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Review

# The crosstalk between fibroblast growth factor 21 (FGF21) system and substance use

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## SUMMARY

**Existing literature indicates that communication between the central nervous system and the peripheral nervous system is disrupted by substance use disorders (SUDs), including alcohol use disorder (AUD). Fibroblast growth factor 21 (FGF21), a liver-brain axis hormone governing energy homeostasis, has been shown to modulate alcohol intake/preference and other substances. To further elucidate the relationship between FGF21, alcohol use, and other substance use, we conducted a scoping review to explore the association between FGF21 and SUDs. Increases in FGF21 reduce alcohol consumption while suppressing FGF21 increases alcohol consumption, demonstrating an inverse relationship. Alcohol elevates FGF21 levels primarily via the liver, subsequently promoting neuronal signals to curb alcohol intake. FGF21 activation engages molecular pathways that defend against alcohol-induced fat accumulation, oxidative stress, and inflammation. Considering the bidirectional association between FGF21 and alcohol, further studies on the FGF21 system as a potential pharmacotherapy for AUD and alcohol-associated liver disease are warranted.**

## INTRODUCTION

### Rationale

Substance use disorder (SUD) remains a leading cause of morbidity and mortality and has a devastating impact on the individual and society as a whole.[1–3] Of further concern is the paucity of effective treatment options for SUDs, which is in part due to the heterogeneity of responses to treatment. Accordingly, the need to develop new medications for SUDs is critical.[3] Addictive substances that have the potential to lead to SUDs and addiction act on both the central nervous system (CNS) as well as the peripheral nervous system (PNS) and peripheral organs (e.g., liver, pancreas, and gut). These substances disrupt the interplay between the CNS and the periphery,[4] such as the neuroendocrine signaling that mediates feeding and satiety. Neuroendocrine pathways are not only crucial for the regulation of metabolism, energy homeostasis, feeding behavior, and consumption of natural rewards like food but are also involved in the regulation of substance use, and growing evidence suggests their role in the development and maintenance of addictive disorders.[5–10] As such, neuroendocrine pathways may be suitable targets to develop novel pharmacotherapeutics of SUDs. The fibroblast growth factor 21 (FGF21) system is one such example that is gaining more traction in the SUD field based on growing evidence from preclinical and clinical research.[11–14]

FGF21 is a 208 amino acid endocrine hormone that is one of many in a family of fibroblast growth factors that have broad mitogenic and cell survival effects.[15] The *FGF21* gene is located on chromosome 19 (19q13.33) in humans.[15] FGF21 signals through a co-receptor complex composed of FGF receptor (FGFR subtype 1–4) and β-Klotho receptor (KLB).[16] Once FGF21 is secreted, it binds the FGFR-KLB receptor complex, initiating autophosphorylation of FGF receptors, which triggers subsequent intracellular signaling pathways including mitogen-activated protein kinases (MAPK signaling),[17,18] the mechanistic/mammalian target of rapamycin complex 1 (mTORC1) signaling,[17] AMP-activated protein kinase (AMPK) signaling,[19] and inhibition of the phosphatidylinositol 3-kinase-Akt kinase-mechanistic/mammalian target of rapamycin (PI3K-Akt-mTOR) pathway.[20] These signaling pathways in general promote cell proliferation, cell growth, energy metabolism, and cell survival. FGF21 is expressed in hepatocytes (greatest expression), pancreatic beta and alpha cells, skeletal muscle cells, white and brown adipose cells, heart cells, intestinal cells, and neurons in brain regions such as the thalamus, hypothalamus, choroid plexus, putamen, amygdala, and hippocampus.[21,22] However, not all these cell types show constitutive expression under normal physiological conditions.[18] The FGF21 co-receptor complex of FGFR1 and β-Klotho has similar expression patterns as FGF21, with most expression in the liver, pancreas, and adipose tissue, but also some expression throughout the brain (similar to FGF21 expression in brain).[21,22] However, there are several brain

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regions which express β-Klotho, but not FGF21, and vice versa. FGF21 serves as a metabolic regulator by stimulating hepatic fatty acid oxidation and ketogenesis, as well as suppressing lipogenesis.[15,23] FGF21 has been shown to produce beneficial health effects by maintaining whole-body energy homeostasis by promoting cellular glucose metabolism and reducing inflammation. FGF21 also protects the liver from the detrimental effects of excessive lipid production and storage by promoting the breakdown of lipids stored in the liver.[15,18,23–25]

Due to the known role FGF21 plays in energy homeostasis, it is not surprising that serum FGF21 levels are increased in patients with type 2 diabetes and obesity.[26] Daily administration of FGF21 to rhesus monkeys with diabetes led to reductions in fasting serum glucose, triglyceride, and insulin levels, in addition to significant improvements in lipoprotein profiles.[27] Therefore, FGF21 holds promise as a therapeutic target for metabolic disorders.[28]

Growing literature suggests a potential role of FGF21 in SUDs, including alcohol use disorder (AUD). Of note, AUD is associated with a host of metabolic changes, such as damage to pancreatic β cells, diminished insulin sensitivity and altered glucose metabolism, liver damage, and increased triglycerides and cholesterol levels.[29] Alcohol produces profound damage to peripheral organs, especially the liver, and to significantly alter immune function.[30–33] FGF21 may counteract some of those changes.[34]

To date, there are a few narrative reviews on FGF21 signaling that touch on aspects of alcohol-related outcomes and FGF21 interplay.[12,18,35,36] However, there is no comprehensive scoping review on the interplay between substance-related outcomes and the FGF21 system. Therefore, a comprehensive and unbiased overview of the current literature has the potential to identify novel themes and gaps in the knowledge base that may guide subsequent research.

### Objectives

The objective of this scoping review is to comprehensively examine and synthesize the current evidence on the interaction between the FGF21 system and SUD in both human studies and animal models.

## RESULTS

### Narrative summary of publications

#### *Genetic variations in FGF21 signaling and alcohol-related outcomes*

Although limited, genetic association studies in humans have found that variants affecting FGF21 signaling are associated with alcohol consumption.[37–39] Søberg et al. showed that the *FGF21* SNP rs838133 was associated with both higher alcohol consumption and sweet intake/preference in humans. Surprisingly, rs838133 was not associated with total energy intake, but was associated with lower waist circumference and lower BMI. They also showed that rs838133 was associated with improved glycemic control.[37] The rs838133 SNP has been shown to result in increased expression of FGF21.[40] They also show that FGF21 is upregulated by sugar intake, similar to the effects of alcohol intake, and sweet disliking individuals had elevated FGF21 levels.[37] Another study by Schumann et al. conducted a genome-wide association meta-analysis and replication study in >105,000 people of European ancestry. They identified the *KLB* gene rs11940694 SNP (minor allele A) was associated with reduced alcohol consumption. There was no significant association between rs11940694 SNP and KLB gene expression.[39] Other studies have explored if variants in the *FGF21* genotype may be associated with alcohol use, smoking, food choices, and aggressive behaviors.[37,41–43] Xu et al. found that the interactions between specific SNPs on *FGF21* and *FGF19* genes were linked to high risk for alcohol dependence.[43] In a Sorb-cohort (indigenous west Slavic ethnic group that is situated in Eastern Germany) study, *FGF21* variants were associated with anthropometric and metabolic parameters, adipokines, food and substance craving, and low-density lipoprotein cholesterol.[41] Another genome-wide association study (GWAS) revealed a genetic variant, rs9914222, located upstream of the *SNHG16* gene and associated with decreased expression, was associated with increased blood levels of FGF21 and increased risk for AUD.[38] These studies show that variants in FGF21 genes and related genes are associated with consumption and craving for both natural rewards as well as addictive substances.

These studies suggest that FGF21 signaling plays a role in homeostatic response to rewarding and reinforcing stimuli, such as sugar and alcohol, by attenuating their rewarding properties. As such, genetic differences that influence FGF21 signaling are associated with alcohol consumption and risk for AUD, as well as consumption of natural rewards like sugar. Animal studies have helped to further elucidate the molecular signaling mechanisms of FGF21 on alcohol and natural rewards.

### Bi-directional relationship between the FGF21 system and alcohol-related behaviors

Experimental manipulation of the FGF21 system impacts alcohol-related behaviors, and alcohol consumption (or administration) impacts endogenous FGF21 signaling. Several animal models demonstrate that increasing FGF21 signaling through administration of human recombinant FGF21 (hrFGF21)[11,39,44,45] administration of the FGF21 mimetic PF-05231023,[11] and genetic overexpression of *FGF21*[44] all result in decreased alcohol consumption. Likewise, decreasing or blocking FGF21 signaling through *FGF21*[11] or *KLB*[39] genetic knock out increases alcohol preference/consumption, suggesting that FGF21 signaling and alcohol consumption are inversely associated. Furthermore, alcohol intake or administration consistently increases endogenous FGF21 signaling, both in the blood as well as its hepatic expression, in animal models and humans.[11,34,46–50] These associations between alcohol and FGF21 suggest that endogenous increases in FGF21 following alcohol exposure promote homeostasis to counteract alcohol-induced biological changes.

Talukdar et al. found that *FGF21*-transgenic mice (overexpression) exhibited reduced alcohol preference [44]. The same group showed that neuron-specific knock out of *KLB* (*KLB*$^{Camk2a}$) produced increased alcohol preference, in a two-bottle choice drinking paradigm, compared to

wild-type (WT) mice.[39] rFGF21 administration by osmotic minipump suppressed alcohol preference in the control but not in the *KLB*$^{Camk2a}$ KO mice. Alcohol metabolism did not differ between *KLB*$^{Camk2a}$ KO and control mice. This work suggests that the effect of FGF21 on suppressing alcohol consumption depends on FGF21 signaling in neurons.[39]

In another comprehensive study, Flippo et al.[11] first showed that knockout of liver-specific *FGF21* gene (*FGF21*-LivKO) in C57BL/6J mice blocked the circulating (blood) upregulation of FGF21 due to alcohol administration (3.5 g/kg, i.g.), demonstrating that alcohol-induced increases in FGF21 primarily originate from increased production and secretion of FGF21 in the liver. Researchers next showed that *FGF21*-LivKO resulted in increased alcohol consumption and preference in a two-bottle choice paradigm. Having demonstrated this bi-directional relationship between liver-produced FGF21 signaling and alcohol consumption, the authors proposed that FGF21 functions as a homeostatic mechanism to decrease alcohol consumption. Congruent findings were observed following intraperitoneal administration of FGF21 or the FGF21 mimetic PF-05231023 (long-acting FGF21 analogue), which both resulted in decreased alcohol consumption in male and female mice using a two-bottle choice paradigm and the Lieber-DeCarli liquid alcohol diet. Administration of intravenous PF-05231023 also reduced alcohol, but not water, consumption in alcohol preferring vervet monkey. Neural mechanisms underlying the effects of FGF21 signaling on reduced alcohol consumption was also investigated in this work. To start, neurons expressing KLB (part of the receptor complex for FGF21 signaling) were fluorescently labeled, and the basolateral amygdala (BLA) demonstrated high expression for KLB. Next, administration of FGF21 or PF-05231023 specifically into the BLA was sufficient to decrease alcohol consumption. *In vitro* electrophysiological recordings from KLB-expressing neurons in the BLA demonstrated that FGF21 bath application increased measures of neuronal excitability and increased the frequency of action potential firing, demonstrating that FGF21 acts directly on neurons. To determine the projection site of the BLA KLB+ (KLB$^{BLA}$) neurons, viral anterograde tracing techniques were used showing that KLB$^{BLA}$ neurons project to the nucleus accumbens (NAc). Further electrophysiology experiments demonstrated that *in vivo* systemic administration of FGF21 (1 mg/kg intraperitoneal injections 3 days prior to recording) increased neuronal excitability specifically in the subpopulation of KLB$^{BLA}$ neurons that also project to the NAc.[11] Notably, BLA→NAc circuitry and dopaminergic signaling in the NAc are involved in reward-seeking and consummatory behaviors,[51–54] which are the result of excitatory projections onto medium spiny neurons (MSNs) in the NAc. Researchers also found that *in vivo* FGF21 treatment results in increased spontaneous post-synaptic currents (sEPSC) frequency on dopamine D2 receptor (D2)-expressing MSNs, but not D1-expressing MSNs. Downregulation of D2 receptors in the striatum (the NAc is part of the striatum) are associated with SUDs.[55] As such, the finding that FGF21 impacts KLB$^{BLA}$ projections to D2 MSNs in the NAc may underlie decreased alcohol consumption by reducing motivation for drinking behavior. Finally, researchers demonstrated that FGF21 signaling in KLB$^{BLA}$→NAc neurons is both necessary and sufficient to produce decreased alcohol consumption, highlighting neural circuitry that contributes to the effects of FGF21 on suppression of alcohol consumption.[11]

FGF21-induced decrease in alcohol consumption may be driven in part by general decreases in reward or reinforcement motivation. Sucrose, like addictive substances, has rewarding properties which rodents and other mammals are highly motivated to consume. Talukdar et al. showed that increasing FGF21 through transgenic overexpression decreased two-bottle choice preference for a sweet solution (saccharin or sucrose) vs. water. The long-acting FGF21 analog PF-05231023 decreased sweet preference in monkeys. Of note, this effect of FGF21 on decreasing sweet preference was shown to be dependent on expression of KLB in neurons. Finally, the researchers showed that FGF21 administration decreased metabolites of dopamine in the NAc in mice.[44] Furthermore, a Genome-Wide Association Study (GWAS) that was previously described in the text above showed an association between the *FGF21* single nucleotide polymorphism (SNP) rs838133 with both candy intake and alcohol consumption in humans.[37] Another study showed that FGF21 suppression of sugar intake and sucrose preference was regulated by hypothalamic glutamate neurons that express the KLB and FGFR1 receptors.[56] This study found that FGF21 signaling on specific glutamatergic neurons in the hypothalamus was both necessary and sufficient to produce the suppression of sucrose intake. Furthermore, they showed that FGF21 signaling directly to dopamine neurons was not required for the suppressive effects on sucrose consumption.[56] Collectively, these findings suggest that FGF21 may blunt the rewarding or reinforcing effects of rewards in general. However, there seems to be divergent neural pathways that underlie the suppressing effects of FGF21 on alcohol consumption compared to natural reward (sucrose) consumption.

Human studies have shown that acute or binge alcohol intake increases circulating FGF21 levels,[45,47,57] consistent with findings from animal experiments. Stankevic et al. found that FGF21 levels in both systemic and hepatic blood were elevated at 60- and 180-min post alcohol administration (nasogastric tube – 2.5 mL/kg, 40% EtOH, over 30-min). Interestingly, several immune and inflammatory markers, except IL-6, were downregulated at these time points following alcohol administration.[58] Two studies found increased serum FGF21 levels in participants engaging in binge drinking at Oktoberfest and Roskilde national festivals.[45,57] Desai et al. conducted parallel human and rodent experiments to elucidate the effects of alcohol on endogenous FGF21 levels over time. In humans, 0.9 g/kg binge-intake of alcohol resulted in a steady increase in serum FGF21 levels starting by 2-h post alcohol that peaked at 6-h post alcohol consumption. FGF21 levels returned nearly to vehicle levels by 10-h post intake. In mice, following a 3.5 g/kg alcohol gavage, serum FGF21 levels were elevated by 3-h, peaked at 6-h, and were identical to that of vehicle by 9-h post alcohol gavage. These findings indicate similar effects of alcohol on circulating FGF21 across mice and humans.[47] In another study, healthy males showed robust increases in FGF21 levels (9-fold) following intragastric or intravenous alcohol administration, with comparable FGF21 pharmacokinetics across the two routes of alcohol administration. These findings suggest that alcohol-induced stimulation of FGF21 is not dependent on the gut.[59] Ho et al. observed a positive association between plasma FGF21 levels and recent alcohol use in patients with AUD.[38] In a follow-up study, they found that plasma FGF21 levels were positively correlated with plasma GABA levels in patients with AUD, and then used iPSC-derived brain organoids to show that FGF21 signaling strongly impacts gene expression related to the GABAergic synaptic pathway.[60] Another study performed a secondary analysis of alcohol cue-reactivity,

alcohol priming, and alcohol self-administration in a bar-like laboratory in non-treatment seeking individuals with AUD. FGF21 levels did not change after cue-reactivity or the priming alcohol drink, but robustly increased after the 2-h alcohol self-administration session and changes in FGF21 levels were trending toward a positive correlation with the amount of alcohol self-administered.[61] Collectively, elevations in endogenous FGF21 following alcohol exposure have been observed across multiple species, including mouse, rat, monkey, and humans.

FGF21 elevations following alcohol consumption may also be involved in counteracting the sedative and intoxicating effects of alcohol.[62] Choi et al. found that after an intoxicating dose of alcohol (5 g/kg, i.g.), mice lacking the *FGF21* gene (global *FGF21*-KO) required on average 1.5-h more to recover their righting reflex (Loss of Righting Reflex test – LoRR) and spent decreased time on a spinning rotarod compared to WT mice. LoRR is a common method used to assess the sedative and anesthetic effects of a substance and measures the amount of time it takes for an animal that has lost consciousness to orient itself in the upright position. The spinning rotarod evaluates motor coordination and is used as a measure of alcohol intoxication. Alcohol concentrations were measured in the brain, demonstrating no difference between *FGF21* KO and WT mice, indicating that differences in LoRR recovery time were not the result of differences in brain alcohol clearance or metabolism. Similar results were observed in liver-specific knockout of FGF21. Next, following a 5 g/kg oral gavage of alcohol, rats were injected with rFGF-21 (i.p.) 1-h later while they were unconscious. rFGF21 administration reduced the time to recover LoRR in both male and female mice – an effect that was dependent on the expression of the *KLB* gene. Furthermore, rFGF21 administration increased time spent on the spinning rotarod.[62] As such, FGF21 signaling has bi-directional effects on alcohol sedation/intoxication, similar to alcohol consumption.[11] Choi et al. then sought to investigate the neural mechanisms that may be underlying these effects. They first showed that 5 g/kg alcohol gavage resulted in increased expression of c-Fos (marker of neuronal activity) in norepinephrine transporter positive ($NET^+$) neurons in the locus coeruleus (LC). This effect was completely absent in the *FGF21*-KO mice, indicating that alcohol increases the activity of NET+ neurons in the LC through increases in FGF21 signaling. Finally, the authors demonstrate that FGF21 acts directly on norepinephrine neurons in the LC to promote arousal that counteracts the sedative effects of alcohol.[62] This furthers the idea that FGF21 acts as a homeostatic regulator to the acute effects of alcohol. Interestingly, sensitivity to the sedative effects of alcohol is a predictor of AUD development and associated with other AUD-related outcomes.[63–67]

Other studies have demonstrated a relationship between FGF21 and alcohol-induced water intake.[46] Song et al. administered a daily bolus of alcohol (3.5 g/kg, i.g.) over the course of 8 days. Starting on day 3, water consumption began to increase in WT mice treated with alcohol compared to water-treated controls, but this effect was not observed in the *FGF21*-KO mice. The researchers then used the $KLB^{Camk2a}$ KO method to knockout *KLB* in neurons specifically. rFGF21 administration by osmotic minipump increased water consumption in control ($KLB^{fl/fl}$) but not in the *KLB*-KO mice. Researchers then crossed the $KLB^{fl/fl}$ mice with Sim1-Cre mice, in which the Cre expression was enriched in the paraventricular nucleus of the hypothalamus (PVN). The effects of FGF21 on water intake was lost in the $KLB^{Sim1}$ mice. Therefore, they concluded that FGF21 exerts its effects on drinking water in part by acting on Sim1+ neurons in the PVN. As such, FGF21 appears to stimulate the behavioral motivation to consume water following alcohol consumption. This effect of FGF21 on water consumption may play a role in counteracting dehydration due to alcohol consumption.[46,68,69]

Existing evidence suggests that increases in FGF21 signaling following alcohol consumption may act as a compensatory mechanism by preventing or decreasing escalated alcohol consumption, attenuating the intoxicating effects of alcohol, and promoting water intake following alcohol consumption. Interestingly, the alcohol-induced increases are largely driven by increased expression of FGF21 in the liver, and the effects of FGF21 on alcohol drinking behavior are dependent on the FGF21 receptor complex expression in neurons in the CNS. Therefore, hepatic increases in FGF21 due to alcohol ingestion may serve to protect the liver from subsequent damage by communicating, through molecular signaling, with the CNS to impact alcohol-seeking behavior and other alcohol-related behaviors.

## Effects of FGF21 on alcohol-induced organ (primarily liver) damage

In addition to involvement in alcohol-related behaviors, FGF21 signaling also modulates the effects of alcohol on the liver and hepatocytes, as well as associated damage from oxidative stress and inflammation. The liver shows the highest expression of FGF21 relative to other organs/tissue.[70] Several studies in humans and animal models point to a link between alcohol-associated liver damage and FGF21 levels, as well as a protective role for FGF21 in alcohol-induced liver damage, oxidative stress, and inflammation.[13,34,48,58] In general, experimental suppression of FGF21 signaling exacerbates the damaging effects of alcohol on the liver, whereas experimental augmentation of FGF21 signaling attenuates these effects.

Christidis et al. showed that patients with alcohol-associated liver disease (ALD) have significant upregulation of serum FGF21 compared to healthy controls.[48] This study also performed parallel animal experiments. Mice were exposed to a 10-day Lieber-DeCarli liquid alcohol diet followed by a 4 g/kg alcohol gavage, and blood and liver were collected 7–9 h after the gavage. The alcohol-exposed group showed increased hepatic FGF21 mRNA and protein levels compared to isocaloric liquid diet, alcohol-naïve controls.[48] Liu et al. showed that FGF21 was six times elevated in patients with alcohol-induced hepatic steatosis compared to controls but those with stable cirrhosis (no fatty liver) had similar FGF21 levels compared to controls.[34] Wagner-Skacel et al. found higher FGF21 levels in patients that had recently consumed alcohol compared to those that did not recently consume alcohol (verified by urinary ethyl glucuronide (ETG) levels which indicated alcohol consumption in the last 12–72 h). However, no statistical differences in FGF21 levels were found between the three groups tested based on liver pathology (alcohol liver cirrhosis - ALC, non-alcohol liver cirrhosis - NALC, and healthy controls).[13] In a recent clinical study, Sak et al. measured serum levels of oxidative stress induced growth inhibitor 1 (OSGIN1) and FGF21 in patients with varying degrees of alcohol-related cirrhosis compared to controls. They found that OSGIN1 and FGF21 levels were elevated in people with ALC, and that levels of OSGIN1 correlated with levels of FGF21. Interestingly, OSGIN1 levels were highest in the cirrhosis group at the Child-Pugh C stage, while FGF21 levels were

highest in the combined Child-Pugh A/B group compared to Child-Pugh C.[71] Another study that measured serum FGF21 levels in people with alcohol-associated hepatitis vs. people with decompensated alcohol-associated cirrhosis found elevated FGF21 levels in the hepatitis group compared to cirrhosis.[72] Together, these data indicate that acute alcohol exposure increases FGF21, and that ALD is associated with elevated FGF21 levels, which may be blunted in more severe forms of ALD in which the liver is cirrhotic and reduced overall functioning.

Many studies demonstrate that the damaging effects of alcohol on the liver are enhanced or exacerbated upon attenuation of FGF21 signaling. The Liu et al. study mentioned earlier found that mice fed a standard Lieber-DeCarli alcohol liquid diet for four weeks showed higher serum and liver FGF21 levels compared to isocaloric liquid diet controls (no alcohol). The alcohol-exposed group also showed increased hepatic triglyceride (TG) levels, histological findings consistent with fatty liver, increased plasma concentrations of the liver enzyme ALT, and increased liver/body weight ratio. These effects were exacerbated in *FGF21*-KO mice compared to WT controls. Furthermore, *FGF21*-KO mice showed more severe hepatic steatosis as a result of alcohol exposure compared to WT controls.[34] Another study showed that *FGF21*-KO mice exhibited exacerbated effects of alcohol exposure on hepatic steatosis, lipotoxicity and inflammation.[73] A study by Xu et al. used mice knocked out for the FGF receptor 1 gene (*FGFR1*) specifically in adipose cells ($fgfr1^{adipoQ\text{-}cre}$) and WT controls. Mice were exposed to the Lieber-DeCarli alcohol liquid diet for 3 weeks, and some mice received WY-14,643 (PPAR-α agonist that induces FGF21) treatment. Liver triglyceride accumulation was induced by chronic alcohol to a greater extent in the $fgfr1^{adipoQ\text{-}cre}$ mice compared to WT mice. Furthermore, liver serum triglyceride accumulation caused by alcohol exposure was blunted by WY-14,643 treatment in the $fgfr1^{adipoQ\text{-}cre}$ mice, but not in WT mice. As such, the FGFR1 receptor expression in adipocytes is necessary for FGF21 signaling to induce its protective effects against alcohol-induced TG accumulation in the liver and serum.[74] A follow up study replicated these effects using a binge-like alcohol drinking model instead of the Lieber-DeCarli alcohol liquid diet.[75] Desai et al. showed that alcohol exposure also increased liver expression of the inflammatory markers tumor necrosis factor α (TNFα), interleukin 6 (IL6), and monocyte chemoattractant protein 1 (MCP1). This hepatic pro-inflammatory effect of alcohol was exacerbated in the *FGF21*-KO group compared to WT controls. Alcohol administration (3.5 g/kg, i.g.) increased serum FGF21; fatty acid synthesis genes were upregulated and fatty acid oxidation genes were downregulated. They also showed that chronic alcohol consumption produces significant liver pathology in *FGF21*-KO mice compared to WT controls, again suggesting that FGF21 protects the liver against long term alcohol-related consequences.[47] A separate group showed that *FGF21*-KO mice had significantly lower blood alcohol concentration (BAC) 0.5-h post alcohol administration (5.0 g/kg, i.g.), slower gastric emptying, and higher glucagon-like peptide-1 (GLP-1) levels compared to WT mice,[76] suggesting that effects of FGF21 on the liver might be related to attenuated alcohol metabolism. However, Desai et al. study found no differences in blood alcohol clearance between WT and *FGF21*-KO mice,[47] suggesting that FGF21 may impact the gastric emptying of alcohol without affecting the activity of alcohol metabolizing enzymes. Another study by Zhao et al. used *FGF21*-KO mice and Lieber-DeCarli alcohol liquid diet (5% w/v, 12-day exposure), combined with a 5.0 g/kg oral alcohol gavage 6-h prior to euthanasia and biospecimen collection. Alcohol exposure resulted in increased expression of FGF21 in blood, liver, and ependymal white adipose tissue (eWAT). Alcohol exposure also resulted in decreased eWAT/body weight ratio, decreased average adipocyte area, increased plasma glycerol and non-esterified fatty acid (NEFA). These effects of alcohol were blunted or completely blocked in *FGF21* KO mice, indicating that alcohol-induced lipolysis is dependent on FGF21 expression.[49] Another study using *FGF21*-LivKO and the Lieber-DeCarli alcohol diet found that greater alcohol-induced fatty liver in the *FGF21*-LivKO compared to WT.[77] In summary, reducing endogenous FGF21 signaling exacerbates the damaging effects of alcohol on the liver independent from and without affecting alcohol metabolism.

Several studies suggest that boosting the FGF21 system attenuates the damaging effects of alcohol on the liver. One study using transgenic overexpression of FGF21 (mostly in liver due to *ApoE* promoter driving overexpression for *FGF21*) were resistant to alcohol-induced fatty liver and metabolic disorders, and had reduced plasma alanine aminotransferase levels, disrupted caspase-3-dependent apoptosis, and blunted pro-inflammatory cytokines.[73] Zhu et al. administered 5 g/kg alcohol by oral gavage to male mice every day over the course of 6 weeks, which produced considerable fatty liver and liver injury. After the 6-week alcohol exposure, mice were treated with FGF21 (2 mg/kg) or vehicle every day for the next 3 weeks. Mice were then sacrificed after an overnight fast and liver and blood samples were collected. FGF21 treatment reversed several markers indicative of alcohol-induced liver injury. Data from this study also suggested that FGF21 counteracts fatty acid accumulation in the liver by promoting AMPK-SIRT1 signaling pathway.[78] Another study by Liu et al. found that following alcohol exposure (Lieber-DeCarli alcohol liquid diet for 4 week (5% alcohol)), hepatic expression and circulating FGF21 levels increased, and that rFGF21 administration (4 mg/kg/day, i.p.) significantly attenuated chronic alcohol-induced hepatic fat accumulation and inflammation in WT mice.[34] Another study showed that genetic knockout of the *Tgr5* gene protected mice from alcohol-induced accumulation of hepatic triglycerides and increased liver and serum FGF21 levels.[79] A different study showed that liver-specific knock out of the Zinc finger protein 36 like 1 gene (*ZFP36L1*) in mice protected against alcohol-induced hepatic steatosis, liver injury, and liver inflammation, and resulted in elevated *FGF21* mRNA levels in the liver.[80] In summary, experimental increases in FGF21 signaling attenuate liver damage produced by alcohol.

In addition to the protective effects of FGF21 on the liver, some studies have shown that FGF21 also protects the heart from alcohol-induced damage. One study examined postmortem cardiac samples from people with a history of heavy alcohol use (≥60 g/day for 10 years) with and without cardiomyopathy, and a healthy control group. FGF21 and β-Klotho levels were elevated in the heavy alcohol groups compared to controls, and protein levels were highest in the group with cardiomyopathy. A subsequent experiment using the Lieber-DeCarli alcohol diet in *FGF21*-KO and WT mice found that FGF21 levels were elevated in blood, liver, and myocardium after alcohol exposure in WT mice. *FGF21*-KO mice developed greater cardiac hypertrophy, fibrosis and cardiac dysfunction compared to WT.[81]

Oxidative stress plays a key role in mediating the in and inflammatory response and liver damage induced by alcohol.[82] Excessive reactive oxygen species (ROS) disrupt homeostasis, resulting in oxidative stress, which triggers hepatic damage by inducing irreversible alteration of lipids.[82] Liver disease-induced oxidative stress can damage extra-hepatic organs, and FGF21 may mitigate this damage, as evidenced in mouse models of ALD where FGF21 suppresses. Preclinical studies in mouse models of ALD show that FGF21 suppresses oxidative stress in peripheral organs such as the liver and cardiac tissue.[78,81,83] Li et al. found that after a high-fat diet and one-week binge alcohol gavage, mice lacking cathelicidin-related antimicrobial peptide (CRAMP), an antimicrobial peptide that helps maintain a balanced microbiota, compared to wild-type mice, had elevated FGF21 levels in epididymal white adipose (eWAT) mass and increased serum adiponectin secretion, which has been shown to alleviate hepatic fat deposition and inflammation.[84] Given that after alcohol exposure, hepatic Camp mRNA significantly increased in WT mice,[85] which has been associated with increased reactive oxygen species production, and that FGF21 inhibits oxidative stress through cAMP response element binding protein (CREB) activation,[86] which regulates adiponectin gene expression,[87] a working hypothesis is that CRAMP deficiency upregulates FGF21/CREB/adiponectin signaling to limit alcohol-induced oxidative stress.

Together, these studies demonstrate that multiple molecular signaling effects of FGF21 counteract alcohol-associated organ damage, e.g., on the liver and heart. FGF21 is upregulated in response to alcohol, which is thought to serve as a protective and homeostatic regulator of alcohol-related damage, including oxidative stress and inflammation. FGF21 emerges as a novel and promising target for the treatment of ALD.

### FGF21 and other substances

Although we have focused until now on the potential link between FGF21 and alcohol-related outcomes, initial work suggests that FGF21 is also involved in the effects of other substances. One study showed that cigarette smokers had higher circulating FGF21 levels than non-smokers, in both male and female participants.[42] The effect of cigarette smoking cessation on circulating FGF21 levels was explored in a clinical study where smokers were treated with either varenicline or a nicotine replacement therapy via a transdermal nicotine patch as part of a 12-week smoking cessation program. There was no significant difference in circulating FGF21 in either the group that quit smoking nor the group that did not.[88] An *in vitro* study found that cannabidiol (CBD) enhanced the levels of *FGF21* mRNA, and other genes involved in browning of white adipocytes in a mouse cell line of adipocytes.[89] CBD administration in mouse model of alcohol-induced steatosis led to less oxidative stress,[90] suggesting that part of the protective and anti-inflammatory effects of CBD may be driven by increased FGF21 expression.[89] In one study, *FGF21* transgenic overexpression resulted in lower morphine preference than WT control mice, demonstrating that FGF21 is involved in preference for morphine reward. This study also found that *FGF21* overexpression blunted the magnitude and rate of acute morphine antinociceptive tolerance development, and blunted acute and chronic morphine physical dependence.[91]

These studies suggest that FGF21 is not only involved in alcohol seeking and other related outcomes such as liver dysfunction but may also interact with other substances. The relative scarcity of research on FGF21 and substances other than alcohol highlights a need for more research on FGF21 and substances other than alcohol. Of note, the basic function of FGF21 signaling in suppressing consumption of calorie rewards to maintain organismal homeostasis suggests that FGF21 plays a role in satiety mechanisms for other drugs and has potential as a therapeutic for other substances by signaling satiety. This mechanistic overlap between reward consumption and satiety signals for food (a "natural reward") and substances of addiction (chemical compound(s) with the biochemical properties that happen to act on reward consumption and satiety signaling) has been highlighted in the literature for decades.[10]

## DISCUSSION

### Summary of evidence

Most publications that met eligibility for inclusion in this scoping review investigated the relationship between the FGF21 system and alcohol. Overall, FGF21 appears to produce compensatory and/or protective mechanisms against the deleterious effects of alcohol and other addictive substances. Alcohol directly acts on the liver to upregulate FGF21 and then to impact different cell types. One effect of elevated FGF21 is decreased motivation for alcohol through signaling in neurons that project to dopamine neurons in the nucleus accumbens (part of the striatum).[11] As such, hepatic increases in FGF21 from alcohol signals through neurons to attenuate continued alcohol use that protect the liver from additional damage. In addition, FGF21 protects against fat accumulation, inflammation, and damage in the liver, decreases alcohol-related inflammation, and attenuates alcohol-related oxidative stress. Other effects of FGF21 signaling include counteracting the sedative and intoxicating effects of alcohol, and increasing motivation for alcohol-induced water consumption, the latter being a potential compensatory effect. Preliminary work suggests that FGF21 may also be involved in the effects of other addictive substances, hence posing the question whether FGF21 may play a role not only in AUD but also in SUDs at large.

## CONCLUSIONS

Together, the published literature suggests a potential dual liver-brain role of FGF21, hence making the FGF21 system a putative pharmacotherapeutic target for people with AUD and ALD comorbidity. In fact, alcohol-induced increase in hepatic FGF21 signaling drives decreased motivation for alcohol that protects the liver and other systems from further damage by alcohol. FGF21 also decreases inflammation and oxidative stress and protects the liver from alcohol-induced fat accumulation and inflammation. Therefore, FGF21 or FGF21 analogs like PF-05231023 hold promise as therapeutics for AUD and ALD.

### Limitations of the study

Many animal studies use cell-type specific genetic knockout mouse lines combined with pharmacological experiments to precisely elucidate the relationship and mechanisms between FGF21 signaling and alcohol effects. However, the human studies are limited in number and nature (i.e., mostly association studies), hence clinical studies that manipulate FGF21 signaling and measure the effects of alcohol consumption are needed to establish causality. Although preclinical studies provide an important platform to elucidate the relationship between FGF21 and alcohol-related behaviors, such information still needs to be confirmed in randomized clinical trials in people with AUD.

The small number of peer-reviewed, published articles on FGF21 signaling and other substance limits our ability to draw robust conclusions. There is a need for more studies that investigate how FGF21 affects other substance-related outcomes, including but not limited to, opioids, cannabis, stimulants, and sedatives. Information relevant to how FGF21 analogues perform in comparison or in combination with other potential medications that are known to treat AUD and/or other SUDs is also of interest and remains to be explored.

There are also some notable potential limitations to FGF21 and FGF21 analogs as therapeutics for AUD and SUDs. Mostly, there are known side effects of FGF21 that need to be taken into account. Primarily, FGF21 may produce a stimulatory effect.[62] Other side effects include bone loss, increased heart rate, and high blood pressure.[28] Like any potential pharmacotherapy, the risk and benefits must be weighed, especially when a new pharmacotherapy is tested in people with different medical conditions. These results will only be obtained through well-controlled clinical trials in people with AUD.

## STAR METHODS

### Resource availability

#### *Lead contact*

Further information and requests should be directed to the lead contact, L.L. (lorenzo.leggio@nih.gov). Reasonable requests will be fulfilled by the lead contact.

#### *Materials availability*

This study did not generate unique reagents.

#### *Data and code availability*

As a review article, this work did not generate data or code.

### Experimental model and study participant details

As a review article, this work did not use study subjects, but summarizes previously published work in human subjects and animal models.

### Method details

#### *Protocol registration and search strategy*

This scoping review followed PRISMA-ScR (Preferred Reporting Items for Systematic Reviews Extension) guidelines. A biomedical research Informationist (DC) performed literature searches in PubMed (National Library of Medicine), Embase (Elsevier), and Web of Science (Clarivate) until August 2022. No language or time restrictions were used in the searches. Our search strategies used controlled vocabulary terms and keywords relevant to "fibroblast growth factor 21"; "alcohol use"; and "substance use". Search strategies for each database are in appendix 1.

#### *Data extraction*

Search results were exported into EndNote (Clarivate Analytics, Philadelphia, PA) and then uploaded to Covidence (Veritas Health Innovation, Melbourne, Australia). Two reviewers (TW, OI) independently screened the title and abstract of the articles in Covidence to include in the scoping review. The same reviewers examined the full texts of the identified inclusion papers to ensure they met the eligibility criteria. Conflicts at each stage of the review were resolved by a third reviewer (MF). Inconsistencies were discussed and resolved through consensus. The following inclusion criteria were used: primary data and peer-reviewed articles on the subject of FGF21, its analogs, or FGFR1/KLB agonists plus the use of alcohol or other substances (e.g., nicotine, cannabis, cocaine, amphetamine); adults ($\geq$18 years) who partook in randomized/nonrandomized trials or observational studies in an inpatient or outpatient settings; animal studies that involved measurement of endogenous FGF21; administration of exogenous FGF21 or FGF21 analogs; *FGF21* knockout (KO); *KLB* KO; and *FGFR1* KO models.

We excluded papers on studies without retrievable full texts such as abstracts and posters. Opinion papers, news/magazine articles, dissertations, conference proceedings, reviews, meta-analyses, and case reviews were excluded. Articles that did not contain information related to alcohol or other substance use were excluded.

#### *Selection of sources of evidence*

See Figure 1 for a schematic of article selection. See Table 1 for a description of each selected publication. Many studies evaluated the FGF21 system in the context of alcohol use and/or alcohol-induced liver injury. Major themes in the literature guided the organization of the review and subsections. Figure 2 is a schematic of major findings from humans.

**The Crosstalk Between Fibroblast Growth Factor 21 (FGF21) and Alcohol and Substance Use Disorders (ASUDs): A Scoping Review**

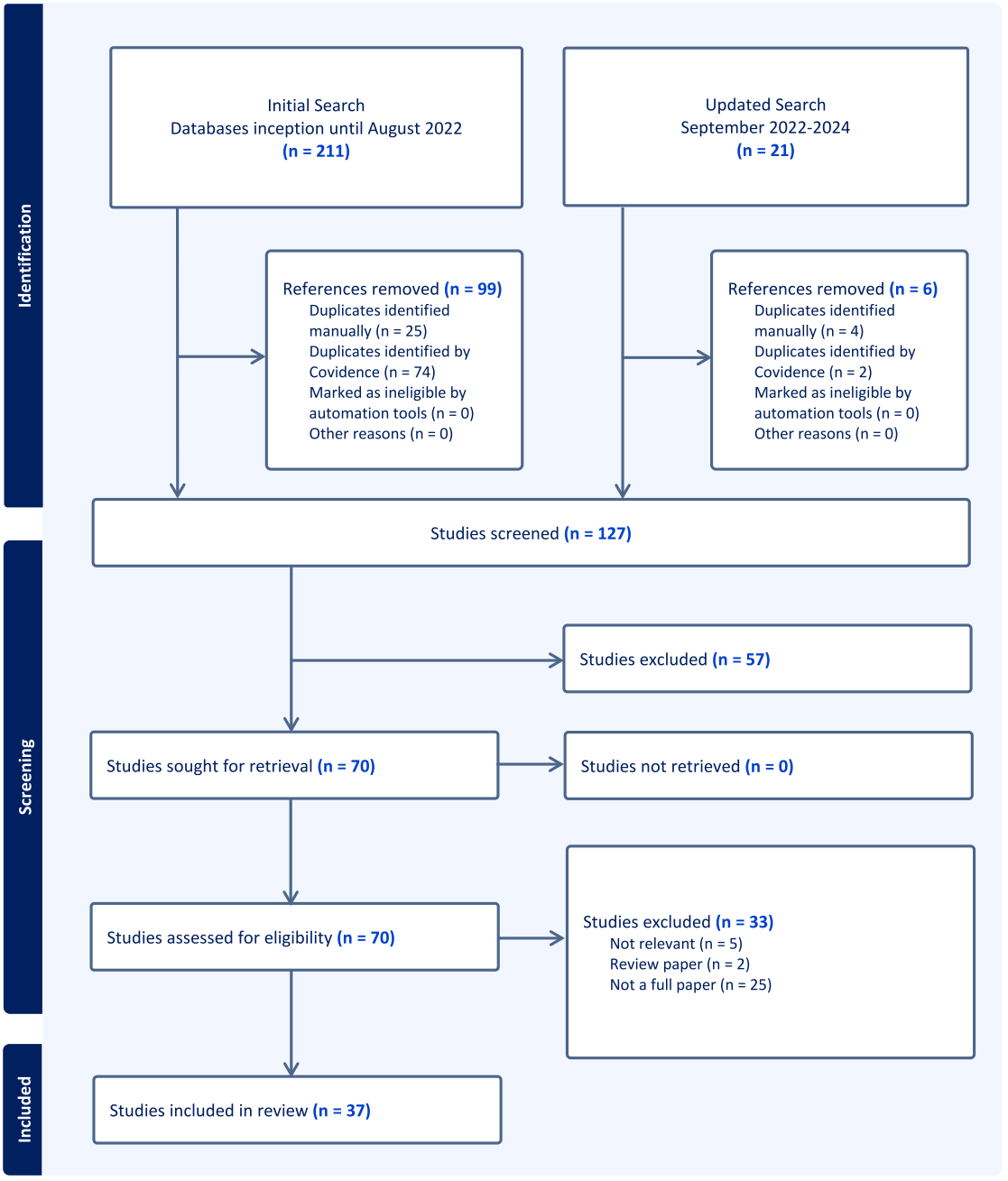


**Figure 1. PRISMA-ScR flow diagram**

The PRISMA-ScR flow diagram details database searches, title and abstract screening, number of full-text articles, and number of articles data was extracted from in this review. A total of 37 publications were included.

**Table 1. Summary of selected publications**

| Substance | Participants/Subjects | Methods | Results | Reference |
|---|---|---|---|---|
| Alcohol | • Mice<br>• Vervet Monkeys | • Alcohol gavage (3.5 g/kg)<br>• Two-bottle choice<br>• Lieber-DeCarli alcohol diet<br>• *FGF21* deletion in liver (LivKO)<br>• FGF21 and PF-05231023 admin.<br>• BLA FGF21/PF-05. admin.<br>• Anterograde tracing in KLB-expressing neurons<br>• Electrophysiology in cell-type and circuit-specific neurons. | • *FGF21* LivKO blocks alcohol-induced increase in FGF21 in mice<br>• *FGF21* LivKO mice consume more alcohol.<br>• FGF21 and PF-05231023 systemic admin. reduced alcohol consumption in mice and monkeys<br>• FGF21 and PF-05. in BLA reduced alcohol consumption in mice<br>• FGF21 suppresses alcohol consumption through $KLB^{BLA}$→NAc circuitry in mice | Flippo et al.[11] |
| Alcohol | Humans | • 3 groups: Alcoholic Liver Cirrhosis (ALC), Non-alcoholic liver cirrhosis (NALC), and healthy controls (HC)<br>• FGF21 levels in blood<br>• urinary ethyl glucuronide (uETG) measured – indicating alcohol consumption in the last 12–72 h | • Participants with elevated ETG levels (both ALC and NALC) showed higher FGF21 levels compared to participants with negative ETG levels | Wagner-Skacel et al.[13] |
| Alcohol | • Humans<br>• Mice | • Humans: FGF21 levels in people with ALD (hepatic steatosis, stable cirrhosis) and healthy controls<br>• Mice: Lieber-DeCarli alcohol diet<br>• *FGF21*-KO<br>• FGF21 admin. in WT mice<br>• Measure FGF21 in liver and blood | • Humans: Hepatic steatosis grp showed highest FGF21 levels – cirrhosis grp similar to HC<br>• Mice: Alcohol diet increased FGF21 in liver and blood<br>• *FGF21*-KO mice showed more severe hepatic steatosis and higher inflammatory markers.<br>• FGF21 admin. decreased alcohol-induced hepatic fat accumulation and inflammation in WT mice | Liu et al.[34] |
| Alcohol | • Humans | • Danish Inter99 cohort<br>• *FGF21* gene variants associated with food and drug intake<br>• Separate clinical study measured FGF21 levels in response to sucrose intake | • FGF21 rs838133 associated with increased candy and alcohol intake<br>• Plasma FGF21 levels upregulated following sucrose intake | Søberg et al.[37] |
| Alcohol | • Humans<br>• *In vitro* | • Humans: AUD grp - GWAS in grp that has alcohol timeline follow back (90-day) and FGF21 levels in blood<br>• *In vitro*: iPSC-derived brain organoids | • Humans: *SNHG16* rs9914222 SNP was associated with higher FGF21 levels and AUD risk<br>• Recent alcohol consumption within 90 days prior to blood sampling was positively correlated with plasma FGF21 levels<br>• *In vitro*: Alcohol induced FGF21 in brain organoids | Ho et al.[38] |
| Alcohol | • Humans<br>• Mice | • Humans: GWAS: >105,000 European Ancestry<br>• Identify SNPs related to alcohol consumption<br>• Mice: Two-bottle choice<br>• FGF21 admin.<br>• Neuron-specific *KLB*-KO mice | • Humans: GWAS identified SNP rs11940694 in *KLB* associated with reduced alcohol drinking<br>• Mice: *KLB*-KO mice have increased alcohol preference and unresponsive to FGF21 admin.<br>• Alcohol metabolism did not differ between *KLB*-KO and WT mice | Schumann et al.[39] |

(*Continued on next page*)

**Table 1. *Continued***

| Substance | Participants/Subjects | Methods | Results | Reference |
|---|---|---|---|---|
| Alcohol | • Humans | • Sorb cohort ($n$ = 1046) – genotyped the *FGF21* locus ±10 kb<br>• Measured blood levels of FGF21 among other markers | • *FGF21* genetic variants associated with anthropometric and metabolic parameters, adipokines, food and substance craving, and low-density lipoprotein cholesterol<br>• FGF21 levels were associated with coffee and alcohol consumption as well as smoking and eating behavior | Epperlein et al.[41] |
| Nicotine | • Humans | • Smokers and never-smokers<br>• Assessed FGF21 and IL-6 | • Serum FGF21 and IL-6 were higher in smokers compared to never-smokers in male and female participants<br>• Serum FGF21 was correlated with total cholesterol, triglycerides, and HbA1c in female participants | Nakanishi et al.[42] |
| Alcohol | • Humans | • Explored the association between *FGF19*, *FGF21* and *FGF23* gene SNPs and occurrence of alcohol dependence (AD) and aggression | • *FGF19* rs948992 TC × *FGF21* rs11665896 GG combination associated with high risk of AD<br>• *FGF19* rs948992 TC × *FGF21* rs11665896 TG × *FGF23* rs11063118 TT combination associated with high level of aggression in AD | Xu et al.[43] |
| Alcohol | • Mice<br>• Cynomolgus Monkey | • Two-bottle choice - sucrose and alcohol<br>• *FGF21* transgenic overexpression (Tg (*FGF21*))<br>• neuron specific KLB-KO ($KLB^{Camk2a}$)<br>• PF-05. tested on sweet preference in monkeys | • Tg (*FGF21*) mice showed reduced alcohol and sucrose preference<br>• FGF21 admin decreases sweet preference in mice<br>• PF-05 decreased sweet preference in monkeys<br>• *KLB*-KO mice do not show the effect of FGF21 on decreased sucrose preference | Talukdar et al.[44] |
| Alcohol | • Humans<br>• Mice | • Humans: Fasted males admin. placebo or alcohol<br>• pre & post Oktoberfest<br>• Mice: Two-bottle choice<br>• FGF21 admin. | • Humans: Acute alcohol ingestion and sustained binge-drinking during Octoberfest increased FGF21 plasma levels<br>• Mice: FGF21 admin. decreased alcohol consumption | Søberg et al.[45] |
| Alcohol | • Humans<br>• Mice | • Humans: alcohol or juice (veh.) oral admin. in healthy controls to measures blood FGF21<br>• Mice: Alcohol bolus over 8-day<br>• *FGF21*-KO<br>• *KLB*-KO in neurons<br>• FGF21 admin.<br>• *KLB*-Sim1 KO (enriched in paraventricular nucleus of the hypothalamus - PVN) | • Humans: Alcohol increased FGF21 compared to vehicle and baseline<br>• Mice: Alcohol increased water intake in WT, but not *FGF21*-KO mice<br>• FGF21 admin. increased water consumption in WT but not in neuron *KLB*-KO<br>• FGF21 effects on water intake may be mediated by Sim1- and KLB-expressing neurons in the PVN | Song et al.[46] |

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**Table 1. *Continued***

| Substance | Participants/Subjects | Methods | Results | Reference |
|---|---|---|---|---|
| Alcohol | • Humans<br>• Mice | • Humans: Alcohol binge intake – measure blood FGF21<br>• Mice: Alcohol i.g. and i.p. admin.<br>• Lieber-DeCarli alcohol diet<br>• Alcohol in drinking water<br>• Two-bottle choice<br>• *FGF21*-KO<br>• *FGF21* overexpression | • Humans: Alcohol increased FGF21 in blood<br>• Mice: Alcohol increased FGF21 in blood and liver<br>• Alcohol diet increased liver expression of pro-inflammatory markers and liver enzymes – exacerbated in *FGF21*-KO<br>• Alcohol diet produced greater hepatic steatosis, fibrosis, inflammatory cytokines, and death, in *FGF21*-KO compared to WT<br>• WT and *FGF21*-KO mice showed preference for alcohol over water<br>• FGF21 overexpression and WT mice injected with FGF21 showed preference for water over alcohol | Desai et al.[47] |
| Alcohol | • Humans<br>• Mice | • Human: Alcohol-related liver disease (ALD) and Healthy controls<br>• Mice: Lieber-DeCarli alcohol diet | • Humans: ALD participants showed higher FGF21 than HC<br>• Mice: Alcohol increased hepatic FGF21 mRNA and protein | Christidis et al.[48] |
| Alcohol | • Mice | • Lieber-DeCarli alcohol diet + alcohol gavage<br>• *FGF21*-KO | • Alcohol increased FGF21 in blood, liver and eWAT, which were attenuated or blocked in *FGF21*-KO mice | Zhao et al.[49] |
| Alcohol | Mice | • Adipocyte specific *Mtor* and *Rptor* knock out mice<br>• Alcohol gavage | • *Mtor* and *Rptor* KO mice showed decreased basal levels of FGF21, which were increased following alcohol gavage | Rodriguez[50] |
| Alcohol | • Humans | • Roskilde festival participants and non-festival grp | • Festival grp engaged in binge drinking<br>• FGF21 elevated after festival (1 week) | Demant et al.[57] |
| Alcohol | • Humans | • Subjects received alcohol via nasogastric tube | • Alcohol increased FGF21 and downregulated inflammatory markers in systemic and hepatic blood samples | Stankevic et al.[58] |
| Alcohol | • Humans | • Intragastric or intravenous alcohol admin. | • Both routes produced robust increases in FGF21 levels with similar pharmacokinetics | Lanng et al.[59] |
| Alcohol | • Humans<br>• *In vitro* | • Follow up analyses of above and additional brain organoid experiments | • Humans: Plasma FGF21 levels positively correlated with plasma GABA levels<br>• In vitro: FGF21 signaling profoundly impacts gene expression related to GABAergic synaptic function | Ho et al.[60] |
| Alcohol | • Humans | • AUD grp undergo bar-lab cue-reactivity, alcohol priming and alcohol self-admin. | • FGF21 levels were elevated after the 2-h alcohol self-admin session | Farokhnia et al.[61] |

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**Table 1. *Continued***

| Substance | Participants/Subjects | Methods | Results | Reference |
|---|---|---|---|---|
| Alcohol | • Mice | • Alcohol admin. (i.g.)<br>• *FGF21*-KO mice<br>• *KLB*-KO mice<br>• Behavioral tests of intoxication and sedation by alcohol<br>• Investigated norepinephrine neurons in the locus coeruleus as mechanism for the anti-intoxicating effects of FGF21 | • FGF21 elevated following alcohol in admin in WT but not *FGF21*-KO<br>• *FGF21*-KO enhanced the sedative and intoxicating effects of alcohol<br>• FGF21 admin. blunted the sedative and intoxicating effects of alcohol<br>• Alcohol increased activity of NET+ (norepinephrine) neurons in the locus coeruleus in WT but absent in *FGF21*-KO<br>• FGF21 produced its anti-intoxicating effects by acting on norepinephrine neurons in the locus coeruleus and promoting arousal | Choi et al.[62] |
| Alcohol | • Humans | • Individuals with varying degrees of alcohol-related cirrhosis using the Child-Pugh score and related categories (P-Ch A, B, and C) and Healthy Controls | • FGF21 levels increased in participants with liver cirrhosis compared to HC<br>• FGF21 levels were highest in P-Ch A and B grp compared to P-Ch C | Sak et al.[71] |
| Alcohol | • Humans | • People with varying degrees of ALD: alcohol-associated hepatitis vs. decompensated alcohol-associated cirrhosis groups.<br>• Serum FGF21 measured in both groups. | • Serum FGF21 levels were higher in the hepatitis vs. the cirrhosis group. | McLean[72] |
| Alcohol | • Mice<br>• Humans | • Mice: chronic plus binge alcohol feeding: Lieber DeCarli Liquid Diet + oral gavage<br>• *FGF21*-Tg mice – overexpression of *FGF21* mostly in liver (*ApoE* promoter)<br>• *FGF21*-KO mice<br>• Humans: postmortem liver samples from healthy controls and from people with ALD | • Mice: FGF21-Tg mice were resistant to alcohol-induced fatty liver and metabolic disorders (reduced hepatic triglyceride overproduction and normalized hyperlipidemia).<br>• FGF21-Tg mice showed reduced plasma alanine aminotransferase levels, disrupted caspase-3-dependent apoptosis, and blunted pro-inflammatory cytokine increases from alcohol<br>• In contrast, FGF21-KO mice showed exacerbated hepatic steatosis, lipotoxicity and inflammation due to alcohol<br>• Humans: SRPK2, pSRp20, and nuclear SREBP-1 were elevated in ALD livers compared to CTRL. | Li et al.[73] |
| Alcohol | • Mice | • Adipocyte specific knock out of *FGFR1* (*fgfr1*$^{adipoQ\text{-}cre}$)<br>• Lieber-DeCarli alcohol diet with or without WY-14,643 - drug induces FGF21 | • Liver and serum triglyceride levels increased by alcohol to a greater extent in the *fgfr1*$^{adipoQ\text{-}cre}$ mice compared to WT.<br>• WY-14,643 blunted the liver triglyceride accumulation in the *fgfr1*$^{adipoQ\text{-}cre}$ mice | Xu et al.[74] |

(*Continued on next page*)

**Table 1. *Continued***

| Substance | Participants/Subjects | Methods | Results | Reference |
|---|---|---|---|---|
| Alcohol | • Mice | • Adipocyte specific knock out of *FGFR1* (*fgfr1*$^{adipoQ\text{-}cre}$)<br>• Binge-like drinking with or without WY-14,643<br>• FGF21 admin. | • Replicated most results of Xu et al. (74) using a binge-like drinking model<br>• Circulating FGF21 was elevated by binge alcohol to a greater extent in *fgfr1*$^{adipoQ\text{-}cre}$ mice<br>• FGF21 admin. induced adipose atrophy, blunted alcohol-induced fatty liver and serum TG elevation to a greater extent in *fgfr1*$^{adipoQ\text{-}cre}$ mice | Xu et al.[75] |
| Alcohol | • Mice | • *FGF21*-KO mice<br>• Alcohol gavage (5 g/kg)<br>• Alcohol concentrations measured over time after gavage<br>• Gastric and Liver tissue collected | • Alcohol concentrations lower in *FGF21*-KO compared to WT 30-min after gavage<br>• No difference in ADH and ALDH2 activity in gastric or liver tissue b/t genotypes<br>• Gastric emptying time was longer, greater intestinal permeability, and higher GLP-1 levels in *FGF21*-KO compared to WT | Wu et al.[76] |
| Alcohol | • Mice | • Lieber-DeCarli alcohol diet<br>• Genetic knockouts of different proteins<br>• KO of Cytochrome P450 2A5 (CYP2A5): *Cyp2a5*-KO<br>• KO of Peroxisome proliferator-activated receptor α (PPARα): *Pparα*-KO<br>• *FGF21*-LivKO (liver-specific KO) | • *Cyp2a5*-KO mice showed higher FGF21 levels and more severe alcoholic fatty liver disease than WT<br>• Alcohol did not elevate FGF21 levels in *Cyp2a5*-KO, but did in WT<br>• *Pparα*-KO mice showed lower FGF21 levels<br>• Alcohol induced FGF21 in WT but not in *Pparα*-KO mice<br>• Alcohol induced hypertriglyceridemia in *Pparα*-KO but not WT. Admin of FGF21 normalized triglyceride levels.<br>• *FGF21*-LivKO showed greater alcohol-induced fatty liver compared to WT | Chen et al.[77] |
| Alcohol | • Mice | • Alcohol (5 g/kg/day i.g.) admin. daily for 6 weeks<br>• FGF21 admin. (2 mg/kg/day) following alcohol exposure for 3 weeks | • Alcohol exposure induced significant fatty liver and injury<br>• FGF21 treatment reduced damage to the liver<br>• FGF21 counteracts fatty liver accumulation by prompting hepatic AMPK-SIRT1 pathway | Zhu et al.[78] |
| Alcohol | Mice | • *Tgr5*-KO mice<br>• Lieber-DeCarli liquid diet + alcohol gavage | • *Tgr5*-KO mice were protected from alcohol-induced accumulation of hepatic triglycerides.<br>• *Tgr5*-KO mice showed elevated liver and serum FGF21 levels following alcohol exposure | Pokhrel[79] |
| Alcohol | Mice | • Liver-specific knockout of *ZFP36L1* gene (*ZFP36L1*-KO)<br>• Lieber-DeCarli alcohol diet | • *ZFP36L1*-KO mice were protected against alcohol-induced hepatic steatosis, liver injury and liver inflammation compared to WT mice.<br>• *FGF21* mRNA was elevated in the liver of *ZFP36L1*-KO mice compared to WT controls | Bathula et al.[80] |

(*Continued on next page*)

**Table 1. *Continued***

| Substance | Participants/Subjects | Methods | Results | Reference |
|---|---|---|---|---|
| Alcohol | • Humans<br>• Mice | • Humans: Postmortem myocardium samples from people with a history of alcohol intake (≥60 g/day for 10 years) with and without alcoholic cardiomyopathy and healthy controls<br>• Mice: *FGF21*-KO<br>• Lieber-DeCarli alcohol diet | • Humans: FGF21 and β-klotho levels were elevated in cardiac samples from people with a history of high alcohol intake and to the greatest extent in those with alcoholic cardiomyopathy<br>• Measures of oxidative stress in the myocardium correlated with FGF21 expression<br>• Mice: FGF21 levels in blood, myocardium and liver were elevated after alcohol exposure in WT<br>• *FGF21*-KO mice developed higher degree of cardiac hypertrophy, fibrosis and cardiac dysfunction | Ferrer-Curriu and Guitart-Mampel et al.[81] |
| Alcohol | • Mice | • Chronic-plus-binge alcohol<br>• Adipocyte-specific KO of *Atg5* ("autophagy related" gene)<br>• Measured levels of circulating FGF21 | • Adipocyte specific *Atg5* KO mice showed increased circulating levels of FGF21 and adiponectin and were resistant to alcohol-induced adipose atrophy and liver injury | Li et al.[83] |
| Nicotine | • Humans | • Smokers treated with varenicline or a transdermal nicotine patch | • Smoking cessation did not affect FGF19 or FGF21 levels compared the unsuccessful smoking cessation group | Kamizono et al.[88] |
| CBD | • Mouse cell line | • 3T3-L1 preadipocytes cultured<br>• adipocytes treated with CBD during differentiation into mature adipocytes<br>• browning of adipocytes was induced in culture | • *FGF21* mRNA was upregulated in response to browning adipocytes and CBD treatment | Parray et al.[89] |
| Morphine | • Mice | • FGF21-transgenic (*FGF21*-Tg) mice<br>• Morphine conditioned place preference<br>• Behavioral measures of analgesic tolerance | • *FGF21*-Tg mice displayed reduced preference for morphine in CPP test<br>• *FGF21*-Tg mice showed blunted magnitude and rate of acute morphine antinociceptive tolerance development<br>• *FGF21*-Tg mice showed blunted acute and chronic morphine physical dependence | Dorval et al.[91] |

The table summarizes the key aspects of each study including substance(s), subjects, methods and results as they relate to FGF21.

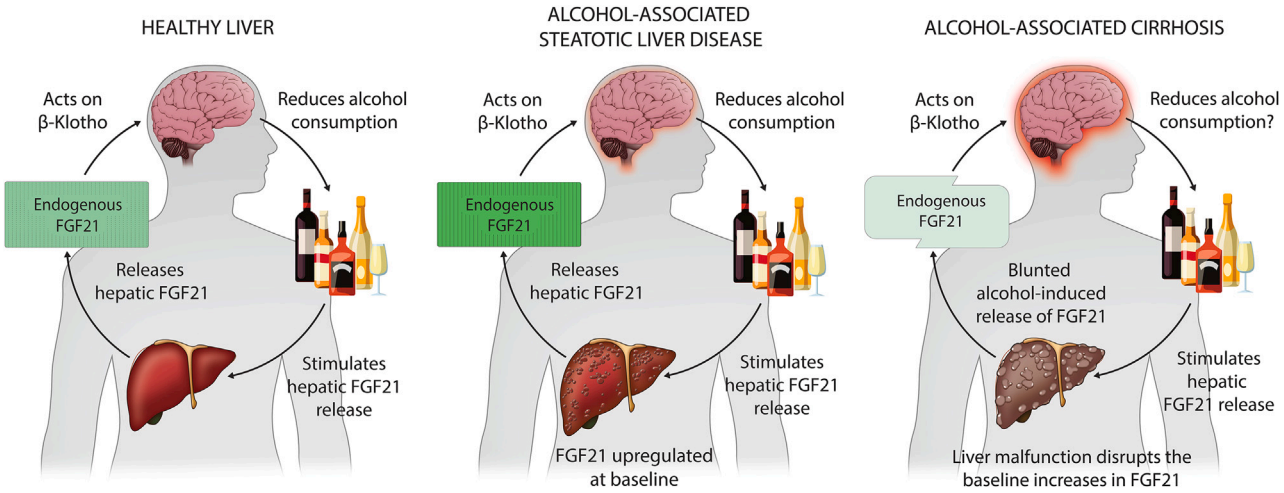


**Figure 2. Relationship between alcohol consumption and the FGF21 system**

In people with healthy livers, alcohol consumption stimulates the release of hepatic FGF21, which binds the receptor complex containing β-Klotho and FGFR1 in neurons to drive decreased alcohol consumption, creating a negative feedback loop to protect against alcohol damage. People with liver steatosis show elevated levels of hepatic FGF21 at baseline, and elevated hepatic FGF21 in response to acute alcohol. In people with liver cirrhosis, FGF21 levels are NOT elevated at baseline and show a blunted response to acute alcohol on hepatic FGF21 levels, which may disrupt the negative feedback loop that drives decreased alcohol consumption.

## Additional resources

Our protocol was pre-registered in Open Science Framework (DOI 10.17605/OSF.IO/VH287).

## APPENDIX 1. DATABASE SEARCH STRATEGIES

### PubMed = 56 citations

("Fibroblast growth factor 21"[tiab] OR FGF21[tiab]) OR ("fibroblast growth factor 21"[tiab] AND (analog[tiab] OR analogue[tiab])) OR "fibroblast growth factor 21-receptor agonists"[tiab] OR "fibroblast growth factor 21 receptor analogues"[tiab] OR "fibroblast growth factor 21 analog"[tiab] OR BMS986171[tiab] OR AMG876[tiab] OR BFKB8488A[tiab] OR NGM313[tiab] OR AKR-001[tiab] OR LY2405319[tiab] OR BMS986036[tiab] OR PF-05231023[tiab] OR BFKB8488A[tiab]) AND ("alcohol-related disorders"[Mesh] OR "alcohol disorder*"[tiab] OR alcoholism[Mesh] OR alcoholism[tiab] OR "alcohol drinking"[Mesh] OR "alcohol drinking"[tiab] OR ethanol[Mesh] OR ethanol[tiab] OR "substance-related disorders"[Mesh] OR "substance related disorder*"[tiab] OR "substance use"[tiab] OR opioids[tiab] OR tobacco[Mesh] OR tobacco[tiab] OR amphetamine[Mesh] OR amphetamine[tiab] OR cannabis[Mesh] OR cannabis[tiab] OR marijuana[tiab] OR cocaine[Mesh] OR cocaine[tiab] OR "crack cocaine"[tiab] OR morphine[Mesh] OR morphine[tiab])

### Embase = 110 citations

('fibroblast growth factor 21':ti,ab OR 'fgf21':ti,ab OR 'fibroblast growth factor 21-receptor agonists':ti,ab OR 'fibroblast growth factor 21 receptor analogues':ti,ab OR 'fibroblast growth factor 21 analog':ti,ab OR 'bms986171':ti,ab OR 'amg876':ti,ab OR 'ngm313':ti,ab OR 'akr 001':ti,ab OR 'ly2405319':ti,ab OR 'bms986036':ti,ab OR 'pf 05231023':ti,ab OR 'bfkb8488a':ti,ab) AND ('alcohol-related disorders'/exp OR 'alcohol-related disorders' OR 'alcohol disorders':ti,ab OR 'alcoholism'/exp OR 'alcoholism' OR 'alcoholism':ti,ab OR 'alcohol drinking'/exp OR 'alcohol drinking' OR 'alcohol drinking':ti,ab OR 'ethanol'/exp OR 'ethanol' OR 'ethanol':ti,ab OR 'substance-related disorders'/exp OR 'substance-related disorders' OR 'substance related disorders':ti,ab OR 'substance use':ti,ab OR 'opioids':ti,ab OR 'tobacco'/exp OR 'tobacco' OR 'tobacco':ti,ab OR 'amphetamine'/exp OR 'amphetamine' OR 'amphetamine':ti,ab OR 'cannabis'/exp OR 'cannabis' OR 'cannabis':ti,ab OR 'marijuana':ti,ab OR 'cocaine'/exp OR 'cocaine' OR 'cocaine':ti,ab OR 'crack cocaine':ti,ab OR 'morphine'/exp OR 'morphine' OR 'morphine':ti,ab)

### Web of Science = 40 citations

(TS="Fibroblast growth factor 21" OR TS=FGF21 OR TS="fibroblast growth factor 21-receptor agonists" OR TS="fibroblast growth factor 21 receptor analogues" OR TS="fibroblast growth factor 21 analog" OR TS=bms986192 OR TS=amg176 OR TS=BFKB8488A OR TS=nem316 OR TS=bms986012 OR TS=PF-05231023 OR TS=BFKB8488A) AND (TS="alcohol-related disorders" OR TS="alcohol disorders" OR TS=alcoholism OR TS ="alcohol drinking" OR TS="alcohol drinking" OR TS=ethanol OR TS="substance-related disorders" OR TS="substance use" OR TS=opioids OR TS=tobacco OR TS=tobacco OR TS=amphetamine OR TS=cannabis OR TS=marijuana OR TS=cocaine OR TS="crack cocaine" OR TS=morphine)

## ACKNOWLEDGMENTS

We would like to thank David Taylor from the NIDA Visual Media team for their help with the Graphical Abstract and Figure 2.

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## AUTHOR CONTRIBUTIONS

Conceptualization: T.W., M.F., and L.L.

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Validation: T.W., R.E.T., O.I., D.C., M.F., and L.L.

Formal Analysis: T.W., R.E.T., O.I., and D.C.

Investigation: T.W., R.E.T., and O.I.

Resources: D.C. and L.L.

Data Curation: T.W., O.I., and D.C.

Writing – Original Draft: T.W., R.E.T., and O.I.

Writing – Review and Editing: T.W., R.E.T., D.C., M.F., and L.L.

Visualization: T.W. and R.E.T.

Supervision: R.E.T., D.C., M.F., and L.L.

Project Administration: D.C., M.F., and L.L.

Funding Acquisition: L.L.

## DECLARATION OF INTERESTS

Outside his federal employment and NIH work, L.L. serves as Editor-in-Chief for Alcohol and Alcoholism for which he receives an honorarium from the UK Medical Council on Alcohol, and he also receives royalties from Routledge for a textbook for which he served as one of the editors. The other authors declare no competing interests.

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