**FGF21: A Novel Regulator of Glucose and Lipid Metabolism and Whole-Body Energy Balance**

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**ABSTRACT**
Fibroblast growth factor (FGF) 21 is a recently recognized metabolic regulator that evokes interest due to its beneficial action of maintaining whole-body energy balance and protecting the liver from excessive triglyceride production and storage. Together with FGF19 and FGF23, FGF21 belongs to the FGF family with hormone-like activity. Serum FGF21 is generated primarily in the liver under nutritional stress stimuli like prolonged fasting or the lipotoxic diet, but also during increased mitochondrial and endoplasmic reticulum stress. FGF21 exerts its endocrine action in the central nervous system and adipose tissue. Acting in the ventromedial hypothalamus, FGF21 diminishes simple sugar intake. In adipose tissue, FGF21 promotes glucose utilization and increases energy expenditure by enhancing adipose tissue insulin sensitivity and brown adipose tissue thermogenesis. Therefore, FGF21 favors glucose consumption for heat production instead of energy storage. Furthermore, FGF21 specifically acts in the liver, where it protects hepatocytes from metabolic stress caused by lipid overload. FGF21 stimulates hepatic fatty acid oxidation and reduces lipid flux into the liver by increasing peripheral lipoprotein catabolism and reducing adipocyte lipolysis. Paradoxically, and despite its beneficial action, FGF21 is elevated in insulin resistance states, that is, fatty liver, obesity, and type 2 diabetes.

**Introduction**
Fibroblast growth factor (FGF) 21 is an important metabolic regulator that controls energy homeostasis. Preservation of constant body mass depends on the balance between food intake and energy expenditure during both physical activity and at rest. Impairment of this balance, in conjunction with unlimited food supply, results in obesity. FGF21 is induced in the liver in response to nutritional signals and secreted into the bloodstream to reach the central nervous system (CNS). There, it exerts its main action of changing food preferences [1–3] and increasing thermogenesis in brown adipose tissue (BAT) by central beta-adrenergic stimulation [4, 5]. FGF21 is a signal that connects the liver with both the CNS and adipose tissue to adjust food preferences and energy expenditure to dietetic changes. Pharmacological treatment with FGF21 exerts many metabolic benefits, for example, weight loss, decreased serum cholesterol and triglycerides (TG), and lowering of plasma glucose while increasing insulin sensitivity (IS) [4, 6–8]. FGF21 decreases serum TG and prevents hepatic lipid deposition, which protects the liver from non-alcoholic fatty liver disease (NAFLD) development [9, 10] (Fig. 1, 2).

FGF21 lowers plasma glucose levels mainly by enhancing BAT insulin sensitivity. In brown and white adipose tissue, it is also locally secreted and exerts auto- and paracrine action. When produced locally in BAT, FGF21 stimulates thermogenesis in response to cold exposure as a downstream effect of central beta-adrenergic signaling [11], while in white adipose tissue (WAT) it suppresses lipolysis [12, 13] and likely stimulates adiponectin secretion [12]. However, while FGF21 exerts many beneficial metabolic effects, it is paradoxically elevated in insulin resistance (IR) states, for example, obesity, type 2 diabetes, and NAFLD [14]. Whether this result is from resistance to its action [15, 16] or increased compensatory secretion [17]...
remains controversial. FGF21 is produced by many different target tissues and exert differential actions via auto-, para-, and endocrine manners resulting in an extremely complicated biological function that in many aspects is still unexplained. Because of its beneficial properties and substantial therapeutic potential, it has evoked enormous interest from academics and pharmaceutical companies.

**Fibroblast growth factors with endocrine actions**

To date, twenty-three FGFs have been isolated, which have been divided into 7 subfamilies. Classical FGFs regulate cellular growth and differentiation, wound healing, angiogenesis, and embryonic development [18] by acting in an auto- and paracrine manner at the site of formation. Endocrine FGFs require the cofactor klotho/β-klotho to achieve ligand-receptor interaction, and the presence of the cofactor determines their action in particular tissues [19, 20].

The endocrine subfamily consists of three FGFs, namely FGF19 (and the mouse counterpart FGF15), FGF21, and FGF23 [20, 21]. Of these three, FGF23 binds klotho, while FGF19 and FGF21 exhibit affinity to the same β-klotho (KLB) cofactor, and the specificity of their action is determined by the activation of different subtypes of four FGF receptors. FGF19 activates FGFR4, whereas FGF21 predominantly binds FGFR1c [21]. FGF19 is produced in the intestine and regulates bile acids synthesis and metabolism. FGF23 is generated in bone and controls phosphate metabolism [22].

**FGF21 signaling**

FGF21 is a protein containing 208 amino acids and is encoded on chromosome 19 (19q13.33). The FGF21 gene is highly conserved between species. Human FGF21 differs by only one amino acid from gorillas and exerts approximately 75% homology with rodents. FGF21 alone exhibits only weak affinity to specific FGFR1c. The presence of KLB is indispensable for interaction with the receptor and determines tissue specificity. To activate the FGF receptor, the C-terminus of the molecule binds KLB, and the N-terminus interacts with FGFR1c [20, 21]. Once bound to its membrane-bound receptor, tyrosine kinase activity is initiated which further activates the mitogen-activated protein kinase pathway (Ras/Raf/MAPK). MAPK induces extracellular signal-related kinase (ERK) 1 and ERK2, which enter the nucleus and stimulate target genes transcription [20, 23, 24]. Besides this, FGF21 also activates the AMPK-SIRT1 pathway which induces posttranslational modification of proteins [25]. However, the exact mechanisms of intracellular FGF21 signaling remain unknown.

Several factors modify FGF21/receptor interaction, which results in changes to its action in vivo. In obesity, excessive TNF-α release from adipocytes suppresses KLB expression which in turn contributes to impaired FGF21 action and FGF21 resistance [26]. Likewise, FGFR1c expression in adipose tissue is reduced in obesity [27]. By contrast, thiazolidinediones [28] and glucagon-like peptide-1 (GLP-1) [29] increase KLB expression which potentiates FGF21 sig-
FGF21 synthesis

FGF21 originates in the liver, both BAT and WAT, the pancreas, and heart muscle. Muscles exhibit very limited expression of FGF21 under physiological conditions. However, its synthesis and release increase dramatically in mitochondrial myopathies as a consequence of excessive oxidative stress [33–37]. Therefore, increased circulating levels of FGF21 likely serve as a marker of mitochondrial disease [38]. Beyond these pathological conditions, serum FGF21 derives primarily, if not exclusively from the liver [39], and molecules generated in adipose tissue, pancreas, and heart muscle act locally in either an auto- or paracrine manner.

FGF21 secretion

The liver is the primary, if not only source of systemic FGF21, and its secretion is induced by nutritional and cellular stress signals. Hepatic FGF21 is generated in response to nutritional stress including extended fasting, a ketogenic diet, amino acid deprivation, or simple sugar consumption. In humans, the most important stimuli are fructose intake [40, 41], prolonged starvation [42] and protein restriction [43]. On the contrary, in rodents FGF21 is mainly secreted in response to extended fasting or consumption of a ketogenic diet.

FGF21 is induced during starvation and consumption of a ketogenic diet

In rodents, FGF21 secretion is induced by free fatty acids (FFA) generated endogenously during adipose tissue lipolysis in the starvation state or delivered with food during high fat consumption [44–46]. Free fatty acids increase FGF21 secretion downstream of nuclear receptor PPARα signaling, which occurs abundantly in the liver and serves as a sensor of cellular energy supply. PPARα is a ligand-activated nuclear receptor that heterodimerizes with nuclear retinoid X receptor (RXR) and stimulates the expression of target genes to induce β-oxidation. Free fatty acids, via PPARα activate FGF21 gene transcription, and subsequently FGF21 increases the expression of the PPARα coactivator-1α (PGC-1α), a key factor that promotes FFA oxidation through mitochondrial biogenesis and function enhancement [47, 48]. PGC-1α directs FFA to the β-oxidation and ketogenesis pathway to generate acetoacetate and beta-hydroxybutyrate, that are subsequently used as energy source. FGF21 also enhances mitochondrial β-oxidation gene expression, that is, CPT-1α and HMGCSC2 [44].

Simultaneously, an alternative pathway of FFA conversion to diacylglycerol and TG is suppressed, which favors increased energy utilization instead of hepatic TG synthesis and fat storage. Because FGF21 stimulates weight loss, and as a consequence increases IS, it would be possible that a decrease in hepatic TG accretion resulted from reduced insulin level. However, this is not the only mechanism involved, as other types of lipotoxic diets stimulate FGF21 expression in the liver as well. Therefore, increased FGF21 secretion occurs in mice consuming different types of lipotoxic diets, such as those deficient in leucine [49], alanine [50], methionine, and choline [51, 52], or a fructose-rich diet, independently of insulin action.

FGF21 is induced by protein restriction

Protein restriction activates FGF21 secretion via transcription factors ATF4 and NRF [43, 53]. In mice, methionine and choline-deficient diets induce hepatic FGF21 mRNA and elevates FGF21 serum levels, while in mice with genetically ablated FGF21 (Fgf21–/-), consumption of this diet leads to hepatic fat accumulation, liver inflammation, and fibrosis [51, 52]. Furthermore, in Fgf21–/-mice, methionine and choline deprivation results in impaired FFA oxidation, and increased expression of genes involved in TG synthesis. In these mice, increased TG storage induces hepatic steatosis, inflammation, and fibrosis that resolves following FGF21 pharmacological treatment [51, 52].

FGF21 is induced by fructose consumption

In humans neither starvation for 48 hours, nor consumption of a ketogenic diet for three months substantially changes FGF21 secretion and unlike rodents, FGF21 is strongly induced by high sugar intake, particularly fructose. This effect is mediated through the carbohydrate response element-binding protein (CHREBP)-dependent pathway [1, 54]. Intravenous injection of 75 g of fructose results in acute FGF21 release. Within 120 minutes following fructose administration, the FGF21 serum level rose on average 3.4-fold (1.5–6.6 fold) in lean individuals and returned to baseline during the subsequent 5 hours. Conversely, after intravenous glucose injection, the FGF21 peak was 40 % smaller and delayed for between 4–5 hours. Furthermore, the FGF21 peak response to fructose injection was 2.5-fold higher, and the area under the curve of secretion 2.7-fold greater in patients with metabolic syndrome than for lean people [40]. FGF21 hepatic expression and plasma levels of the protein are induced by simple sugar downstream of CHREBP signaling, which is strongly activated by fructose and to a lesser extent by glucose [1, 54–56]. CHREBP activates hexose metabolism by de novo lipogenesis, which triggers simple sugar transformation into FFA. Although glucose and fructose exhibit the same caloric value, they are not metabolically equivalent. Fructose is 90 % absorbed during the first pass through the liver, where it primarily enters de novo lipogenesis and is metabolized to FFA, whereas glucose is preferentially captured and used in peripheral tissues. Although fructose strongly activates hepatic CHREBP, which induces genes involved in de novo lipogenesis, concomitantly, CHREBP stimulates FGF21 gene expression, which favors β-oxidation of FFA instead of their conversion to TG and further accretion in the liver. This action is seen in Fgf21–/- mice, which when fed with the fructose-rich diet for 8 weeks, exert histological traits of hepatic steatosis, inflammation, and fibrosis. This transcriptional mecha-
FGF21 decreases hepatic triglyceride accumulation

FGF21 is generated primarily in the liver and protects other tissues against metabolic and nutritional stress. In addition, it acts on the liver itself to prevent inflammation and fibrosis induced by excessive lipid deposition. FGF21 analogues reduce hepatic fat and normalize biochemical markers of hepatic cirrhosis in patients with obesity, type 2 diabetes, and non-alcoholic steatohepatitis (NASH) [57]. Administration of the FGF21 analogue pegbelfermin in persons with NASH reduces hepatic fat content when assessed with MRI on average from 6–8 to 1–3 % [10]. FGF21 exerts complex actions in the liver, which encompasses a probable direct intrahepatic paracrine effect, but also general action through modification of adipose tissue signaling. As mentioned in the previous section, FGF21 increases the expression of PGC1-α in the liver, which through mitochondrial enhancement induces FFA oxidation and prevents their conversion into TG and further accumulation [47]. Furthermore, FGF21 reduces NAFLD, and obesity-related endoplasmic reticulum and oxidative stress linked to excessive hepatic lipid deposition [58, 59]. Additionally, decreased hepatic lipogenesis results in reduction of TG serum concentration. Moreover, FGF21 lowers serum TG levels through suppression of WAT lipolysis [9] and increases in lipoprotein lipase activity and lipoprotein catabolism [60]. In a number of clinical trials, FGF21 analogues, as well as monoclonal antibodies against the FGFR1-KLB complex, lower TG serum level by up to 70 % [8, 9].

Although some studies suggest a direct action of FGF21 in the liver [48, 61], others demonstrate divergent results [47, 62, 63]. Interestingly, primary hepatocytes do not express FGFR1c, the most important FGF21 receptor [20, 64]. However, they show KLB activity, and expression of FGF21 receptors FGFR2 and FGFR3, although these are less specific for FGF21 [20, 64]. It is also possible that FGFR1c expression in hepatocytes emerges during NASH, in a similar manner to immortalized HepG2 hepatocytes which express FGFR1c unlike healthy hepatocytes [65]. Furthermore, selective KLB ablation in mouse hepatocytes do not affect carbohydrate and lipid metabolism, suggesting that a direct effect in the liver is dispensable for FGF21 function [66]. Therefore, the beneficial action of reversing liver inflammation and fibrosis may occur indirectly with the participation of adipose tissue. It has been suggested that adiponectin may be an important player in this issue. According to some studies, pharmacological administration of FGF21 stimulates adiponectin secretion in adipose tissue, and adiponectin acts reciprocally in the liver, where it suppresses intracellular lipids accumulation and lipotoxicity [67, 68], although these results are controversial [69]. Furthermore, the surge of adiponectin may be induced by pharmacological doses of FGF21 or FGF21 analogues [9, 10, 70], and whether this occurs under physiological conditions in vivo remains to be determined. Paradoxically, despite beneficial action FGF21 serum concentration is elevated in humans with hepatic steatosis [71]. Augmented FGF21 mRNA expression within liver biopsies and increased FGF21 serum levels occur in NAFLD and increased hepatic fat content in 1H-MRI correlate well with FGF21 serum concentration [71, 72]. The possible explanation of this phenomenon is that in NAFLD-associated states like insulin resistance, obesity, and type 2 diabetes, the surplus of nutritional factors, or increased endoplasmic reticulum and oxidative stress activate compensatory FGF21 release. Thereafter, secondary resistance to FGF21 action may develop, which triggers a further surge of its secretion. Evidence of this can be seen in mice with diet-induced obesity, in which FGFR resistance in the liver, as well as in adipose tissue, was observed. It has manifested by diminished receptor-dependent ERK1/2 kinases phosphorylation and decreased transcription of target genes after exogenous FGF21 administration. Receptor resistance was overcome by higher pharmacological doses of FGF21 [16].

FGF21 increases insulin sensitivity of adipose tissue

In 2005, during a screening of several novel proteins of unknown function to identify molecules able to act as an insulin sensitizer, FGF21 appeared to induce glucose uptake in 3T3-L1 adipocytes independently of insulin action with a magnitude of effect comparable to insulin [22]. Further studies demonstrated that this effect required FGF21-induced upregulation of the insulin-independent glucose transporter GLUT-1 [73]. This was the first identified function of the molecule since its discovery in 2000. However, according to further research, glucose uptake with GLUT 1 is not meaningful in vivo, and FGF21 exerts its glucose-lowering properties through the potent and acute insulin-sensitizing effect on peripheral tissues.

In diet-induced obese mice, a single injection of FGF21 resulted in an acute decrease in plasma glucose by more than 50 %, which occurred within one hour following administration, an effect dependent on increased IS [74]. Likewise, intraperitoneal injection of FGF21 alone to wild-type ad libitum fed mice does not affect plasma glucose levels, whereas co-administration with insulin substantially enhances insulin-dependent plasma glucose disposal, an effect exceeding that of insulin alone [69]. An acute glucose-lowering effect depends primarily on FGF21 to enhance peripheral glucose disposal in BAT [69, 74, 75]. Interestingly, adipose tissue-devoid lipodystrophic mice do not exhibit the insulin-sensitizing effect of FGF21 [76]. Similarly, ablation of KLB from adipocytes completely abolished the hypoglycemic action of FGF21, whereas KLB removal from the liver does not [66, 69]. This suggests that FGF21 exerts its hypoglycemic properties primarily by enhancing peripheral glucose disposal, and to a lesser extent influencing hepatic glucose production. BAT dissipates energy as heat, unlike WAT that functions to store energy. This is achieved through the expression of uncoupling protein-1 (UCP-1) that destroys the inner mitochondrial membrane hydrogen gradient, allowing protons to penetrate the inside of the mitochondria with their electrochemical energy dissipated as heat. BAT in rodents [77] and humans [78] is
extremely insulin sensitive and exhibits a high capacity for glucose uptake, which is used to produce heat. Moreover, the ability of BAT to dispose of glucose and increase IS is similar in extent to skeletal muscle [78]. FGF21 exerts its acute insulin-sensitizing action primarily in brown adipocytes by inducing UCP-1 expression [69, 79]. This is shown by experiments using either mice with the KLB gene removed or mice with genetically ablated UCP-1, as neither are able to exert the acute hypoglycemic action of FGF21 [69, 80]. Conversely to BAT, white adipose tissue is not important for the FGF21 glucose-lowering effect. However, the administration of FGF21 enhances IS of white adipocytes resulting in suppression of lipolysis [13, 69, 81].

Although the principal hypoglycemic action of FGF21 is accomplished by an acute increase in peripheral insulin sensitivity of BAT, suppression of hepatic glucose production has also been observed [82]. It has been suggested that this effect on hepatic IS might be an indirect action in concert with adiponectin secretion. However, the data demonstrate divergent results on this issue. As mentioned previously, pharmacological doses of FGF21 induce adiponectin secretion in WAT [67, 68]. Adiponectin secretion is markedly enhanced in transgenic mice overexpressing the FGF21 gene (fgf21Tg) and suppressed in Fgf21−/− mice [23]. Furthermore, mice with an abolished FGF1 (Fgfr1−/−) do not demonstrate the rise in adiponectin secretion in response to FGF21 administration when compared to wild-type mice [83]. Adiponectin reduces intercellular lipids accumulation, primarily ceramides, in insulin-sensitive tissues [67]. Intrahepatic ceramide accumulation contributes to lipotoxicity and insulin resistance (IR). It has been suggested that FGF21 enhances hepatic IS indirectly, through induction of adiponectin secretion in adipose tissue and the beneficial effect of the adipokine in the liver. Moreover, clinical studies of FGF21 analogues demonstrate that FGF21 strongly elevates plasma adiponectin levels in patients with obesity and type 2 diabetes [8–10, 70].

However, it has been demonstrated that the hypoglycemic action of FGF21 remains unchanged in adiponectin knock-out mice (Adipoq-KO) [69]. Recent studies have provided different results, however, suggesting that FGF21 is induced locally and acts in a paracrine manner in adipocyte tissue, where it stimulates adiponectin secretion into the bloodstream, which further enhances FGF21 production in the liver in a feed-forward manner [84].

The interrelationship between FGF21 and adiponectin was confirmed in both cell culture and following the administration of pharmacological doses of FGF21 analogues in humans. These studies demonstrate conflicting results and do not fully reflect the physiology. Therefore, further research is indispensable to evaluate the interdependence of FGF21 and adiponectin in physiological conditions in vivo.

**FGF21 as a metabolic regulator**

Serum FGF21 is generated in the liver and released into the bloodstream in response to nutritional stimuli, providing information to the brain about systemic nutrient status, making the CNS the main target of the endocrine function of FGF21. The signal is processed in the ventromedial hypothalamus (VMH) glutamatergic neurons which suppress sucrose intake in response to increased plasma glucose concentration [85] and dorsal vagal complex of the medulla oblongata [86]. The CNS coordinates further actions of FGF21 including a suppressed preference for sugar and alcohol intake, increased physical activity, regulation of circadian rhythm and an increase in activity time, enhancement in sympathetic-nerve activity from the brainstem to BAT, which promotes thermogenesis, increases liver IS and reduces hepatic TG deposition. This complex action results in protection against weight gain by adjusting appetite and resting energy expenditure (REE) to macronutrient intake. However, there is likely some interspecies variability and plasticity of FGF21 action in the CNS, which customizes its action to be the most effective in the particular situation. This can be evidenced in mice, where enhanced thermogenesis is crucial to maintain stable body mass, whereas in humans, suppression of sugar intake is the most effective way to prevent weight gain [87]. However, when a certain mechanism is not effective, the alternative pathway may also be triggered. In mice with genetically ablated UCP-1, there was the same bodyweight reduction as in their wild-type counterparts, due to either suppressed food intake [88] or increased physical activity [89].

FGF21 controls macronutrient preference by suppressing the appetite for simple sugar, and genome-wide association studies (GWAS) identified single nucleotide polymorphisms (SNP) in the FGF21 gene associated with increased sweet taste preference [90–92]. In humans, the main stimulus for FGF21 hepatic secretion is simple sugar intake, especially fructose as mentioned previously. Circulating FGF21 then exerts its action in the VMH, decreasing the excitability of glutamatergic neurons sensible to high glucose serum levels, which in turn diminishes sweet taste preference and decreases sucrose intake [85].

Besides suppressing simple sugar intake, FGF21 increases the resting metabolic rate through intensifying BAT thermogenesis, which activates weight loss and maintains core body temperature. In mouse pups, consumption of fatty acids contained in maternal milk resulted in increased hepatic FGF21 synthesis, and in turn enhanced expression of brown adipocytes thermogenesis genes (UCP-1, PGC1α, Dio-2) enabling core body temperature maintenance [79]. Besides endocrine action in BAT, cold exposure stimulates local production of FGF21 in adipocytes. In mice, FGF21 is generated locally in BAT in response to cold exposure, which stimulates non-shivering thermogenesis to maintain core body temperature [93, 94] and this effect is essential in rodents, but to a lesser degree also occurs in humans [95]. As mentioned above, FGF21 secreted from the liver or generated locally in adipose tissue enhances thermogenesis genes in brown adipocytes, including UCP-1 controlling energy dissipation as heat [79] and PGC1α activating mitochondrial biogenesis and intensifying their function [25, 96]. FGF21 also induces browning of white adipocytes and generation of dispersed brown-like adipocytes in white adipose depots [96]. Therefore, adipose tissue is indispensable for systemic FGF21 action. In mice with Fgfr1 knock-out (FR1KO) selective for adipose tissue, which lack FGF21 signaling in adipocytes, the majority of systemic action of FGF21 is abolished including plasma glucose, TG and insulin lowering and increase in REE [83]. FGF21 expression in adipocytes is stimulated through the transcription factor PPARy that is activated by FFA, and induces adipocytes differentiation and TG storage, preventing their ectopic accumulation. FGF21 augments in a feed-forward manner PPARy function, by diminishing
FGF21 in future therapy

Beneficial FGF21 function has encouraged many attempts to use it as a therapeutic agent to treat obesity-related comorbidities including type 2 diabetes mellitus, dyslipidemia, NAFLD, and NASH. Native FGF21 is not appropriate for therapeutic use, because of poor pharmacodynamic properties, including a short half-life of 0.5–1 hour, proteolytic cleavage by serum proteases, and tendency to precipitate into insoluble aggregates. Therefore, long-acting analogues conjugated to PEG or immunoglobulins, which are resistant to aggregation or proteolytic cleavage have been synthesized. Another group of potential therapeutic agents represents FGF21 receptor agonists including bispecific monoclonal antibodies which bind to the FGF1-KLB complex, and also avimers (avidity multimers) that are artificially constructed proteins that bind specific antigens, which activate FGF1 and KLB [57]. FGF21 analogues were intended to treat type 2 diabetes, because of their profound hypoglycemic effect in mice and non-human primates. However, they did not induce a significant fall in plasma glucose levels in humans. Instead, they decrease serum lipids, increase serum adiponectin levels, and exert a diverse effect on weight reduction. In different species, particular analogues differ in hypoglycemic, lipid-lowering, and weight-reducing properties, which results from interspecies differences in FGF21 action and divergent biological activity.

At present, FGF21 analogues and mimetics appear to be an effective strategy in NAFLD and NASH treatment. In a phase IIa clinical trial using the FGF21 analogue pegbelfermin in patients with NASH, no suppression of HbA1c was noted in the serum, which was the primary endpoint of the study, but there was a significant reduction in N-terminal type III collagen propeptide (PRO-C3) serum level, a marker of hepatic fibrosis [98]. Another multi-center clinical trial using pegbelfermin, in patients with biopsy confirmed NASH, revealed a decrease in hepatic fat content determined by the proton density-weighted MRI fat fraction by 30% in over 50% of subjects. Improvement in biochemical hepatic fibrosis and injury markers (Pro-C3, ALT, AST), and increased adiponectin serum levels were also noted [11]. Subsequent studies of the safety and efficacy of FGF21 mimetic AKR-001 and NGM-313 in NASH are also underway [57, 99]. Concomitantly, there are safety concerns about side effects of FGF21 analogues, drugs intended for prolonged, and potentially lifelong treatment. Administration of one discontinued analogue evoked a rise in heart rate and blood pressure [8]. However, this effect was specifically attributable to a certain analogue, not related to them all. Furthermore, administration of pharmacological doses of FGF21 in mice induces bone loss, which raises concerns about the effects on bone metabolism [100]. Indeed, in humans increases in bone turnover markers were observed following injection of certain analogues, but not others [8] and this effect may be secondary to the induced weight loss. A further problem is the immunogenicity of analogues that arises from the induction of FGF21-antibodies, which have been observed in 50% of pegbelfermin treated patients [98]. However, FGF21 analogues are generally well-tolerated, and most side-effects are gastrointestinal, related to their interaction with FGF19 and bile acid metabolism.

The complex and still unresolved biological action of FGF21 has given rise to new insight into FGF21 analogues and their therapeutic potential. Initially trialed as antidiabetic drugs, these are finally recognized as a successful strategy to treat other obesity-related comorbidities such as NAFLD and NASH. Until the present time, specific treatment was not available to these patients, other than mildly-effective diet therapy. Therefore, in the future FGF21 analogues may become a successful strategy to treat the cluster of obesity-related diseases, as a complement to antidiabetic drugs. However, further clinical trials with larger sample sizes are needed to evaluate whether prolonged administration of FGF21 analogues by decreasing hepatic and plasma lipid levels can reduce NASH progression and cardiovascular risk.

FGF21: current knowledge and controversies

Current knowledge only partially elucidates the complicated biology of FGF21. Although target tissues have been identified, exact intracellular pathways of FGF21 signaling within these tissues remain unknown. Their recognition is crucial for future targeted therapies designed for selective action in specific tissues and identifying modifiers that may amplify FGF21 function. Furthermore, the mechanism of elevated FGF21 serum levels in obesity and IR remains unclear. Although some animal studies suggest there is resistance to FGF21 action in obese states, this is not verified in humans. It is not elucidated whether elevated serum levels of FGF21 in obesity result from resistance to its action or compensatory increased secretion. Furthermore, human studies following administration of FGF21 analogues do not reflect physiological conditions. The principal FGF21 biological action of reducing hepatic fat content, either by direct paracrine action in the liver or indirectly by crosstalk with adipose tissue is still unresolved. The way of coordinating whole-body energy balance including food preferences, circadian cycle, physical activity, and resting energy expenditure is an important unanswered question. Although KLB expression has been documented in many dispersed neurons of the brain, the exact action of FGF21 in the central nervous system remains unrevealed. Therefore, this molecule, since discovery in 2000, still continues to evoke interest, leaving many questions for further research.

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Conflict of Interest
The authors declare that they have no conflict of interest.
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