

Fibroblast Growth Factor-21 as a Potential Therapeutic Target of Nonalcoholic Fatty Liver Disease

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Abstract: Nonalcoholic fatty liver disease (NAFLD) is a highly prevalent disease without any approved treatment to-date despite intensive research efforts by researchers and pharmaceutical industry. Fibroblast growth factor (FGF)-21 has been gaining increasing attention as a possible contributing factor and thus therapeutic target for obesity-related metabolic disorders, including NAFLD, mainly due to its effects on lipid and carbohydrate metabolism. Most animal and human observational studies have shown higher FGF-21 concentrations in NAFLD than non-NAFLD, implying that FGF-21 may be increased to counteract hepatic steatosis and inflammation. However, although Mendelian Randomization studies have revealed that variations of FGF-21 levels within the physiological range may have effects in hyperlipidemia and possibly nonalcoholic steatohepatitis, they also indicate that FGF-21, in physiological concentrations, may fail to reverse NAFLD and may not be able to control obesity and other diseases, indicating a state of FGF-21 resistance or insensitivity that could not respond to administration of FGF-21 in supraphysiological concentrations. Interventional studies with FGF-21 analogs (eg, pegbelfermin, efruxifermin, BOS-580) in humans have provided some favorable results in Phase 1 and Phase 2 studies. However, the definite effect of FGF-21 on NAFLD may be clarified after the completion of the ongoing clinical trials with paired liver biopsies and histological endpoints. The aim of this review is to critically summarize experimental and clinical data of FGF-21 in NAFLD, in an attempt to highlight existing knowledge and areas of uncertainty, and subsequently, to focus on the potential therapeutic effects of FGF-21 and its analogs in NAFLD.

Keywords: hepatic fibrosis, hepatic inflammation, hepatic steatosis, hepatokine, nonalcoholic steatohepatitis, treatment

Introduction

Nonalcoholic fatty liver disease (NAFLD) has been closely associated with the metabolic syndrome (MetS); this has led to the recommendation of change in the disease nomenclature including a change to simply Fatty Liver Disease (FLD) or to metabolic (dysfunction)-associated fatty liver disease (MAFLD).^{1,2} In the setting of increasing prevalence of obesity and metabolic dysregulation in western societies, there has been a global increase in the prevalence of NAFLD, in parallel with type 2 diabetes mellitus (T2DM) and obesity epidemics. Notably, NAFLD may often remain under-diagnosed, provoking undesirable individual and public consequences.³ NAFLD demonstrates a wide histological spectrum, gradually ranging from simple nonalcoholic fatty liver (NAFL), to nonalcoholic steatohepatitis (NASH), hepatic fibrosis, liver cirrhosis and hepatocellular carcinoma (HCC) in a minority of patients, which appears in the absence of significant alcohol consumption, viral infection or any other specific hepatic disease.⁴ It is expected that NAFLD may possibly become the leading cause of liver transplantation in the near future.⁵ Despite its high prevalence and the extensive research in the field, the treatment of NAFLD remains an unmet medical need, since there is to-date no medication specifically licensed for NAFLD.⁶

NAFL starts as a state of imbalance between factors favoring fat export vs factors favoring fat accumulation in the liver.⁴ Insulin resistance (IR) interplays with systemic and hepatic inflammation, through various adipokines, cytokines, hepatokines and other mediators, to increase the load of free fatty acids that divert from adipose tissue to the liver, but also hepatic de novo lipogenesis, thus favoring fat accumulation in the liver.⁷ Adipokines, cytokines, hepatokines and other mediators seem to contribute to the pathogenesis of the disease, thus being appealing therapeutic targets.⁸ Fibroblast growth factors (FGF) comprise a family of peptidic molecules, which are widely expressed; by activating FGF receptors (FGFR), FGFs contribute to the repair and regeneration of tissues, but also to metabolic homeostasis.⁹ FGF-21 has been gaining increasing interest, as a possible therapeutic target of obesity-related metabolic disorders, including NAFLD, owing to its impact on lipid and carbohydrate metabolism.¹⁰ FGF-21 is a hepatokine with pleiotropic properties.⁶ It mainly acts through FGFR1, which is a tyrosine kinase receptor and requires β -Klotho, a single-pass transmembrane glycoprotein, as co-receptor for its activation.¹¹ FGF-21 improves insulin sensitivity by increasing glucose uptake in the skeletal muscle and brown adipose tissue. In the liver, FGF-21 enhances fatty acid β -oxidation and decreases hepatic de novo lipogenesis (Figure 1).⁶

In this review, we critically summarized experimental and clinical data of FGF-21 in NAFLD, trying to highlight existing knowledge and areas of uncertainty, and subsequently, we focus on the potential therapeutic effects of FGF-21 and its analogs in NAFLD.

Search Strategy

We performed a computerized literature search using the PubMed, not limited by publication time. A query was created by combining MeSH terms and non-MeSH terms: ((“non-alcoholic fatty liver disease”[Mesh]) OR (nonalcoholic fatty liver disease) OR (non-alcoholic fatty liver disease) OR (nonalcoholic steatohepatitis) OR (non-alcoholic steatohepatitis) OR NASH OR NAFL* OR MAFLD*) AND ((fibroblast growth factor-21) OR (fibroblast growth factor 21) OR FGF-21

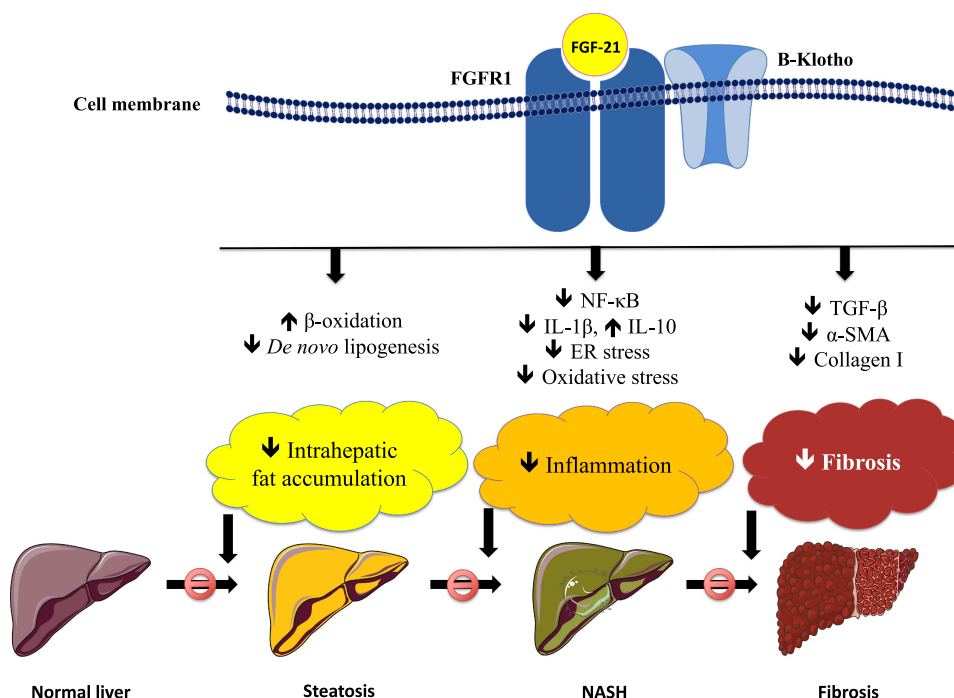


Figure 1 Potential mechanisms of action of FGF-21 in the liver. FGF-21 acts through a transmembrane tyrosine kinase receptor, FGFR1, which requires a co-receptor (β -Klotho) for its activation. FGF-21 may be beneficial for hepatic steatosis and inflammation, and possibly fibrosis. Regarding steatosis, FGF-21 decreases hepatic de novo lipogenesis and increases the mitochondrial β -oxidation of fatty acids, thus decreasing intrahepatic lipid accumulation. FGF-21 also decreases hepatic inflammation, by inhibiting NF- κ B pathway, decreasing pro-inflammatory cytokines (eg. IL-1 β) and increasing anti-inflammatory cytokines (eg. IL-10), as well as decreasing intrahepatic oxidative stress and ER stress. Limited data support that FGF-21 may possibly decrease hepatic fibrosis, via decreasing the hepatic expression of TGF- β , α -SMA and collagen I.

Abbreviations: ER, endoplasmic reticulum; FGF-21, fibroblast growth factor-21; FGFR1, fibroblast growth factor receptor 1; IL, interleukin; NF- κ B, nuclear factor kappaB; NASH, nonalcoholic steatohepatitis; SMA, smooth muscle actin; TGF, tumor growth factor.

OR FGF21 OR (FGF 21)). This query provided 329 results (last update: October 15, 2022). Subsequently, articles were selected based on the principles of evidence-based medicine, focusing mainly on original articles, preclinical and clinical. Search was extended to the reference list of some of the selected articles. Since this was a narrative review, some more articles were added on the discretion of the authors, when this was regarded as necessary for the flow of this review.

Experimental Studies

In the liver, the activation of FGF-21 pathway results in higher expression of genes involved in mitochondrial β -oxidation and in lower expression of genes involved in de novo lipogenesis (Figure 1). Therefore, by decreasing hepatic lipogenesis and rendering β -oxidation of fatty acids (FAs) more efficient, intrahepatic lipid accumulation is decreased.¹² Furthermore, FGF-21 has been proposed to decrease hepatic inflammation by downregulating the nuclear factor kappaB pathway¹³ and decreasing pro-inflammatory cytokines (eg, interleukin-1 β) and increasing anti-inflammatory cytokines (eg, interleukin-10).¹⁴ Moreover, FGF-21 seems to lessen intrahepatic oxidative stress and endoplasmic reticulum (ER) stress,¹⁵ which interplay with oxidative stress. Additionally, limited data reported that FGF-21 decreases the hepatic expression of tumor growth factor- β , α -smooth muscle actin and collagen I, implying a potentially beneficial effect on hepatic fibrosis (Figure 1). Some of these actions have been proposed to be partly mediated by the activation of AMP-activated protein kinase (AMPK)¹⁶ and peroxisome proliferator-activated receptor (PPAR)- α intracellular pathways.¹⁷

Further in vitro studies showed that treatment of mouse primary hepatocytes with palmitic acid upregulated FGF-21 mRNA, reportedly through increasing lipid-induced ER stress.¹⁸ Previous studies have also shown that ER stress may positively and directly induce the expression and secretion of FGF-21 in adipocytes,¹⁹ but also its expression in H4IIE hepatoma cells and in isolated rat hepatocytes.²⁰ Consistently, treatment of human hepatocellular carcinoma (HepG2) cells with the ER stressors tunicamycin or thapsigargin increased FGF-21 mRNA and protein.¹⁵ More recently, sulforaphane, a sulfur-rich compound found in cruciferous vegetables, increased FGF-21 hepatic expression and attenuated the adverse effects of palmitic or oleic acid on HepG2 cells.²¹ Furthermore, lipopolysaccharide (LPS) administration in mice Hep1-6 cell line reduced the expression of FGFR1 in a dose-dependent manner.²² In this regard, administration of *Bifidobacterium adolescentis*, a species residing in human intestine, reduced the gut microbiota-derived LPS, increased hepatic FGFR1 and alleviated NAFL and NASH in mice on a choline-deficient high-fat (HF) diet (Table 1).²²

The key findings of animal studies investigating the effects of relevant interventions on FGF-21 or the effect of FGF21 treatment on NAFLD are summarized in Table 1. Generally, higher circulating levels and hepatic expression of FGF-21 were observed in obese than lean rodents, those on HF diet (HFD) than chow diet, as well as those with than without NAFLD.^{23–30} Regarding specific histological lesions, higher circulating and/or hepatic FGF-21 were observed in more severe hepatic steatosis, inflammation and/or fibrosis.^{18,27,31} FGF-21 levels were also positively associated with hepatic fat content (HFC).^{26,30} FGF-21 treatment or FGF-21 analog treatment (eg, LY2405319, B1344, BMS-986171) or genetic FGF-21 upregulation (eg, adenovirus- or plasmid-mediated) improved hepatic histology, including steatosis, inflammation and/or fibrosis in most studies.^{22,27,31–37} More interestingly, other authors reported that monotherapy with FGF-21 or a glucagon-like peptide-1 receptor agonist (GLP-1RA) had an inferior effect on hepatic histology than a GLP-1-Fc-FGF-21 dual agonist,³⁵ that the combination of an FGF-21 analog with a C-C motif chemokine receptor 2/5 (CCR2/5) inhibitor had also greater effect on hepatic steatosis and NASH than either monotherapy,³⁸ and that a chimeric FGF-21/FGF-19 analog reduced liver weight more than FGF-21 monotherapy.³⁹

Both exercise and chow diet (vs HFD or methionine-choline deficient [MCD] diet) were shown to reduce circulating and hepatic FGF-21.^{28–30,32,33,40,41} Limited relevant data also showed that: a) calorie restriction more efficiently increased hepatic FGF-21 than exercise;²⁵ b) through upregulating FGF-21, which subsequently exerted its effects partly through the browning of white adipose tissue, female mice were protected more than male ones, when fed on a MCD diet;²⁸ c) HFD accelerated the progression to NASH, independently from diabetes, especially in an FGF-21 deficient state;²⁹ however, more studies are needed to consolidate these conclusions. Weight loss through bariatric surgery was also reported to decrease circulating and/or hepatic FGF-21 in line with the improvement in hepatic histology.^{42,43} Other authors reported that FGF-21 upregulation and improvement of hepatic histology were observed after treatment with exendin-4 (exenatide), which is a GLP-1RA,²⁴ hydroxytyrosol, which is a phenolic antioxidant,⁴⁴ a PPAR pan-agonist

Table I Animal Studies Investigating the Effect of Relevant Intervention on FGF-21 or the Effect of FGE-21 Treatment on NAFLD

First Author (Year) ^{Reference*}	Animal Model	Intervention(s)	Duration (Weeks)	Main Findings
Fisher (2010) ²³	Male C57BL/6 DIO mice	FGF-21 administration	22	Higher hepatic mRNA and circulating FGF-21 in DIO mice than lean controls.
Samson (2011) ²⁴	Male C57BL/6 DIO mice	Exendin-4 administration	8	Higher circulating FGF-21 in mice fed HFD than controls. Reduced HFC, hepatic FGF-21 mRNA, and hepatic FGF-21 protein following exendin-4 administration.
Fletcher (2012) ²⁵	Male OLETF rats	Wheel running exercise or caloric restriction	36	Higher hepatic FGF-21 mRNA in obese rats. Higher FGF-21 hepatic protein after caloric restriction than after exercise. Higher circulating FGF-21 and hepatic FGF-21 mRNA in hepatic steatosis. Obesity prevention and circulating FGF-21 reduction after daily exercise and caloric restriction.
Garcia-Caraballo (2013) ²⁶	Male C57BL/6 mice	HFD/HPD vs HFD/LPD vs LFD/HPD	4	Higher circulating FGF-21 and hepatic FGF-21 mRNA were positively associated with HFC. HPD reversed HFD-induced hepatic steatosis.
Yu (2013) ⁴⁸	Male C57BL/6J mice on HFD	Bitter melon extract administration	12	Bitter melon attenuated hepatic steatosis through beneficial effect on FGF-21 signaling.
Fisher (2014) ²⁷	Male C57BL/6, FGF-21-KO mice	a) HFD diet b) MCD diet followed by exogenous FGF-21 administration	16	More severe hepatic steatosis, inflammation and fibrosis in FGF-21-KO mice after the MCD diet. Reduced steatosis, NASH, hepatic lipid peroxidative damage, pro-inflammatory and pro-fibrotic protein concentrations after FGF-21 treatment.
Markan (2014) ⁴¹	Male hepatic- or adipose-specific FGF-21-KO mice	HFD diet	6	Hepatic FGF-21 production is a potential insulin sensitizer in acute refeeding and overfeeding states, through promoting glucose uptake in peripheral tissues. Higher HFC in FGF-21-liver-KO mice on a HFD compared to FGF-21-adipose tissue-KO mice on a HFD and to controls.
Tanaka (2015) ¹⁸	Male C57BL/6J, FGF-21-KO mice	MCD	1	Higher HFC, more severe steatosis and greater ER stress after MCD diet on a FGF-21-deficient state compared to a FGF-21-sufficient state.
Gong (2016) ³²	Male C57BL/6 mice on MCD	FGF-21 administration	4	Reduced steatosis, hepatocellular ballooning, perisinusoidal fibrosis and hepatic injury markers after FGF-21 administration.

Lee (2016) ²⁸	Male and female C57BL/6N mice	MCD diet	2	MCD induced more severe NAFLD in male than female mice. Higher circulating FGF-21 and hepatic FGF-21 mRNA in female than male mice. FGF-21 lead to the browning of white adipose tissue in female, but not in male mice.
Lee (2016) ³¹	Male ob/ob mice on MCD diet	LY2405319 administration	4	Treatment with LY2405319 prevented hepatic inflammation and fibrosis and attenuated hepatic injury markers.
Liu (2016) ²⁹	Male C57BL/6J FGF-21-KO mice, with streptozotocin-induced diabetes	HFD	32	Higher circulating FGF-21 in NASH. Acceleration of NASH progression in both diabetic and in non-diabetic mice on a FGF-21-deficient state.
Mosinski (2016) ⁴²	Male obese SD rats on HFD	RYGB surgery	24	Reduced hepatic steatosis and inflammation after RYGB surgery. Normalization of circulating FGF-21 and FGF-21 mRNA after RYGB surgery.
Park (2016) ³³	Male CREBH-deficient mice	Recombinant adenovirus-mediated human FGF-21 overexpression	NA	Reduced steatosis, inflammation and hepatic injury markers after adenovirus-mediated FGF-21 overexpression.
Pirozzi (2016) ⁴⁴	Male SD rats on HFD	Hydroxytyrosol administration	6	Reduced hepatic steatosis, hepatocyte ballooning, inflammation and higher hepatic FGF-21 mRNA after hydroxytyrosol treatment.
Rusli (2016) ³⁰	Male C57BL/6J mice	Calorie-restricted diet or MFD	28	Higher circulating FGF-21 in MFD compared to calorie-restricted diet at all ages. Higher circulating FGF-21 and hepatic FGF-21 mRNA are associated with higher HFC at middle and old, but not at young age.
Xiao (2016) ⁴⁰	Male C57BL/6 mice on HFD	Exercise	16	Reduced steatosis and markers of hepatic injury, together with higher hepatic FGF-21 protein after exercise.
Abd Elwahab (2017) ⁴⁹	Male albino rats on cafeteria diet	Taurine administration	12	Reduced hepatic inflammatory and apoptotic markers, as well as circulating and hepatic FGF-21 after taurine treatment.
An (2017) ⁴⁵	Male C57BLKS/J mice	PPAR pan-agonist (MHY2013) administration	3	Reduced steatosis and obesity-induced IR, through increase in FGF-21, after MHY2013 treatment.
Chukijrungrat (2017) ⁵⁰	Male, female (with or without ovariectomy) SD rats on high fructose/HFD	Estrogen administration in the ovariectomized group	12	Higher hepatic FGF-21 mRNA only in male on high fructose/HFD, but not in female mice. Reduced hepatic steatosis and higher hepatic FGF-21 mRNA after estrogen supplementation in the ovariectomized group.
An (2018) ⁴⁶	Male SD rats	PPAR pan-agonist (MHY2013) administration	4	Reduced hepatic steatosis and inflammation and higher circulating FGF-21 after MHY2013 administration.

(Continued)

Table I (Continued).

First Author (Year) ^{Reference*}	Animal Model	Intervention(s)	Duration (Weeks)	Main Findings
Cunningham (2018) ⁴⁷	Female Wistar rats on Western diet	Curcumin administration	12	Reduced hepatic steatosis, inflammation and hepatic FGF-21 protein after curcumin administration. Curcumin reduced the diet-induced high hepatic FGF-21 protein, serving as a potential FGF-21 resistance inhibitor.
Pei (2018) ⁴³	Male Wistar rats on HFD	Sleeve gastrectomy	20	Reduced HFC and circulating FGF-21 after sleeve gastrectomy.
Cui (2020) ³⁴	Male C57BL/6 mice on MCD	Long-acting PEGylated FGF-21 analog (B1344) administration	4–6	Reduced HFC, lobular inflammation, hepatocellular ballooning, lipotoxicity and fibrosis after B1344 treatment.
Cui (2020) ³⁴	Male obese cynomolgus monkeys with NAFLD	Long-acting PEGylated FGF-21 analog (B1344) administration	15	Reduced hepatic steatosis and fibrosis and moderately reduced inflammation and hepatocellular ballooning after B1344 administration.
Long (2021) ²²	Male C57BL/6j mice on choline-deficient HFD	Live or heat-killed <i>B.a.</i> , administration followed by FGF-21 administration	24	Reduced hepatic steatosis and fibrosis and higher sensitivity to FGF-21 after live <i>B.a.</i> administration. Weaker effects of heat-killed <i>B.a.</i> compared to live <i>B.a.</i>
Pan (2021) ³⁵	a) Male C57BL/6 mice on HFD b) SD rats	GLP-1-Fc-FGF-21 dual agonist vs GLP-1 vs FGF-21 monoagonist administration	6	Greater reduction in steatosis, inflammation, hepatocellular ballooning and NAS after GLP-1-Fc-FGF-21 compared to GLP-1 or FGF-21.
Klaebel (2022) ³⁹	Female guinea pigs on HFD	Recombinant native human FGF-21 vs chimeric human recombinant analog FGF-21/19	2–6	Reduced liver weight and attenuation of dyslipidemia after treatment with the chimeric agonist compared to native FGF-21.
Puengel (2022) ³⁸	Male C57BL/6j mice on CDAHFD	CCR2/5 inhibitor (BMS-687681-02-020) vs PEGylated FGF-21 agonist (BMS-986171) vs their combination	12	Reduced HFC and NAS, but no significant changes in fibrosis with the FGF-21 agonist. Greater reduction in hepatocyte ballooning, NASH, NAS and fibrosis after the combination treatment than each monotherapy.
Tang (2022) ³⁶	Male C57BL/6j mice on CDAHFD	FGF-21 analog (PEGylated FGF-21v) administration	12	Reduced HFC and liver stiffness after treatment with the FGF-21 analog.
Wu (2022) ²¹	Male C57BL/6j mice on HFD	Sulforaphane administration	6	Reduced steatosis and inflammation and higher hepatic FGFR1 and FGF-21 protein after sulforaphane administration.
Yano (2022) ³⁷	Male C57/BL6j mice on HFD	Plasmids of hepatocyte-specific promoter of FGF21 expression administration	12	Reduced hepatic steatosis after treatment. Higher hepatic lipid oxidation and ketone synthesis as possible compensatory mechanisms to decrease hepatic steatosis.

Note: *References are sorted according to the year of publication (primarily) and the surname of the first author (secondarily).

Abbreviations: *B.a.*, *Bifidobacterium adolescentis*; CCR2/5, C-C motif chemokine receptor 2/5; CDAHFD, choline-deficient, L-amino acid-defined, high-fat diet; CREBH, cyclic AMP-responsive element-binding protein H; DIO, diet-induced obesity; ER, endoplasmic reticulum; fc, fragment crystallizable; FGF-21, fibroblast growth factor-21; FGF-21v, fibroblast growth factor-21 variant; FGFR1, fibroblast growth factor-21-Receptor 1; GLP-1, glucagon-like peptide-1; HFC, hepatic fat content; HFD, high-fat diet; HPD, high-protein diet; IR, insulin resistance; KO, knock-out; LFD, low-fat diet; LPD, low-protein diet; MCD, methionine-choline deficient diet; MFD, medium-fat diet; NA, not available; NAFLD, non-alcoholic fatty liver disease; NAS, nonalcoholic fatty liver disease activity score; NASH, non-alcoholic steatohepatitis; ob, obese; OLETF, Otsuka Long-Evans Tokushima Fatty; PEGylated, polyethylene glycol modified; PPAR, peroxisome proliferator-activated receptor; RYGB, Roux-en-Y gastric bypass; SD, Sprague-Dawley.

(MHY2013),^{45,46} curcumin, which is a member of the ginger family,⁴⁷ bitter melon extract,⁴⁸ sulforaphane, which is an organosulfur found in cruciferous vegetables,²¹ and estrogen (in ovariectomized mice).⁵⁰ Taurine, which is an essential amino acid, was shown to reduce hepatic inflammation together with FGF-21.⁴⁹ However, more mechanistic studies are required to consolidate these findings and show whether any of the reported effects of these compounds are mediated by FGF-21, thus elucidating the specific molecular pathways.

Considering the above, *in vitro* studies have shown that FGF-21 is upregulated in various hepatic cells and adipocytes, after treatment with long-chain FAs and this is partly mediated through ER stress. This has been validated in animal studies and may possibly represent a counteracting mechanism targeting to protect the liver from the overflow of FAs. For example, it has been supported that FGF-21 could serve as an insulin sensitizer, and this action may also be specific for the liver, where FGF-21 is mainly produced, but minimal for other tissues, eg, the adipose tissue.⁴¹ However, resistance or insensitivity to FGF-21 action in obesity and possibly related disorders, including NAFLD, has been speculated.²³ This represents a state that the upregulated FGF-21 cannot exert its fully beneficial actions, eg, it is inadequate to counteract the diet-induced negative metabolic effects. In line, obese mice, after treatment with exogenous FGF-21, demonstrated an impairment of downstream FGF-21 molecular pathways, implying a resistant state.²³ One potential mechanism for this supposedly FGF-21 resistance or insensitivity may be the above mentioned downregulation of FGFR1.²² Of course, further mechanistic studies are required to further investigate and validate FGF-21 resistance or insensitivity and to investigate potential ways to attenuate this.

Clinical Studies

Several observational studies evaluating the association between FGF-21 and NAFLD-related parameters (Table 2), and interventional studies, evaluating the effect of lifestyle or certain medications on FGF-21 in NAFLD patients (Table 3) or the effects of FGF-21 analogs on NAFLD-related parameters (Table 4) have been recently published. Ditto for ongoing interventional studies (Table 5).

Observational Studies

Clinical observational studies investigating the association between NAFLD and FGF-21 are summarized in Table 2. These are mainly cross-sectional and case-control studies, but there are also two cohort studies.

Most,^{51–57} but not all,^{58,59} cross-sectional and case-control studies reported higher circulating and/or hepatic FGF-21 concentrations in patients with than without NAFLD. However, a limitation of many of these studies was that NAFLD patients had higher body mass index (BMI) and/or waist circumference than controls, which may have distorted the association between FGF-21 and NAFLD, since FGF-21 is also upregulated in obesity.^{52,54,58,60} On the other hand, limited data from patients with NAFLD and T2DM or IR showed that FGF-21 may not be largely affected by T2DM⁵³ or IR⁶⁰ specifically in NAFLD patients, which may imply that NAFLD itself is a more important drive than T2DM or IR for FGF-21 upregulation. Other authors also speculated that FGF-21 is more closely associated with lipid metabolism than glucose metabolism and IR.⁵² However, more studies are needed to validate these observations and mechanistic studies to quantify the separate magnitude of NAFLD, obesity and T2DM on hepatic FGF-21 upregulation.

Most imaging-based studies also showed a positive association between FGF-21 concentrations and HFC.^{54,61} On the contrary, data derived from biopsy-proven studies on FGF-21 concentrations between NAFL and NASH are conflicting: some authors reported higher FGF-21 in NASH than NAFL,^{55,62} whereas others similar concentrations between NAFL and NASH.^{38,63,64} There is also one study showing lower FGF-21 concentration in children with NASH than non-NASH.⁵⁹ Limited data on particular histological lesions are also conflicting. More specifically, some authors showed higher FGF-21 concentrations in more severe hepatic steatosis,^{63–65} inflammation,⁶² or fibrosis.⁶² However, other authors reported similar FGF-21 levels among different degrees of steatosis,⁶² inflammation^{64,65} and fibrosis.^{38,64,65}

There is also a study investigating the association between single nucleotide polymorphisms (SNPs) of the *FGF-21* gene with NAFLD: the rs499765 of *FGF-21* gene was associated with higher circulating FGF-21 and the presence of NAFLD, whereas the rs2071699 and rs838136 were not associated with NAFLD.⁶⁶ In a more recent genome-wide association study (GWAS) with large sample size, the rs2548957 of *FGF-21* gene was shown to be associated with

Table 2 Main Characteristics and Outcomes of Clinical Observational Studies

First Author (Year) ^{Reference*}	Study Type	Study Groups (n)	Main Findings
Dushay (2010) ⁵¹	Case-control	Morbidly obese with biopsy-defined NAFL (6) or NASH (9) vs lean non-NAFLD controls	Higher circulating FGF-21 in NAFL and NASH compared to controls. Higher hepatic FGF-21 mRNA in NAFL, but not in NASH compared to controls.
Li (2010) ⁵²	Case-control	Patients with US-defined NAFLD (224) vs non-NAFLD controls (124)	Higher hepatic FGF-21 mRNA, hepatic FGF-21 protein and circulating FGF-21 in NAFLD than controls. Higher circulating FGF-21 with higher hepatic FGF-21 protein.
Yilmaz (2010) ⁶³	Case-control	Patients with biopsy-defined NAFLD (82) vs non-NAFLD controls (77)	Higher circulating FGF-21 in greater steatosis stages. Similar circulating FGF-21 between NAFL and definite NASH.
Li (2011) ⁵³	Case-control	Newly diagnosed patients with T2DM, without NAFLD (32) vs T2DM with US-defined mild NAFLD (20) or with US-defined NAFLD (17) vs healthy controls (30)	Higher circulating FGF-21 in T2DM patients with mild NAFLD or NAFLD compared to both non-NAFLD T2DM patients and controls.
Yan (2011) ⁶¹	Cross-sectional	Patients with US-defined and ¹ H-MRS- defined NAFLD and abnormal glucose metabolism (138)	Circulating FGF-21 was positively correlated with HFC.
Reinehr (2012) ⁵⁸	Cross-sectional	Obese (60) and lean (40) children with US-defined NAFLD	Higher circulating FGF-21 in obese than lean children. Similar circulating FGF-21 in children with and without NAFLD.
Alisi (2013) ⁵⁹	Case-control	Children with biopsy-defined NAFL (84), NASH (32) and NAFLD-related fibrosis (59) vs children without NAFLD (23)	Reduced circulating FGF-21 in NAFL, NASH and fibrosis compared to non-NAFLD. Reduced circulating FGF-21 in NASH than non-NASH.
Giannini (2013) ⁵⁴	Case-control	Obese adolescents with MRI-defined and biopsy-defined NAFLD (79), without NAFLD (107) vs lean controls (31)	Higher circulating FGF-21 in obese than lean children, especially those with NAFLD. Higher circulating FGF-21 was positively correlated with NASH, HFC, visceral fat and cytokeratin-18.
Li (2013) ⁶⁸	Prospective cohort	a) Baseline: patients with US-defined NAFLD (159) vs without NAFLD (553). b) Prospective arm: patients with US-defined NAFLD (123) vs without NAFLD (442) at baseline were followed-up for 3 years.	Higher baseline circulating FGF-21 in patients who developed NAFLD at follow-up. High baseline circulating FGF-21 was independently associated with NAFLD. FGF-21 was increased during follow-up compared to baseline in patients who developed NAFLD.
Jiang (2014) ⁶⁶	Case-control	Patients with US-defined NAFLD (65) vs non-NAFLD controls (275)	SNP rs499765 of <i>FGF-21</i> gene was associated with the presence of NAFLD and with higher circulating FGF-21.
Mutanen (2014) ⁶⁵	Case-control	Children with intestinal failure with or without biopsy-defined NAFLD (35) vs children without intestinal failure (59)	Higher circulating FGF-21 in patients with or without NAFLD than controls. Higher circulating FGF-21 in patients with higher steatosis grade. No association between circulating FGF-21 and hepatic inflammation or fibrosis.
Koliaki (2015) ⁵⁵	Case-control	Obese individuals with or without biopsy-defined NAFLD (41) vs lean non-NAFLD controls (12)	Higher circulating FGF-21 in NAFL and NASH compared to non-NAFLD controls. Higher circulating FGF-21 in NAFL than NASH.

(Continued)

Table 2 (Continued).

First Author (Year) ^{Reference*}	Study Type	Study Groups (n)	Main Findings
Zhan (2015) ⁶⁹	Prospective cohort	Individuals followed-up for 5 years (352)	Higher circulating FGF-21 in subjects with NAFLD compared to non-NAFLD individuals. Circulating FGF-21 was independently associated with the development of NAFLD.
Zhang (2015) ⁵⁶	Cross-sectional	T2DM with LEAD (294) vs without LEAD (210) with or without US-defined NAFLD	Higher circulating FGF-21 in NAFLD than non-NAFLD. Higher circulating FGF-21 in women, but not in men, with LEAD compared to those without LEAD.
Sodhi (2016) ⁶⁰	Cross-sectional	Obese children with IR (27) or without IR (16) vs lean controls without IR (28)	Higher circulating FGF-21 in obese children with or without IR compared to lean controls.
Waluga (2017) ⁶⁴	Cross-sectional	Women with severe obesity and biopsy-defined NAFLD or non-NAFLD during bariatric surgery (56)	Similar circulating FGF-21 and hepatic FGF-21 mRNA in different degree of NAS. Similar circulating FGF-21 and hepatic FGF-21 mRNA in different severity of hepatocyte ballooning, lobular inflammation and fibrosis. Higher circulating FGF-21 in steatosis grades 2–3 compared to 0–1.
Barb (2019) ⁶²	Case-control	Overweight or obese patients with ¹ H-MRS-defined and biopsy-defined NAFL (52) and NASH (94) vs non-NAFLD controls (41)	Higher circulating FGF-21 in NASH compared to NAFL and controls. Higher circulating FGF-21 with increasing severity of hepatic inflammation and fibrosis. Similar circulating FGF-21 in different steatosis grades.
Flisiak-Jackiewicz (2019) ⁵⁷	Case-control	Obese children with US-defined, MRI-defined and ¹ H-MRS-defined NAFLD (34) or non-NAFLD (52) vs lean children (24)	Higher circulating FGF-21 in NAFLD obese children compared to non-NAFLD obese children and lean controls.
Puengel (2022) ³⁸	Case-control	Patients with biopsy-defined NAFL (31) or NASH (54); of them, 12 patients had advanced hepatic fibrosis	Similar circulating FGF-21 between NAFL and NASH. Similar circulating FGF-21 between different hepatic fibrosis stages.
Larsson (2022) ⁶⁷	Cross-sectional	Genome-wide association study in adults (6259); replication in women (4483)	SNP rs2548957 of <i>FGF-21</i> gene was associated with higher circulating FGF-21. SNP rs2548957 of <i>FGF-21</i> gene was associated with lower GGT, but not HFC or the presence of NAFLD. SNP rs2548957 of <i>FGF-21</i> gene was also associated with lower alcohol consumption, lower LDL-C, CRP and galectin.

Note: *References are sorted according to the year of publication (primarily) and the surname of the first author (secondarily).

Abbreviations: CCL2, chemokine (C-C motif) ligand 2; CRP, C-reactive protein; FGF-21, fibroblast growth factor-21; GGT, gamma glutamyltransferase; ¹H-MRS, proton-magnetic resonance spectroscopy; HFC, hepatic fat content; IR, insulin resistance; LDL-C, low-density lipoprotein-cholesterol; LEAD, lower extremity atherosclerotic disease; MRI, magnetic resonance imaging; NAFL, nonalcoholic fatty liver; NAFLD, nonalcoholic fatty liver disease; NAS, nonalcoholic fatty liver disease activity score; NASH, nonalcoholic steatohepatitis; SNP, single nucleotide polymorphism; T2DM, type 2 diabetes mellitus; US, ultrasonography.

circulating FGF-21 concentrations, as well as with lower gamma-glutamyltransferase (GGT), but not HFC or NAFLD.⁶⁷ Of course, more studies are needed to validate these findings and investigate whether they are of clinical implication.

The two cohort studies have also provided valuable information. In a 3-year prospective cohort study, FGF-21 concentrations at baseline were independently higher in individuals who developed NAFLD during the follow-up.⁶⁸ Furthermore, an increase in FGF-21 concentrations during the follow-up (compared to baseline) was also associated with the development of NAFLD.⁶⁸ Another prospective cohort study also showed that FGF-21 concentrations were positively

Table 3 Main Characteristics and Outcomes of Interventional Studies Evaluating the Effect of Lifestyle or Medications on Circulating FGF-21 in Patients with NAFLD

First Author (Year) ^{Reference*}	Design	Intervention Group (n)	Control (Placebo or Active) Group (n)	Intervention(s)	Duration	Liver Biopsy	Outcome(s) of Interest	Findings of Interest
Dushay (2010) ⁵¹	Prospective (short-term), open-label, uncontrolled	a) Healthy nonobese individuals (20) b) Healthy, nonobese individuals (4) d) Healthy, nonobese individuals (11)	No control group	a) 75 g OGTT b) Breakfast, followed by fasting c) Ketogenic diet	a) 16 h b) 72 h (fasting) c) 13 days	No	Circulating FGF-21	a) Circulating FGF-21 did not change after OGTT. b) Circulating FGF-21 did not change after a 16-h fasting, but tended to decrease after a 72-h fast. c) Circulating FGF-21 did not change after ketogenic diet.
Samson (2011) ²⁴	Open-label RCT with active control group	T2DM patients on diet and/or metformin (11)	T2DM patients on diet and/or metformin (10)	Pioglitazone (45mg/d) and exenatide (10 µg/bid) vs pioglitazone (45 mg) monotherapy	12 months	No	Effect of exenatide on circulating FGF-21 and HFC.	Reduced circulating FGF-21 in the combination, but not in the monotherapy group. Greater reduction of HFC in the combination group compared to monotherapy group.
Matikainen (2012) ⁷⁰	Prospective (short-term), open-label, uncontrolled	Healthy individuals (47)	No control group	Oral fat load	12 h	No	Effect of oral fat load on circulating FGF-21 and HFC.	Reduced circulating FGF-21 4-h after fat load. Individuals with HFC >5% had impaired suppression of circulating FGF-21 after oral fat load.
Reinehr (2012) ⁵⁸	Prospective, uncontrolled	Obese children with or without US-defined NAFLD (60)	No control group	Lifestyle modification (exercise, diet and behavioral therapy)	1 year	No	Effect of lifestyle modification on circulating FGF-21 and NAFLD.	Change in circulating FGF-21 was positively correlated with changes in BMI and circulating leptin. No association between changes in circulating FGF-21 and NAFLD.

Markova (2016) ⁷¹	Prospective, open-label, with active control group	Patients with T2DM and ¹ H-MRS-defined NAFLD (18)	Patients with T2DM and ¹ H-MRS-defined NAFLD (19)	Animal vs plant HPD	21 months	No	Effect of high protein diet on circulating FGF-21 and HFC.	Similarly reduced circulating FGF-21 and HFC in both groups. Change in circulating FGF-21 was positively associated with change in HFC.
Taniguchi (2016) ⁷³	Crossover RCT	Elderly men (33)	Elderly men (33); crossover design	Endurance exercise vs no exercise	10 (5+5) weeks	No	Effect of endurance exercise on HFC and circulating FGF-21.	Reduced circulating FGF-21 and HFC after exercise, without weight loss. Change in circulating FGF-21 was positively associated with change in HFC.
Braun (2017) ⁸¹	Double-blind RCT	HIV patients with visceral obesity (28)	HIV patients with visceral obesity (22)	Tesamorelin (2 mg/d) vs placebo	6 months	No	Effect of tesamorelin on circulating FGF-21 and HFC.	Tesamorelin did not reduce FGF-21 more than placebo. Change in circulating FGF-21 was positively associated with changes in HFC and FIB-4.
Li (2021) ⁷⁹	Prospective, uncontrolled	Overweight patients with newly diagnosed T2DM and MRI-defined NAFLD (20)	No control group prospectively	Liraglutide 0.6–1.8 mg/d	12 weeks	No	Effect of liraglutide on circulating FGF-21 and HFC.	Reduced circulating FGF21 only in patients with significant decrease in HFC (≥29%). Change in circulating FGF-21 was positively associated with change in HFC.
Asghari (2022) ⁷⁴	Open-label RCT	Overweight/obese with US-defined NAFLD (30)	Overweight/obese with US-defined NAFLD (30)	Calorie-restricted diet vs “healthy” diet	12 weeks	No	Effect of calorie-restricted diet on circulating FGF-21 and NAFLD.	Circulating FGF-21 and hepatic steatosis remained unaffected after either diet.

Note: *References are sorted according to the year of publication (primarily) and the surname of the first author (secondarily).

Abbreviations: BMI, body mass index; FIB-4, fibrosis-4 index; FGF-21, fibroblast growth factor-21; ¹H-MRS, proton-magnetic resonance spectroscopy; HFC, hepatic fat content; HIV, human immunodeficiency virus; HPD, high-protein diet; MRI, magnetic resonance imaging; NAFLD, nonalcoholic fatty liver disease; OGTT, oral glucose tolerance test; RCT, randomized controlled trial; T2DM, type 2 diabetes mellitus; US, ultrasound.

Table 4 Main Characteristics and Outcomes of the Interventional Studies Evaluating the Effects of FGF-21 Analogs on NAFLD

First Author (Year) ^{Reference*}	Design	Intervention Group (n)	Control (Placebo or Active) Group (n)	Intervention(s)	Duration	Liver Biopsy	Outcome(s) of Interest	Findings of Interest
Sanyal (2018) ⁸³	Phase 2a, double-blind, placebo-controlled RCT	Overweight/obese patients with biopsy-defined NASH (fibrosis stage 1–3) and MRI-PDFF-defined HFC≥10% (51)	Overweight/obese patients with biopsy-defined NASH (fibrosis stage 1–3) and MRI-PDFF-defined HFC≥10% (26)	Pegbelfermin (10mg/d or 20mg/week) vs placebo	16 weeks	Yes (baseline only)	Safety and efficacy of pegbelfermin on HFC.	Reduced HFC, improved liver function tests and increase in adiponectin in both treatment groups compared to placebo. No effect of pegbelfermin on body weight. Mild adverse events, mainly diarrhea and nausea.
Charles (2019) ⁸²	Phase 2, double-blind placebo-controlled RCT	Patients with T2DM and obesity (96)	Patients with T2DM and obesity (24)	Pegbelfermin (1 mg/d or 5 mg/d or 20mg/d or 20 mg/week) vs placebo	12 weeks	No	Effect of pegbelfermin on HFC.	Reduced circulating fibrosis marker (PRO-C3) in 20mg/d and 20mg/week groups compared to placebo. Increase in adiponectin in all treatment groups compared to placebo. No effect of pegbelfermin on body weight and liver function tests after treatment.
Harisson (2021) ⁸⁴	Phase 2a, double-blind, placebo controlled RCT (BALANCED)	Patients with biopsy-defined NASH (fibrosis stage 1–3 and NAS≥4) (59).	Patients with biopsy-defined NASH (fibrosis stage 1–3 and NAS≥4) (21)	Efruxifermin (28mg/d, 50mg/d or 70mg/d) vs placebo	16 weeks	Yes (paired in 42 patients)	Effect of efruxifermin on HFC, liver function tests and hepatic histology.	Reduced HFC and improved liver function tests in all treatment groups compared to placebo. Reduced NAS after treatment. Body weight was decreased only in 70mg/d group compared with placebo.
Rader (2022) ⁸⁵	Phase 1b, double-blind, placebo controlled RCT	Obese with hypertriglyceridemia (30)	Obese with hypertriglyceridemia (31)	LLF580/ BOS-580 (300mg/4 weeks) vs placebo	12 weeks	No	Effect of LLF580 on HFC, liver function tests and noninvasive fibrosis indices.	Reduced HFC, liver function tests, PRO-C3 and enhanced liver fibrosis score after LLF580 compared to placebo. Increase in adiponectin compared to placebo. No effect of LLF580 on body weight.

Note: *References are sorted according to the year of publication.

Abbreviations: FGF-21, fibroblast growth factor-21; HFC, hepatic fat content; MRI-PDFF, magnetic resonance imaging-proton density fat fraction; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; NASH, nonalcoholic steatohepatitis; PRO-C3, N-terminal type III collagen propeptide; RCT, randomized controlled trial; T2DM, type 2 diabetes mellitus.

Table 5 Main Characteristics and Primary Outcomes of Ongoing Clinical Trials Targeting FGF-21 or Evaluating the Effects of FGF-21 Analogs on NAFLD

Clinical Trial ID, Date of Registration*	Design	Intervention Group (n)	Comparator (Control or Active) Group (n)	Intervention(s)	Duration	Liver Biopsy	Primary Outcome(s)
IRCT201511233664N16, 08/02/2016	Placebo-controlled RCT	Patients with US-defined NAFLD (NA)	Patients with US-defined NAFLD (NA)	Resveratrol 300mg twice daily vs placebo vs calorie restriction diet	12 weeks	No	Circulating FGF-21, liver function tests, hepatic steatosis (among multiple primary outcomes).
NCT03486899 (FALCON 1), 03/04/2018	Phase 2b, multicenter, double-blind, placebo-controlled RCT	Patients with biopsy-proven NASH and stage 3 hepatic fibrosis (120)	Patients with biopsy-proven NASH and stage 3 hepatic fibrosis (40)	Pegbelfermin (BMS-986036) (10mg, 20mg, 40mg/week) vs placebo	24 weeks	Yes (paired)	Proportion of patients achieving \geq 1 fibrosis stage improvement without worsening in NAS or NAS improvement without worsening of fibrosis.
NCT03486912 (FALCON 1), 03/04/2018	Phase 2b, multicenter, double-blind, placebo-controlled RCT	Patients with biopsy-proven NASH and compensated cirrhosis (75)	Adults with biopsy-proven NASH and compensated cirrhosis (25)	Pegbelfermin (BMS-986036) (10mg, 20mg, 40mg/week) vs placebo	48 weeks	Yes (paired)	Proportion of patients achieving \geq 1 fibrosis stage improvement without worsening in NAS.
IRCT20140107016123N14, 04/12/2019	Double-blinded, placebo-controlled RCT	Obese patients with US-defined NAFLD (moderate-to-severe hepatic steatosis)(50)	Obese patients with US-defined NAFLD (moderate-to-severe hepatic steatosis) (50)	Pomegranate extract 225mg twice daily vs placebo	12 weeks	No	Circulating FGF-21, liver function tests, hepatic steatosis (among multiple primary outcomes).
NCT04880031, 10/05/2021	Phase 2a, multicenter, double-blind, placebo-controlled RCT	Obese patients with MRI-PDFF \geq 10% and LSM 7–9.9kPa (NA) [†]	Obese patients with MRI-PDFF \geq 10% and LSM 7–9.9kPa (NA) [†]	BOS-580 (5 different doses; NA)	12 weeks	No	Safety outcomes: change in blood pressure, heart rate, laboratory abnormalities and emergent adverse events.

Notes: *References are sorted according to the date of registration. [†]The total number of participants is expected to be 88, but their assignment per group was not specifically reported.

Abbreviations: FGF-21, fibroblast growth factor-21; MRI-PDFF, magnetic resonance imaging-proton density fat fraction; NA, not available; NAFLD, nonalcoholic fatty liver disease; NAS, nonalcoholic fatty liver disease score; NASH, nonalcoholic steatohepatitis; RCT, randomized controlled trial; US, ultrasonography.

and independently associated with the development of NAFLD.⁶⁹ Both these cohort studies were performed in Chinese populations, thus the extrapolation of their results to other ethnic groups should be cautiously considered.

All the above considering, most observational studies showed higher circulating FGF-21 concentrations in patients with than without NAFLD and an association between FGF-21 with HFC. More importantly, baseline circulating FGF-21 and its changes in the long-term were also shown to be associated with the development of NAFLD. However, FGF-21 was not clearly associated with histological characteristics of more severe disease, since relevant studies provided conflicting data. This may be, at least partly, attributed to small sample sizes of biopsy-proven studies (Table 2), thus possibly being underpowered to show differences between NAFL and NASH or between different degrees or stages of severity of particular histological lesions (eg, hepatic inflammation, fibrosis). One appealing hypothesis, which however remains to be elucidated, would be that, in accordance with animal studies, FGF-21 is upregulated in human NAFLD as a potential counterbalancing mechanism; nonetheless, it remains unclear whether the hepatic FGF-21 upregulation is sufficient to counteract the progression of the disease to inflammation, fibrosis and cirrhosis, which requires long-term studies with large sample size, paired liver biopsies and histological endpoints.

Interventional Studies Investigating the Effect of Lifestyle or Medications on Circulating FGF-21

The main characteristics and outcomes of the interventional studies investigating the effect of lifestyle or medications on circulating FGF-21 in patients with NAFLD are summarized in Table 3. In one of the above mentioned studies showing higher circulating and hepatic FGF-21 in NAFLD patients than lean controls in a case-control fashion (Table 2),⁵¹ the authors also investigated the effect of fasting glucose load (oral glucose tolerance test) and ketogenic diet on circulating FGF-21.⁵¹ They practically showed no effect of these short-term interventions on FGF-21,⁵¹ which, however, need cautious interpretation, due to the small sample size and the uncontrolled nature of this study. Other authors reported that oral fat load reduced circulating FGF-21 in apparently healthy individuals, but this reduction was impaired in those with HFC>5% (ie, those with presumable NAFLD),⁷⁰ possibly implying a type of FGF-21 resistance or intolerance in individuals with NAFLD. Taken together, these two studies suggest that a different effect of glucose⁵¹ or fat load⁷⁰ on FGF-21 may be hypothesized, which, however, requires larger, directly comparative studies to be definitely shown. Notably, high protein diets, either animal or plant-based, reduced both FGF-21 and HFC in patients with T2DM and NAFLD.⁷¹ Nonetheless, this potential beneficial effect of high protein diets should be cautiously considered in the light of the potential adverse effect of high protein diet, especially those containing processed proteins and red meat, on other parameters of T2DM and NAFLD.⁷²

Limited data from studies with lifestyle intervention provided conflicting results. A program combining exercise, diet and behavioral therapy in children,⁵⁸ or another one with endurance exercise in elderly men⁷³ were reported to decrease circulating FGF-21. This decrease of FGF-21 was positively associated with the reduction in HFC in the latter,⁵⁸ but was not associated with NAFLD in the former study, despite its positive association with the decline in BMI.⁷⁰ On the contrary, an open-label randomized controlled trial (RCT) in overweight/obese individuals with NAFLD reported no effect of a calorie-restricted diet or a common (characterized as “healthy”) diet on both circulating FGF-21 and NAFLD.⁷⁴ These results warrant further research to definitely show whether the beneficial effect of lifestyle modifications on NAFLD⁷⁵ may be partly mediated by FGF-21, as well as whether there are distinct effects in different ages.

There are also a few studies investigating the effect of relevant medications on FGF-21 and NAFLD. In an RCT comparing the combined effect of exenatide and pioglitazone vs pioglitazone monotherapy on circulating FGF-21 and HFC in patients with T2DM inadequately controlled with metformin, the combination treatment more effectively decreased both HFC and circulating FGF-21 compared with pioglitazone monotherapy.²⁴ This may imply an additive effect of exenatide on pioglitazone treatment. It must be emphasized that pioglitazone has been recommended by most guidelines as an off-label treatment of NASH with significant fibrosis, although it has certain adverse effects, including weight gain.⁷⁶ In this regard, it is important to note that exenatide attenuated the adverse effect of pioglitazone on weight gain in this study,²⁴ highlighting the importance of the combination treatment in some patients with NAFLD.⁷⁷ Another uncontrolled study, exploring the effect of liraglutide, an anti-diabetic medication with beneficial impact on hepatic

steatosis and inflammation,⁷⁸ on FGF-21, supported that circulating FGF-21 decreased only in patients with significant decrease in hepatic steatosis.⁷⁹ Notably, there was positive association between change in FGF-21 and HFC in this study. However, whether FGF-21 partly mediates the beneficial effect of liraglutide on HFC needs studies of different design. It is also of note that tesamorelin, a synthetic growth hormone releasing hormone analog used in human immunodeficiency virus (HIV)-infected patients with lipodystrophy,⁸⁰ marginally did not decrease FGF-21, but any change in FGF-21 was positively associated with change in HFC and fibrosis-4 (FIB-4), a non-invasive index of hepatic fibrosis.⁸¹ Of course, the design of this study cannot show a causative link between FGF-21 and improvement in hepatic steatosis or fibrosis. It should be noted that tesamorelin has not yet approved for the treatment of central obesity in non-HIV populations.

Considering the above, studies on lifestyle modification have not clearly shown whether FGF-21 may partly mediate their beneficial effect on NAFLD. The same also remains to be elucidated for pioglitazone and liraglutide, medications that have demonstrated beneficial effects on NAFLD. A distinct effect of glucose and fat load on FGF-21 in NAFLD may be implied by the existing data, but existing studies are considered too limited to draw definitive conclusions.

Interventional Studies Investigating the Effect of FGF-21 Analogs on NAFLD

The main characteristics and outcomes of the interventional studies investigating the effect of FGF-21 analogs on NAFLD are summarized in Table 4. There are two RCTs on the effect of pegbelfermin (BMS-986036), which is a recombinant PEGylated human FGF-21 analog, on NAFLD/NASH. Endogenous FGF-21 has short half-life, so the use of FGF-21 analogs seems to be a therapeutic alternative aiming at providing a more prolonged action.⁸² In a phase 2 RCT, pegbelfermin subcutaneous injection (1 mg/d, 5 mg/d, 20mg/d or 20 mg/week) in patients with T2DM and obesity, of whom 97% with presumable NAFLD, showed that the dose of 20 mg/d and 20 mg/week decreased N-terminal type III collagen propeptide (PRO-C3), regarded as a non-invasive index of hepatic fibrosis.⁸² Notably, pegbelfermin increased circulating adiponectin, but it failed to improve alanine aminotransferase (ALT) and aspartate aminotransferase (AST) more than placebo in this study, which, however, were not their primary endpoints. Pegbelfermin was shown to be safe and well tolerated, with the most common adverse effects being bruising (5%) and reactions (4%) at injection sites. Diarrhea, nausea and dyspepsia were also more frequent in the pegbelfermin groups than placebo and were mild to moderate in intensity.⁸²

In a phase 2a RCT, pegbelfermin (10 mg/d or 20 mg/week) was subcutaneously administered in overweight/obese patients with biopsy-proven NASH and fibrosis stage F1-F3.⁸³ After 16 weeks of treatment, hepatic fat fraction (evaluated by magnetic resonance imaging-proton density fat fraction) was similarly reduced in both treatment arms compared with placebo.⁸³ Furthermore, treatment with both doses similarly reduced ALT, AST and PRO-C3 and increased adiponectin.⁸³ This study did not show a beneficial effect of pegbelfermin on liver stiffness (evaluated by magnetic resonance elastography). However, this was measured in a subset of patients, thus being possibly underpowered toward this aim and the duration of the study may be short to show difference in liver stiffness, regarded as a non-invasive index of hepatic fibrosis. To this aim, there are two ongoing RCTs with NASH patients and F3 or cirrhosis (F4) and paired liver biopsies (Table 5), which are expected to clarify the effect of pegbelfermin on hepatic histology, including fibrosis.

Apart from pegbelfermin, efruxifermin (AKR-001), a long-acting Fc-FGF21 fusion protein, was investigated in NASH patients with NAS \geq 4 and F1-F3 in another phase 2a RCT (BALANCED).⁸⁴ All doses of efruxifermin improved liver function tests and HFC compared with placebo. NAFLD activity score was also shown to improve after treatment, and there were also favorable effects on hepatic fibrosis (in patients with greater improvement in HFC).⁸⁴ However, these secondary histological results should be cautiously interpreted, because liver biopsy was performed only in a subset of patients and was not performed in patients on placebo. As for pegbelfermin, most adverse events were considered to be mild-to-moderate, with gastrointestinal ones (eg, diarrhea, nausea, vomiting) being more frequent in the efruxifermin groups than placebo.⁸⁴

LLF580, later renamed to BOS-580, is another long-acting Fc-FGF21 fusion protein, introduced to have longer duration of action compared with pegbelfermin and efruxifermin.⁸⁵ In a phase 1 RCT with obese patients with hypertriglyceridemia, BOS-580 provided favorable results, ie, it improved HFC, liver function tests and markers of

liver fibrosis, and increased adiponectin.⁸⁵ As with other FGF-21 analogs, gastrointestinal adverse events were more frequent in BOS-580 groups than placebo.

It is also important to note that FGF-21 analogs showed a favorable effect on lipid profile in all the above mentioned trials. More specifically, FGF-21 analogs reduced total cholesterol, low-density lipoprotein cholesterol and triglyceride, whereas increased high-density lipoprotein cholesterol concentrations.^{82–85} This is regarded as important effect, because improvement in lipid profile may be associated with decrease in cardiovascular risk, which is high in patients with NAFLD.⁸⁶

In summary, FGF-21 analogs have provided favorable results in phase 1 and phase 2 clinical trials. Of note, the hepatic effects of FGF-21 were observed without substantial decrease in body weight in most studies. Crucially, adiponectin was increased, despite unaffected body weight, at least after treatment with pegbelfermin and BOS-580. This is considered important, because adiponectin, which has been proposed to have beneficial role in the disease,⁸⁷ may partly mediate the effects of FGF-21 in NASH. Of course, RCTs with paired liver biopsies are expected to clarify the histological effects of FGF-21 analogs in the liver.

Ongoing Interventional Studies

Based on favorable data from clinical trials with FGF-21 analogs in NASH, there are several RCTs that are ongoing, whose main characteristics and primary outcomes are summarized in Table 5. FALCON 1 and 2 are two phase 2b RCTs with paired liver biopsies, investigating the histological effects of pegbelfermin in NASH patients with F3 and F4 (compensated cirrhosis), respectively. Both studies seem to be completed and their results are expected to be published soon, elucidating the therapeutic effectiveness of pegbelfermin in patients with NASH. A phase 2a RCT is also ongoing, investigating mainly the safety of LLF580 (BOS-580) in obese participants with presumable NASH and fibrosis, after the favorable results of the phase 1 RCT.⁸⁷ There are also two relevant RCTs, investigating the effects of resveratrol and pomegranate extract, respectively, on circulating FGF-21 and NAFLD (Table 5). In an era with no approved treatment for NAFLD,^{NaN} the results of these studies are anticipated with considerable interest.

Discussion

NAFLD is a highly prevalent disease with global distribution without any approved medical treatment.⁴ This renders research for its pharmacological treatment appealing for both researchers and pharmaceutical industry. In this setting, research on FGF-21 has provided some promising results, which was the main focus of this review. Briefly, it seems that hepatic FGF-21 is upregulated in animal and human NAFLD (Tables 1 and 2), possibly as a counteracting mechanism against hepatic lipid accumulation. This mechanism may work under normal conditions, but may fail, when NAFLD is not reversed and its duration is prolonged, implying either a state of resistance/insensitivity or tolerance to FGF-21 action in obesity and possibly related disorders, including NAFLD, but this remains to be shown.²³ Some existing early-phase trials provided favorable results, which may partly be mediated through adiponectin upregulation in the adipose tissue (Table 4); in this regard, clinical trials with histological endpoints, especially hepatic inflammation and fibrosis, would be needed to show with a level of certainty any definitive effect of FGF-21 analogs on the liver. Results of these trials are expected (Table 5). It should be underlined that adiponectin was early considered to be a beneficial adipokine for NAFLD;⁸⁷ however, it is difficult to produce functionally active recombinant adiponectin, because its molecule needs complex post-translational modifications to be active.⁸⁹ For this reason, medications that upregulate endogenous adiponectin are considered important for NAFLD. It should be also highlighted that there are also other FGF-21 analogs or FGF-21 receptor agonists or FGFR1/ β -Klotho co-agonists in various preclinical stages of investigation, as they are elsewhere summarized in detail.¹¹

It is of interest that hepatic FGF-21 and circulating FGF-21 are well correlated in rodent²⁶ and human⁵² studies, implying that circulating FGF-21 concentrations reflect its hepatic concentrations. This is important and may have diagnostic implications, since liver biopsy, the current gold standard for the diagnosis and staging of the disease, cannot be performed in all NAFLD patients: the high prevalence of the disease and the low but existent rates of morbidity and mortality of liver biopsy are the main deterrent factors for the use of liver biopsy in a wide scale.⁹⁰ In this regard, diagnostic accuracy studies showed a moderate accuracy of FGF-21 concentrations for the non-invasive diagnosis of

NASH, which, however, increased, when a two-step approach⁹¹ or combination of FGF-21 with other parameters⁹² were used. Along these lines, in a recent meta-analysis of diagnostic accuracy studies, FGF-21 concentrations alone showed moderate sensitivity and specificity (0.62 and 0.78, respectively) for diagnosing NASH.⁹³ However, both sensitivity and specificity increased (0.92 and 0.85, respectively), when FGF-21 concentrations were combined with other parameters, including cytokeratin-18.⁹³ The above considering, more studies may possibly be needed to identify better combinations of parameters for the non-invasive diagnosis of NASH and, possibly more importantly, of hepatic fibrosis.

Although FGF-21 seems an appealing therapeutic target for NAFLD, there are also significant considerations, ie, bone and cardiovascular side effects. More specifically, increased bone density was shown in FGF21-null, whereas decreased trabecular bone volume was shown in FGF21-transgenic mice and diet-induced obese mice treated with FGF21.⁹⁴ FGF21 was reported to switch the differentiation of bone marrow precursors to adipocytes rather than osteoblasts, thus resulting to impaired bone formation, as well as to induce the activation of osteoclasts, thus resulting to bone loss.⁹⁴ However, data from clinical studies currently remain controversial, as elsewhere summarized in detail.⁹⁵ Regarding cardiovascular safety, which is a major concern for patients with NAFLD, since cardiovascular diseases represent the first cause of death of NAFLD patients,⁸⁶ there are limited data reporting an increase in heart rate and blood pressure after treatment with some FGF-21 analogs, which, however, were not shown by other authors.^{96,97} The clinical significance of these cardiovascular effects remains to be shown, as well as whether these effects outweigh the above mentioned beneficial effect of FGF-21 analogs on lipid profile, in terms of cardiovascular risk. These observations warrant further studies to evaluate the safety of FGF-21 analogs, focusing on cardiovascular and bone-related side effects. Until then, patients with metabolic bone diseases (eg, osteoporosis) or relevant cardiovascular diseases (eg, uncontrolled blood pressure or heart arrhythmias) may be reasonable to be excluded from the relevant clinical trials with FGF-21 analogs. An interesting concept may also be the co-administration of FGF-21 analogs together with GLP-1RA, since the latter have shown beneficial effects on NAFLD, but also on cardiovascular and bone metabolism.⁷⁷ By this way, FGF-21 and GLP-1RA may have an additive or synergistic effect on the liver, and GLP-1RA may alleviate the possible adverse effects of FGF-21 on bone and cardiovascular diseases.

In conclusion, the hepatokine FGF-21 seems to play a role in the physiology of lipid metabolism, which renders it an appealing candidate for the treatment of some patients with NASH. There are favoring experimental and clinical results in phase 2 clinical trials. These have led to Phase 3 clinical trials, whose results may clarify whether FGF-21 analogs are beneficial in liver histology, especially in hepatic fibrosis. The cardiovascular and skeletal safety of FGF-21 analogs should be also further evaluated in clinical studies, in order to conclude whether any potential benefit outweighs possible risks.

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