the therapeutic effects of resveratrol for NAFLD. Molecular hydrogen has been reported to improve oxidative stress, reduce body weight and plasma glucose levels and improve insulin sensitivity [79]. Drinking molecular hydrogen-water significantly alleviates fatty liver in db/db mice and diet-induced fatty liver in wild-type mice [79]. A recent study demonstrated that molecular hydrogen improves metabolism dysfunction by inducing hepatic FGF21 in db/db mice [79].

More and more researchers want to investigate whether FGF21 is a potential candidate for the treatment of NAFLD. In diabetic rhesus monkeys, FGF21 administration for 6 weeks improves insulin sensitivity and decreases fasting plasma glucose [8]. Significant improvements in lipid profiles are observed in FGF21-treated diabetic rhesus monkeys [8]. The subcutaneous administration of LY2405319, a novel FGF21 variant, reduced plasma glucose and body weight in ob/ob and diet-induced obese mice [97]. However, a short circulating half-life limits the use of FGF21 in clinical application. The site-specific PEGylation of human FGF21 shows a dramatically prolonged half-life and enhanced efficacy in db/db mice [5]. The twice-weekly dosing of human PEGylated FGF21 reduced the hepatic lipid content and improved plasma lipid profiles, and exerts some beneficial effects on glucose metabolism [5]. There is still an urgent need for more animal studies and further clinical research studies to assess the safety and effectiveness of FGF21-related biological agents.

6. Summary

FGF21 is a novel metabolic regulator with beneficial effects on the regulation of energy homeostasis, glucose-lipid metabolism and insulin sensitivity. Several strategies can reverse simple fatty liver and NASH by activating FGF21 signaling pathway. Many researchers are interested in investigating whether FGF21 is a potential candidate for the treatment of NAFLD. Although FGF21 administration has some therapeutic effects for NAFLD in animal studies, there is still an urgent need for more animal studies and further clinical research to assess the safety and effectiveness of FGF21-related biological agents.

Author Contributions

G. W. planned the review. J. L. reviewed papers and wrote the manuscript. Y. X. and Y. J. H. provided advice on planning the review.

Acknowledgments

This work was supported by grants from the Major National Basic Research Program of P. R. China (2011CB503904) and the Chinese National Natural Science Foundation (81270369; 81070244) to Guang Wang and the Capital Clinical Research Foundation of Beijing Municipal Commission of Science and Technology (Z131107002130204) to Yuan Xu.

REFERENCES


